Childhood BMI and Pubertal Timing

Changes over time and associations with adult cardiovascular mortality

Maria Bygdell

Department of Internal Medicine and Clinical Nutrition
Institute of Medicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

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maria.bygdell@gu.se

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ABSTRACT

Background and Aim

Puberty in boys has long been an under-investigated area of research mainly due to the lack of an easily available pubertal marker, corresponding to menarche for girls. Using a unique, large, population-based cohort, this thesis targets some of the knowledge gaps regarding puberty and BMI change during puberty in boys. The aims of this thesis were to describe the long-term trend for pubertal timing and childhood BMI since the 1940s until today, and to characterize the association between childhood BMI and pubertal timing. In addition, the relative contribution of childhood BMI before puberty and BMI change during puberty for risk of adult cardiovascular mortality was evaluated.

Subjects and Methods

School has been mandatory in Sweden during the study period and with a near complete participation in the free school health care program with repeated and recorded standardized measurements of height and weight, the school health care material could be considered population-based. We collected information on height and weight throughout childhood until young adulthood for all included subjects from archived school health records and from the military conscription register, into the BMI Epidemiology study (BEST) cohort. Using these measurements, we calculated prepubertal childhood BMI (8 years of age), post pubertal young adult BMI (20 years of age), and BMI change during puberty. We also estimated age at the pubertal growth spurt, Peak Height Velocity (PHV) which is an objective assessment of pubertal timing, using a curve-fitting method. Through linkage with a high-quality register we obtained a near-complete follow-up time for subjects in the study and their underlying cause of death from the Cause of Death Register at the Swedish National Board of Health and Welfare.

Results

Childhood BMI and the prevalence of overweight and obesity among 8-year-old boys have overall increased substantially since birth year 1946 until birth year 2006. However, after a significant increase in BMI starting in the 1970s and with a peak at birth year 1991, we observed a moderate but significant decrease (Paper I). Furthermore, we have demonstrated that childhood BMI was inversely associated with pubertal timing in normal weight but not in overweight boys (Paper II). Age at PHV displayed a clear secular trend towards earlier puberty among boys. Since the 1940s until birth year 1996, the pubertal growth spurt was 1.5 months earlier per decade, and this trend could only slightly be explained by the coinciding increase in childhood BMI (Paper III). Moreover, we have made the novel observation that childhood BMI and BMI change during puberty is only marginally correlated (r=0.06) indicating that these developmental parameters may provide non-overlapping information for prediction of risk of adult disease (Paper IV). We demonstrated that BMI change during puberty, but not childhood BMI, was independently associated with the risk of adult cardiovascular mortality. The association between BMI change during puberty and cardiovascular mortality was non-linear, with 22% increased risk of cardiovascular mortality per additional increase in BMI units for individuals in the highest quartile. (Paper IV).

Conclusions

In conclusion, the measurements in school health care that has been ongoing for almost 100 years and included essentially all children in Gothenburg have together with information from the military conscription register provided data on height and weight both before and after puberty in a large cohort of boys. This unique material has enabled us to identify long-term trends of childhood BMI and pubertal timing. We identified a large BMI increase during puberty as a novel independent risk marker for increased risk of adult cardiovascular mortality. The results from this thesis have the potential to transmit directly into benefits for the society through adjustments of the school health care program for improved identification of individuals at risk. Our findings suggest that BMI should be monitored closely during the pubertal years.

Keywords: BMI Epidemiology Study, childhood BMI, BMI change during puberty, Peak Height Velocity, pubertal timing, cardiovascular mortality

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Barndoms-BMI och pubertet

Förändringar över tid och associationer med kardiovaskulär mortalitet

Bakgrund och Syfte

Kunskapen om pojkars pubertet är bristfällig, troligtvis på grund av avsaknaden av lättillgängliga pubertetsmarkörer. Medan det hos flickor har funnits en tydlig pubertetsmarkör, menarche, har forskningen försvårats av att motsvarande saknats hos pojkar. Genom att använda en unik och omfattande populationsbaserad kohort har målet med denna avhandling varit att utöka kunskapen rörande pojkars pubertet. Syftet med denna avhandling har varit (1) att beskriva långsiktiga trender för pubertetstid, barndoms-BMI och övervikt/fetma från 1940-talet fram till nutid, (2) att karaktärisera associationen mellan barndoms-BMI och pubertetstid, samt (3) att undersöka associationen mellan barndoms-BMI, BMI-förändring under puberteten och risk för kardiovaskulär mortalitet i vuxen ålder.

Metod

Under hela avhandlingens studieperiod har skolplikten utgjort obligatorisk skolgång för hela Sveriges befolkning. Då i princip alla elever tagit del av den kostnadsfria skolhälsovården, med upprepade standardiserade och sedermera arkiverade mätningar av längd och vikt, utgör skolhälsovårdsmaterialet ett populationsbaserat dataunderlag. Till BEST-kohorten har vi samlat in information om längd och vikt genom barndomen och fram till ung vuxen ålder från arkiverade skolhälsojournaler och mönstringsregistret. Från dessa mätningar har vi för varje individ beräknat prepubertalt barndoms-BMI (8 år), postpubertalt BMI (20 år) och BMI-förändring under puberteten. Genom kurvanpassning av längdmätningar, har vi kunnat beräkna en ålder då tillväxthastigheten under tillväxtspurten är som högst (Peak Height Velocity, PHV) vilket är ett objektivt mått på pubertetstid. Vi har länkat ihop BEST-kohorten med Dödsorsaksregistret hos Socialstyrelsen och på så sätt erhållit information om dödsorsaker.

Resultat

Barndoms-BMI och prevalens av övervikt och fetma hos 8-åriga pojkar har ökat markant från 1946 till nutid. Denna statistiskt signifikanta ökning började på 1970-talet och nådde en topp 1991 för att därefter sjunka till en något lägre nivå (studie I). Vi har också visat att barndoms-BMI är omvänt associerat med pubertetstid hos normalviktiga, men inte hos överviktiga, pojkar (studie II). Hos pojkar har vi även funnit en tydlig sekulär trend mot tidigare pubertetstid. Från 1940-talet fram till nutid har pubertetstillväxtspurten inträffat 1,5 månader tidigare per årtionde. Denna trend kan bara till viss del förklaras av den samtidiga ökningen av barndoms-BMI (studie III). Vidare har vi visat att BMI-förändring under puberteten, men dock inte barndoms-BMI, är självständigt associerat med risk för kardiovaskulär mortalitet i vuxen ålder. Associationen mellan BMI-förändring under pubertet och kardiovaskulär mortalitet var icke-linjär med 22% ökad risk för kardiovaskulär död per ökad BMI-enhet för individer i den högsta kvartilen. Vi visar även att barndoms-BMI och BMI-förändring under pubertet bara korrelerar marginellt ($r=0.06$), vilket indikerar att dessa två parametrar kan ge icke-överlappande information om risker för vuxen sjukdom (studie IV).

Slutsatser

Mätningarna inom skolhälsovården har pågått i över 100 år och har omfattat i stort sett alla skolbarn i Göteborg. Vi har genom dessa mätningar etablerat en stor, världsunik kohort med information om pojkars längd och vikt såväl före som efter puberteten. Detta unika material möjliggör studier av trender för BMI under barndomen och pubertet över långa tidsperioder. Vidare har vi för första gången kunnat isolera betydelsen av prepubertalt respektive postpubertalt BMI för kardiovaskulär dödlighet i vuxen ålder. Dessa resultat har potential att omvandlas till direkt tillämpad samhällsnytta som ett underlag för justeringar av skolhälsovårdsprogrammen för förbättrad identifikation av individer med risk för kardiovaskulär dödlighet i vuxen ålder. Studiens resultat antyder att BMI bör följas noggrant under pubertetsåren.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Bygdell M*, Ohlsson C*, Célind J, Saternus J, Sondén A, Kindblom JM. **The rise and recent decline of childhood obesity in Swedish boys: The BEST cohort**. *International Journal of Obesity* (Lond). 2017;May;41(5):807-812.

II. Bygdell M*, Kindblom JM*, Celind J, Nethander M, Ohlsson C. **Childhood body mass index is inversely associated with pubertal timing in normal weight but not in overweight boys**. *American Journal of Clinical Nutrition*. 2018;Dec;108(6):1259-1263.

III. Ohlsson C*, Bygdell M*, Celind J, Sondén A, Tidblad A, Sävendahl L, Kindblom JM. **A secular trend for earlier pubertal timing in boys born 1947-1996**. *Submitted manuscript.*

IV. Ohlsson C, Bygdell M, Sondén A, Rosengren A, Kindblom JM. **Association between excessive BMI increase during puberty and risk of cardiovascular mortality in adult men: a population-based cohort study**. *The Lancet Diabetes & Endocrinology*. 2016;4(12):1017-2104.

*Contributed equally

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CONTENT

ABBREVIATIONS

BEST	BMI Epidemiology Study
BMI	Body Mass Index
CDC	Centers for Disease Control and prevention
CI	Confidence Interval
DOHAD	Developmental Origins of Health and Disease
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
GOOD	Gothenburg Osteoporosis and Obesity Determinants (study)
HR	Hazard Ratio
ICD	International Classification of Diseases
ICP	Infancy-Childhood-Puberty (model)
IOTF	International Obesity Task Force
LDL	Low-Density Lipoprotein
LH	Luteinizing Hormone
LISA	Longitudinal Integration Database for Health Insurance and Labor Market Studies
PHV	Peak Height Velocity
PIN	Personal Identification Number
SCAPIS	Swedish CardioPulmonary bioImage Study
SD	Standard Deviation
WHO	World Health Organization

1 INTRODUCTION

1.1 Epidemiology

1.1.1 History of epidemiology

The medical sciences use different types of studies to investigate health and disease on individual and population levels. One such approach, epidemiology, is defined as:

"*The study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems.*" (1)

In 1854, the London physician John Snow[1] performed some of the earliest classical epidemiological work. While investigating an outbreak of cholera in London, he recorded all cholera deaths and additional variables, including the source of drinking water. He discovered that the majority of the cholera afflicted obtained their drinking water from pumps operated by a water company that fetched sewage-contaminated water from downstream the river Thames. People that were less affected by cholera obtained their drinking water from a company that fetched uncontaminated water from upstream the Thames. Both companies served drinking water to households of all kinds – rich and poor, large and small houses, people with differing conditions and occupations. Snow collected data on 350,000 men and women of all ages. This exemplifies a "natural experiment" wherein different water supplies were assigned to different households without their choice or knowledge. Snow showed among other things that there were 14 times more cholera fatalities in households with water from one company than in households with water from the other company. The contaminated water pumps were later closed, and the outbreak of cholera stopped. This initial work from Snow illustrates the nature of epidemiology, using a large amount of data to reveal patterns and associations to further the understanding of health and disease. From this initial work by Snow and other 19th century pioneers, epidemiology has evolved and developed into a well-used approach in medical sciences (2, 3).

[1] Not to be confused with his namesake Jon Snow of Winterfell fame.

1.1.2 Life course epidemiology

The idea that prenatal life has impact on adult health and disease originates from findings made by the late Professor David Barker (1938-2013) (4). He proposed the fetal origins hypothesis which states that undernutrition *in utero* permanently programs the metabolism, structure, and function of the human body on an adverse trajectory and through this, increases the risk of adult diseases (5-12). The concept of Developmental Origins of Health and Disease (DOHAD) has since then been extended to include growth in the entire developmental period, from fetal stages through infancy, childhood and puberty to adult age, and their associations with risk of different diseases. Barker used historical birth records and showed that low birth weight was associated with an increased risk of diseases such as adult cardiovascular disease and diabetes (13-15).

Another aspect of life course epidemiology is to describe changes in developmental risk factors over time, such as birth weight, childhood BMI, and pubertal timing. In trend studies it is necessary to have information from several time points over a relatively large period of time to be able to distinguish sustained trends from periodical fluctuations. Descriptive trend studies of developmental risk factors may indicate rising incidence of diseases before this potential increased incidence is established. Limitations of this type of research design include bias due to changes over time regarding other factors of importance e.g. nutrition, demographic, socioeconomic spectra, infectious disease epidemics, drug exposure, and other lifestyle factors. Confounding due to these changes over time are sometimes possible to adjust for but sometimes not.

1.1.3 Study designs in clinical research

Several different study designs are used in clinical research. Analytical studies are used to investigate causes or determinants of diseases or associations between exposures and outcomes. Descriptive studies can be used to study the distribution of exposure and disease (Figure 1). In an experimental study the researcher allocates the study exposure (intervention), often using randomization, thus enabling estimation of the true causal effect of the exposure. However, many studies cannot be performed using this design since it would be unethical and sometimes impossible to assign exposures to subjects, e.g. pubertal timing or high childhood BMI. In an observational study the exposure has not been randomized and therefore we cannot exclude the effect of potential confounders. Observational studies are usually divided into cohort studies, case-control studies, and cross-sectional studies (2). There are

also Mendelian randomization studies that use genetic variations to evaluate the causal effect of a modifiable (non-genetic) exposure on disease (16).

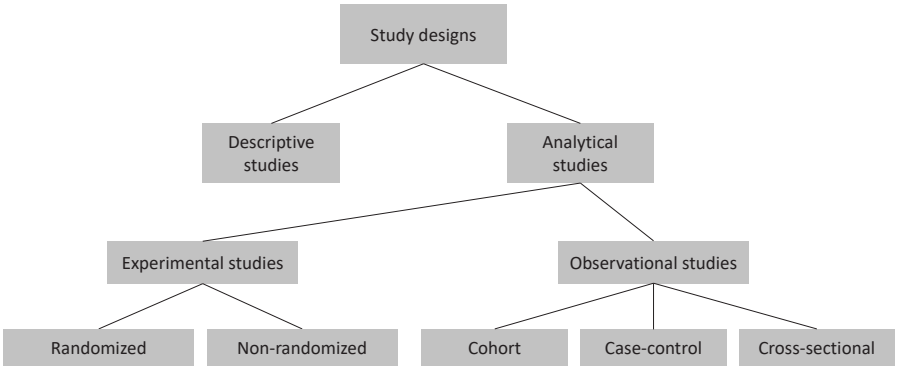


Figure 1. Study designs in clinical research.

1.1.4 Cohort studies

In a cohort study, a defined group of people is followed from the time of exposure until the outcome or the end of the study. The outcome is ascertained through follow-up using either direct contact (i.e. examination, clinical visit), indirect contact (self-report, questionnaires etc.) or registers. This design is advantageous for investigating rare exposures, especially in a register-based cohort study. In a retrospective cohort study, the exposures are in the past and a possible weakness is that the quality of data could be questionable. In a prospective cohort study, the exposures are in the present and the events in the future, where possible weaknesses include difficulties to capture rare diseases (if not using registers), expensiveness, and requires a long follow-up period (2).

1.1.5 Epidemiology using Swedish registers and personal identification numbers

Since 1947, all citizens in Sweden have been assigned a personal identification number (PIN) at birth or immigration. This number follows every citizen through life until death, and is used extensively throughout all areas of society: hospital visits and admissions for various disorders, diseases recorded in quality registers, education, migration, marriage, passports, income, military conscription, child birth, and deaths (17). Diseases and deaths are recorded in registers held by the National Board of Health and Welfare. By using data from registers, researchers are able to study health and disease in the Swedish

population over a long period of time and in a large study population. Data from these registers are population-based and having access to this source of secondary data (data that the researcher has not collected for the purpose of the study) is highly advantageous (18-20). Considering these advantages, Sweden offers a unique possibility to conduct register-based research.

There are several different high-quality population-based registers held at the National Board of Health and Welfare and at Statistics Sweden (21):

- Cause of Death register
- National Patient register
- Cancer register
- Swedish Prescribed Drug register
- Swedish Medical Birth register
- Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA)
- Register of the Total Population

There are also quality registers such as the Swedish National Diabetes register held by other organizations (22).

1.2 Overweight and obesity

1.2.1 Adipose tissue

The human body can be divided into two different compartments, fat mass and fat-free mass. The fat-free mass consists mainly of lean mass (mostly skeletal muscles) and bone mineral content (23). Adipose tissue is an innervated and vascularized organ that mainly consists of adipocytes, but also connective tissue matrix and immune cells (24). A prominent feature is its ability to increase in size. There are different forms of adipocytes: white, brown, and the recently discovered beige/brite and pink adipocytes (25, 26). The white adipocytes contain one large lipid droplet (approximately 90% of their volume) and their function is to store energy for the release of fatty acids during fasting (25, 27). Brown adipocytes contain many small lipid droplets and several mitochondria; their function is to burn energy for thermogenesis (25, 28). It has generally been believed that brown adipocytes only are present and functional in infants and young children, but studies have shown that adults also have brown adipose tissue depots (29). The plasticity of adipose tissue involves the browning of white adipocytes in response to cold temperatures, and whitening of brown adipocytes during a positive energy balance (30).

Adipose tissue is mostly present in two compartments of the body – the subcutaneous depot and the visceral depot (27), where large white adipocytes are present in subcutaneous depots and small white adipocytes in visceral depots. Fat storage in the upper body is both visceral and subcutaneous; the visceral fat storage surrounds the internal organs and the subcutaneous fat depot is located directly under the skin. In the lower body, the largest fat depots are subcutaneous, mainly femoral and gluteal (31).

Adipose tissue has long been considered a passive organ, but this view has changed. During the past decades several studies have elicited the many endocrine functions of adipose tissue, and have shown that it is in fact an endocrine organ. Adipose tissue responds to both nervous and hormonal signals and secretes hormones (32). Moreover, adipose tissue is, in addition to the adrenal gland and the gonads, a major contributor in the metabolism of steroid hormones (32, 33). The two most well-known hormones secreted by adipose tissue are leptin and adiponectin, although an array of molecules is secreted (32). Leptin, a part of the signaling pathway to the central nervous system, was discovered in the early 1990s. Leptin is secreted in direct proportion to the adipose tissue mass, thus reflecting the amount of energy stores in the body and is involved in the regulation of appetite and energy expenditure (34, 35). Adiponectin has an antiatherogenic effect; low adiponectin levels are associated with insulin resistance and inflammation (36). Both adiponectin and leptin are expressed more abundantly in subcutaneous fat than in visceral fat. Thus, the adipose tissue is a highly active tissue that not only produces a number of molecules, but also expresses several receptors and therefore is responsive to both the nervous and the hormonal system (32).

1.2.2 Measurement of overweight and obesity

For an absolute measurement of fat mass, magnetic resonance imaging can be used (37). However, this technique is not used on a regular basis in the clinical practice and is inconvenient in large epidemiological studies (38). There are other methods for the indirect estimation of both total and visceral fat mass: bioelectrical impedance analysis, dual-energy X-ray absorptiometry, waist circumference, waist/hip-ratio, skinfold thickness, and Body Mass Index (BMI). BMI is an easily available estimate that can be calculated from a single height and weight measurement (39).

$$Body\ mass\ index = \frac{weight}{height^2} = kg/m^2$$

BMI is extensively used in epidemiological studies and is the basis for categorizing overweight and obesity. For adults, the BMI cut-offs for normal weight, overweight, and obesity are established (Table 1) but evidence suggest that different ethnic populations might require different BMI cut-offs. It has been proposed that the South Asian population have a different body composition with more adipose tissue at a given BMI value compared to the Caucasian population (40). This is consistent with study results showing that South Asians have an increased risk for type 2 diabetes at a lower BMI level than Caucasians (41).

Table 1. The different BMI cut-offs according to CDC, IOTF, and WHO for overweight and obesity in boys at 8 years of age and in adults above 18 years of age.

	Overweight boys (kg/m^2)	**Obesity boys** (kg/m^2)	**Overweight adults** (kg/m^2)	**Obesity adults** (kg/m^2)
CDC	17.9	20.0		
IOTF	18.4	21.6		
WHO	17.4	19.7	25.0	30.0

Centers for Disease Control and prevention (CDC), International Obesity Task Force (IOTF), World Health Organization (WHO)

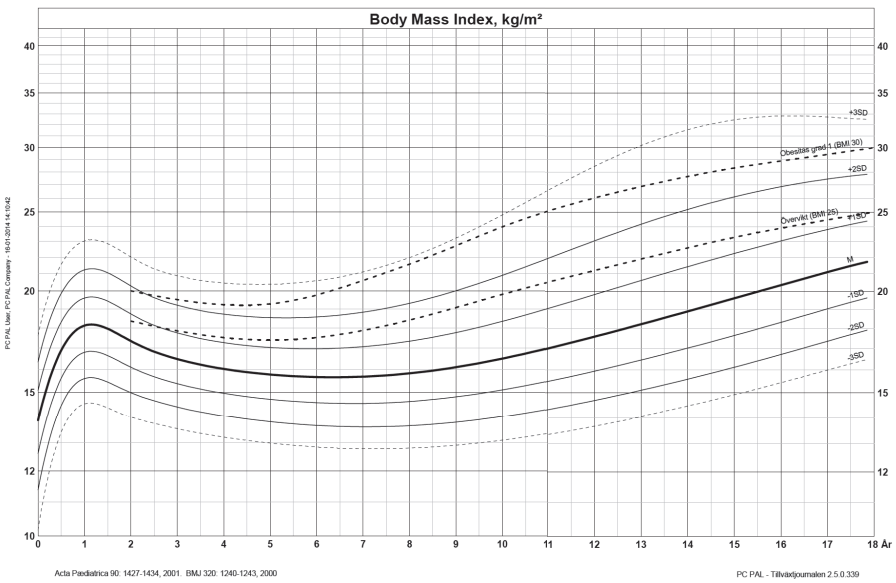


Figure 2. The Swedish BMI curves during infancy, childhood, and adolescence for boys. Age on the x-axis and BMI on the y-axis. Figure from Rikshandboken Barnhälsovård.

Children have a natural change in BMI during development, and consequently, have different BMI reference values at different ages. After adiposity rebound, the time point after infancy when BMI starts to increase again after its nadir between three and seven years of age, children increase in BMI (42, 43). Therefore, there are different BMI cut-offs for overweight and obesity at different ages during childhood and adolescence (Figure 2 and Table 1) (44-46).

1.2.3 Trends of overweight and obesity

In 2016, 1.9 billion adults were overweight and of these 650 million were obese, according to the World Health Organization. This corresponds to 39% of the worlds' population being overweight and 13% obese (47). These high rates are the result of a global obesity epidemic observed during the last three decades (48). In a similar manner, high rates of overweight and obesity and increasing BMI are demonstrated in children: 380 million children were overweight or obese in 2016 (47, 49-51). Recently, indications of a stabilization of the childhood obesity epidemic have been reported (52-54). However, studies on changes of childhood overweight and obesity, typically rely on a short period of time and include few birth cohorts (49). In order to analyze whether trends are sustained, an approach with a longer perspective is needed.

1.2.4 Risks and causes of overweight and obesity

Overweight and obesity constitute a substantial risk factor for a variety of diseases, including cardiovascular disease, diabetes, musculoskeletal disorders, and some forms of cancer (e.g. breast, colon) (55, 56). BMI above 25 kg/m^2 in adults is associated with increased risk of mortality according to a prospective study collaboration including over 10 million participants and spanning four continents (57). In 2010, overweight was estimated to account for 3.4 million deaths globally (58).

Overweight and obesity are conditions caused by multiple factors: genetic, environmental, (environment, education, and socioeconomic factors) and behavioral (diet, physical activity, and medication) (59). Overweight and obesity can be defined as storage of excess fat that is associated with increased risk of morbidity and mortality (60). White adipose tissue expands during a positive energy balance through hypertrophy (increase of adipocyte size) and hyperplasia (increase in adipocyte number) (27, 61, 62). When energy balance is positive the risk for overweight and obesity is highly increased. Adverse health outcomes from overweight and obesity, diseases like cardiovascular disease and cancer, are mainly associated with visceral fat (63-68). In the

Gothenburg Osteoporosis and Obesity Determinants (GOOD) study it has been shown that BMI increase during childhood is especially associated with the amount of subcutaneous fat in young adult age, while BMI increase during puberty is also associated with the amount of visceral fat (69).

1.3 Puberty

Puberty is a period of transition from childhood into adulthood ending with the attainment of reproductive capacity. Puberty is controlled by the hypothalamic-pituitary-gonadal-axis and involves a complex cascade of hormones and developmental events. Gonadotropin-releasing hormone (GnRH) is secreted from GnRH neurons in the hypothalamus in a pulsatile manner; it controls the pituitary-gonadal axis and subsequently the gonads and their development during puberty (Figure 3). For the fetus *in utero*, the pulsatile release of GnRH is initiated shortly after the GnRH neurons are developed in the hypothalamus. This pulsatile action continues during infancy but is suppressed in late infancy and during childhood. Spontaneously, or triggered by an unknown factor, the inhibition stops and GnRH is again released in a pulsatile manner. This marks the start of the pubertal process. The mechanisms behind the initiation and inhibition of this GnRH release, and ultimately pubertal initiation, have long been an enigma and are still not fully understood (70-72). GnRH stimulates the anterior pituitary gland to start the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH regulate gonadal function e.g. secretion of sex hormones, spermatogenesis, ovulation, and folliculogenesis. Sex hormones stimulate the hypothalamus in a negative feed-back loop inhibiting expression and further release of GnRH (72-74).

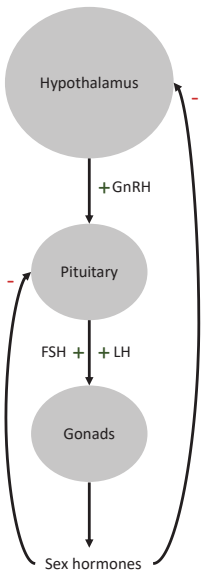


Figure 3. Schematic simplified model of the hypothalamus-pituitary-gonadal-axis.

1.3.1 BMI and puberty

The inverse association between childhood BMI and menarcheal age is established, and a systematic review recently confirmed that higher childhood BMI was associated with earlier menarche (75-77). Leptin has been proposed

to be the link between adipose tissue and puberty in girls (78). In 1970, Frisch et al proposed a hypothesis stating that a critical level of body mass was needed for the initiation of puberty. In 181 girls, they observed that both late and early maturing girls reached menarche at the same mean weight but not the same mean height (79). This hypothesis was one of the first linking nutrition and body size to pubertal events. Whether or not the increasing BMI and earlier initiation of puberty are mechanistically connected is unknown. For boys, contradicting results with both positive and negative associations between prepubertal BMI and pubertal timing have been reported (80-82).

1.3.2 Pubertal series of events

The pubertal period is defined by a series of events and acquirement of secondary sex characteristics. The sequence of pubertal events for boys, preceded by a fat spurt, is growth of testes and scrotum, growth of pubic hair, growth of the penis, height spurt, and muscular development (Figure 4). The pubertal process in girls starts with the development of breast buds and growth of pubic hair, followed by a height spurt and menarche. For both boys and girls, the pubertal phase ends with the closing of epiphyses (72, 83).

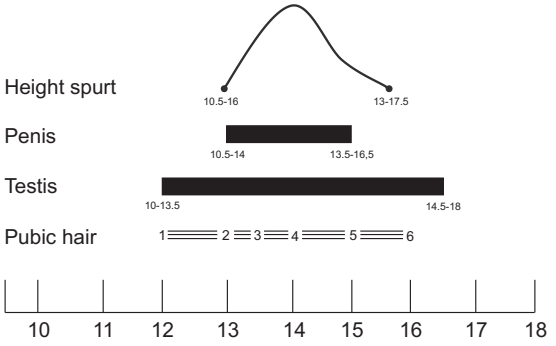


Figure 4. Series of pubertal events in boys. Age on the x-axis. Figure adapted from J.M. Tanner, Growth of the human at the time of adolescence (1953).

1.3.3 Assessment of pubertal timing

The different events during puberty can be used as assessments of pubertal timing. Physical examination using Tanner staging and estimation of testicular volume and pubic hair is used in clinical practice and in cross-sectional and prospective studies (84). In large population studies, self-report of pubertal demarcations is commonly used, i.e. recalled age at menarche for girls but for boys a corresponding easily available demarcation is lacking. Self-reported recalled age at voice breaking has been used to some extent but it has not been

validated for retrospective use (85, 86). Access to retrospective data of repeated height measurements enables the use of the adolescent growth spurt as a marker for pubertal timing. Age at Peak Height Velocity (PHV), the maximum velocity during growth spurt, is derived from direct measurements of height producing an objective estimate of pubertal timing. Age at PHV shows a strong correlation with pubertal timing retrieved from detailed longitudinal physical examination of secondary sex characteristics (r=0.8) (87). Self-reported pubertal timing in men display a slightly lower correlation with pubertal timing retrieved from secondary sex characteristics (88). In addition, age at PHV has the advantage of being easily estimated in a large number of individuals.

1.3.4 Secular trends of pubertal timing

Since the mid-19th century, a secular trend for earlier age at menarche has been observed both in Europe and the US (76, 89-94). This is partly the result of coinciding increase of BMI and improvements in living conditions, nutrition, and hygiene (91, 95).

For boys, large studies of a potential secular trend of earlier pubertal timing are scarce. One reason for this might be that for boys there is no clear, easily recalled pubertal demarcation corresponding to menarcheal age for girls. However, a large Danish cohort study used growth data for assessment of age at PHV for boys born 1935-1969 and found a secular trend for earlier pubertal timing (80, 81). Although, this study did not have information on height after age 15 years, and therefore boys with late puberty were not included.

1.4 Cardiovascular disease

Cardiovascular disease is the umbrella term for diseases of the heart and circulation, such as myocardial infarction, angina, stroke, and heart failure. The mechanisms underlying cardiovascular disease are silently ongoing for decades before acute manifestation. The most important mechanism is atherosclerosis, a complex process involving many different cell types. Key steps in the atherosclerotic process include hypercholesterolemia and the retention of low-density lipoprotein (LDL) particles in the vascular wall, oxidative modification of LDL, activation of endothelial cells, adhesion and migration of leukocytes, differentiation of monocytes into macrophages and lipid uptake of macrophages. Activation of macrophages and T-cells results in inflammation within the atherosclerotic lesion and contributes, together with proliferation of vascular smooth muscle cells, to the formation of an

atherosclerotic plaque. Rupture of an atherosclerotic plaque is the most important cause of myocardial infarction (96).

Cardiovascular disease is the leading cause of death worldwide, according to the 2017 Global Burden of Disease study. Globally, 17.8 million people died in 2017 from cardiovascular diseases. The total number of cardiovascular deaths have increased over the latest decades mainly due to an ageing population, nonetheless, the age-standardized death rates have decreased mainly due to better treatments and prevention (97). Risk factors for cardiovascular disease include smoking, overweight/obesity, diabetes, age, hypertension, and hypercholesterolemia (98).

A high BMI in adulthood has been shown to associate with increased risk of cardiovascular diseases as well as with high childhood and adolescent BMI (99-105). In a large cohort from Israel, high BMI in late adolescence was associated with increased risk for coronary heart disease, independent of adult BMI (103). This was consistent with findings from the Harvard Growth Study that suggested that the association between adolescent overweight and incidence of adult coronary heart disease was independent of adult BMI (102). In contrast to these findings, a study pooling four cohorts by Jounala et al. revealed a clear increased risk of cardiometabolic disease for overweight adults whereas no independent increased risk for subjects with childhood overweight was observed (106). Another Israeli study showed that overweight as well as BMI in the upper end of the normal range during late adolescence was associated with increased risk for cardiovascular mortality (104). Finally, a study from the Swedish Conscript register in men aged 18 at baseline shows an increase in risk of acute myocardial infarction, stroke, and cardiovascular death that starts already at high normal BMI levels and increases markedly at very high levels of BMI, so that the risk of cardiovascular death (after adjustments) was nearly six-fold for individuals with a BMI ≥35, compared to that of lean men (107).

1.5 Knowledge gaps

Puberty in boys has long been an under-investigated area of research, mainly due to the lack of an easily available pubertal marker, comparable to menarche for girls. Neither an association between childhood BMI and pubertal timing nor a secular trend for earlier pubertal timing among boys have therefore been established. When investigating trends of childhood BMI and pubertal timing in boys, earlier studies have mainly used small cohorts and few data points over a relatively short period of time.

In life course epidemiology, studies have investigated the association between childhood or adolescent BMI and adult mortality and morbidity. A large Danish cohort of old school health records has been used to investigate the association between childhood BMI, age 7 to 13 years, and risk of different adult diseases e.g. cancer and coronary heart disease (99, 108-113). Some studies have shown that childhood overweight and obesity have a high degree of tracking into adult overweight and obesity (114) while others have found weaker correlations (115). Nonetheless, it is not clear whether or not childhood obesity affects adult morbidity and mortality indirectly, via tracking into adult overweight, or directly via other pathways related to a potential metabolic burden of childhood obesity. To investigate the consequences of childhood overweight and obesity, studies with information on childhood exposures and adult outcomes are needed. The challenges of investigating these life course research questions are the need for extremely long follow-up, up to 50-60 years, and the statistical power needed for studies on mortality and diseases. Many of the studies conducted so far have included a heterogeneous sample of children, pooling individuals of various ages from early childhood to young adulthood and often with only one BMI measurement. The relative contribution of high BMI during the different distinct developmental periods (pre-natal, infancy, childhood, puberty) is therefore largely unknown.

2 AIM

This thesis targets some of the knowledge gaps regarding puberty, childhood BMI and BMI change during puberty in boys.

The specific aims in the four papers in this thesis are:

I. To investigate the changes over time of childhood BMI in boys born 1946-2006.

II. To investigate the association between childhood BMI and pubertal timing in boys.

III. To investigate the changes over time of pubertal timing in boys born 1947-1996.

IV. To investigate how childhood BMI and BMI change during puberty associate with cardiovascular mortality.

3 SUBJECTS AND METHODS

3.1 The BMI Epidemiology Study cohort

3.1.1 Child health care and school health care in Sweden

In the early 20th century, children born in underprivileged families could be admitted to a nationwide organization, Mjölkdroppen, by deacons and physicians. The nurses and physicians employed by Mjölkdroppen helped feeding the babies either by distributing cow's milk or by a small financial contribution for the mothers to be able to stay at home and continue breastfeeding. In return, mothers complied to measure the babies' weight in order to monitor their growth and general health. During the organization's early years, the objective of Mjölkdroppen was to prevent infant mortality. Later, the objectives expanded to include general medical supervision and guidance. In Gothenburg, Mjölkdroppen initially operated as a charity but in 1945 the activities were included in the social welfare of the municipality and its services were offered to all families over the entire socioeconomic spectrum. This laid the foundation of today's child health care service. While

Figure 5. One of the numerous lanes of shelves with archived school health records at the Archives of the City of Gothenburg and Region Västra Götaland.

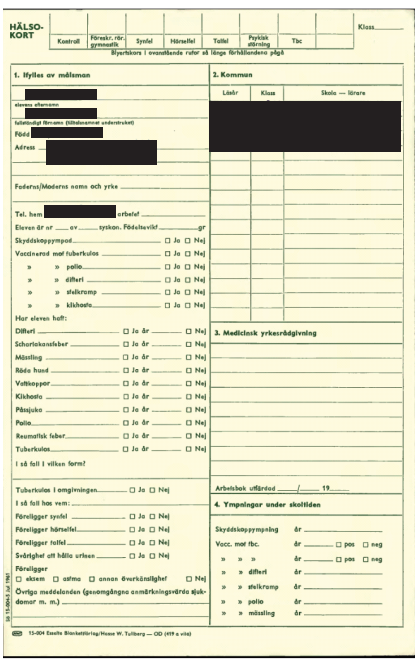
initially only including infants, the organization soon expanded to include children until the age of six, when they started school (116-118).

In parallel to the establishment of child health care, special school nurses and physicians were employed at all schools with the directive to follow the pupils' health, to vaccinate them, and to measure their height and weight regularly. The measurements were recorded in special health records that followed the pupils throughout childhood until graduation, even if they changed school. Initially, the schools managed this health work locally, but in 1944-1945 state funding and central management was established (119, 120).

Consequently, for more than 100 years nearly every child in Sweden, approximately 99% in child health care and 98.5% in school health care (119), had their height and weight measured in a standardized manner throughout infancy, childhood and adolescence by professional nurses. In Gothenburg, these health records have been archived in the Archives of the City of Gothenburg and Region Västra Götaland, both in paper form and from the 1990s in digital form. The total number of archived records has been estimated to ≈400,000 dating from the early 20th century and onwards (Figure 5).

3.1.2 Data collection from records

We have initiated the digitalization of the school health records in Gothenburg and computerized height and weight data into the BMI Epidemiology Study (BEST) cohort for individuals with a health record containing a complete PIN, or who could be matched to a complete PIN in population registers (Figure 6). Approximately 98% of all records had a complete PIN or could be matched in population registers. Therefore, the BEST cohort is a population-based cohort. Furthermore, we have added information on young adult height and weight, mortality, and country of birth from national registers into the BEST cohort (Figure 7).


HÄLSO-KORT

Kontroll | Förvakt. rör. gymnastik | Synfel | Hörselfel | Tallel | Psykisk störning | Tbc | Klass

Blyertskors i ovanstående rutor så länge förhållandena pågå

1. Hyllies av målsman

elevens efternamn

fullständigt förnamn (tilltalsnamnet understruket)

Född

Adress

Faderns/Moderns namn och yrke

Tel. hem arbetet

Eleven är nr av tyskan. Födelseviktgr

Skyddsoppympad ☐ Ja ☐ Nej

Vaccinerad mot tuberkulos ☐ Ja ☐ Nej

» » polio ☐ Ja ☐ Nej

» » difteri ☐ Ja ☐ Nej

» » stelkramp ☐ Ja ☐ Nej

» » kikhosta ☐ Ja ☐ Nej

Har eleven haft:

Difteri ☐ Ja år ☐ Nej

Scharlakansfeber ☐ Ja år ☐ Nej

Mässling ☐ Ja år ☐ Nej

Röda hund ☐ Ja år ☐ Nej

Vattkoppor ☐ Ja år ☐ Nej

Kikhosta ☐ Ja år ☐ Nej

Pärsjuka ☐ Ja år ☐ Nej

Polio ☐ Ja år ☐ Nej

Reumatisk feber ☐ Ja år ☐ Nej

Tuberkulos ☐ Ja år ☐ Nej

I så fall i vilken form?

Tuberkulos i omgivningen ☐ Ja ☐ Nej

I så fall hos vem:

Föreligger synfel ☐ Ja ☐ Nej

Föreligger hörselfel ☐ Ja ☐ Nej

Föreligger tallel ☐ Ja ☐ Nej

Svårighet att hålla urinen ☐ Ja ☐ Nej

Föreligger

☐ eksem ☐ astma ☐ annan överkänslighet ☐ Nej

Övriga meddelanden (genomgångna anmärkningsvärda sjukdomar m. m.)

2. Kommun

Läsår	Klass	Skola — lärare

3. Medicinsk yrkesrådgivning

Arbetsbok utfärdad / 19

4. Ympningar under skoltiden

Skyddsoppympning år

Vacc. mot tbc. år ☐ pos ☐ neg

» » » år ☐ pos ☐ neg

» » difteri år

» » stelkramp år

» » polio år

» » mässling år

Figure 6. An old school health record from the Archives of the City of Gothenburg and Region Västra Götaland.

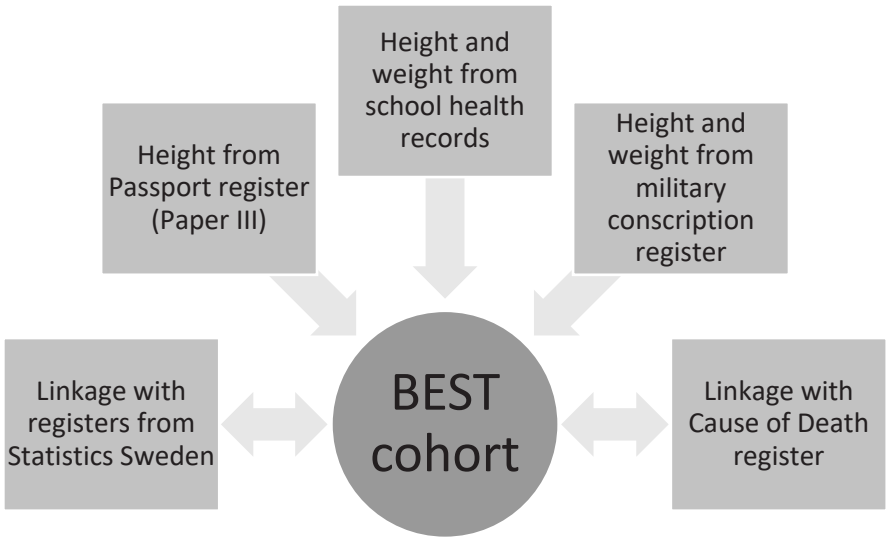


Figure 7. Data included in the BEST cohort. BMI Epidemiology Study (BEST)

3.1.3 Linkage with registers

Using the unique PINs, we retrieved measurements of height and weight in late adolescence through linkage with the Swedish conscription register, and height through linkage with the Swedish passport register (only Paper III).

To retrieve information on causes of death in the study population and demographic variables for adjustments in the statistical analyses, country of birth, height, and weight data during childhood and adolescence were linked, using PINs, with registers held by the Swedish National Board of Health and Welfare and Statistics Sweden. The study participants have been followed in these registers until December 31th, 2013 in the papers included in this thesis.

Conscription register

In Sweden, military conscription was compulsory until 2010, although less rigorous during the 1990s (121). At conscription, men (approximately 18-20 years old) were enlisted and assessed for military service, including medical examination and measurement of height and weight. For men born 1951 and onwards height, weight, and other data from the examination and assessment are preserved in digital registers held at The Military Archives of Sweden and at the Swedish Defense Recruitment Agency (before 2011 called the National Service Administration). For men born before 1951, height and weight data have been archived in paper records and these data were manually retrieved and transcribed.

Passport register

The Passport register contains heights that have been collected through self-reporting or measurement at the time of application for a passport. The Passport register contains heights for all citizens that have applied for a passport from 1991 and onwards.

Cause of Death register

The Cause of Death register contains information on all causes of death of all deceased citizens in Sweden, where causes of death have been recorded since 1911. For register-based research, data on causes of death are electronically available since 1952 with retrospectively collected data from paper records between 1952-1961. The register uses the International Classification of Diseases (ICD) system for classification of cause of death. When an individual in Sweden dies, a physician must submit a medical death certificate to the National Board of Health and Welfare within 3 weeks. This information is then recorded into the Cause of Death register, which is updated annually. Included in the medical death certificate is information on underlying cause of death defined according to the international World Health Organization's ICD:

"*The disease or injury which initiated the train of morbid events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury.*" (122)

In addition to underlying cause of death, the physician can also report information on contributing causes of deaths. While the underlying cause of death is the actual cause of death, the contributing cause of death contributed to the outcome but was not the direct cause of death.

Practically all deaths before 1991 were reported with the underlying cause of death to the Cause of Death register. The legal obligation to send in the medical death certificate was removed in 1991. Nonetheless, nearly all underlying causes of death are still reported to the register. This can be illustrated by that in 2015, 99.1% of all deaths were reported with an underlying cause of death, and of these only 2.7% had insufficient information for registration in the register (19). The quality of the register is reported to be good and the causes of death accurate for cardiovascular disease (123).

Registers from Statistics Sweden

We used the LISA database to retrieve information on participants' country of birth and migration. The LISA database was established in 1990 and is updated annually. It contains information on all people aged 16 and older that were

registered in Sweden on December 31st of the year of update. The information originates from several different registers, e.g. the Register of the Total Population. Established in 1968, the Register of the Total Population, collects information from the Swedish Tax Agency and contains for example information on country of birth (124).

3.2 Determination of variables

3.2.1 Childhood BMI and young adult BMI

Childhood BMI was calculated for every included individual using all paired height and weight measurements between 6.5 to 9.5 years of age. This age interval was selected to represent the childhood period after infancy but before the confounding effect of puberty on body composition and BMI in boys. Since BMI is age dependent also within the selected childhood period, BMI was adjusted to an exact age within the interval. Under the assumption that BMI increase during this age interval is linear, we used all BMI observations within the interval in a linear regression, to estimate the mean size and increase of BMI. Further, assuming that all individuals follow the estimated BMI increase we could translate each observation into the expected value at exactly 8 years of age. For individuals with more than one observation within the age interval, the mean of the BMI estimations at eight years was used. Young adult BMI was calculated using the same principle but with the age interval 17.5 to 22 years of age with BMI age-adjusted to 20 years of age.

3.2.2 BMI change during puberty

For each subject, a BMI change during puberty was calculated as the difference between young adult BMI and childhood BMI:

$$BMI\ change\ during\ puberty = young\ adult\ BMI - childhood\ BMI$$

3.2.3 Assessment of pubertal timing

Age at PHV is the age at the maximum growth spurt during puberty and can be used as an assessment of pubertal timing. Repeated measurements of height are needed to be able to determine age at PHV. Our research group has previously developed a method to estimate age at PHV by curve-fitting of repeated height measurements using a modified Infancy-Childhood-Puberty (ICP) model (125-127).

3.2.4 Curve-fit of height measurements and calculation of age at PHV

The modified ICP model fits human growth data to three mathematical functions (Figure 8). The infancy part is represented by an exponential function, the childhood part by a quadratic function, and the pubertal part by a logistic function. The curve-fitting program merges the different mathematical functions in every growth phase. For each individual with sufficient height data in the growth phases, the model is fitted by minimizing the sum of squares using a modification of the Levenberg-Marquardt algorithm. Visual inspection was used as an additional quality ascertainment (128). Age at PHV was defined as the age at maximum growth velocity during puberty.

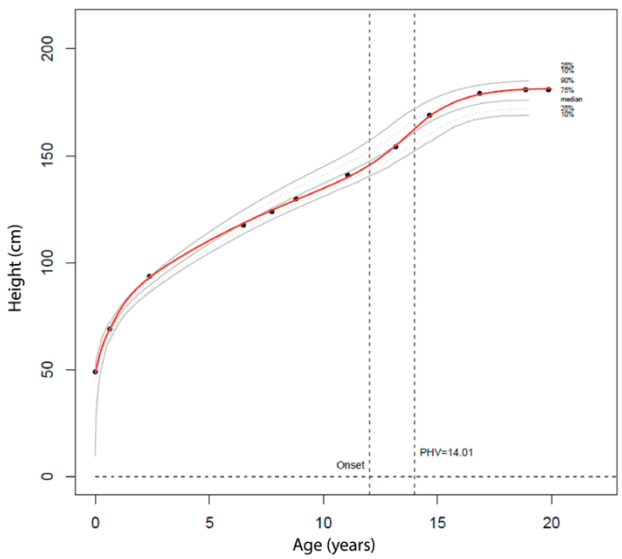


Figure 8. Example of a growth curve modelled from repeated height measurements using a modified Infancy-Childhood-Puberty model. Black dots represent actual measurements, red line represent the height estimation from the curve fitting-method. Peak Height Velocity (PHV)

3.3 Cardiovascular disease mortality

Using the Cause of Death register and the underlying cause of death, we defined cardiovascular disease mortality as diagnostic codes I00-I99 in ICD-10 and as codes 390-459 in ICD-8 and 9. For the analyses of the separate underlying causes of death the following definitions were used: Coronary heart disease I20-25 (ICD-10) and 410-414 (ICD-8 and 9), Stroke I61, I63, I64 (ICD-10) and 431, 433, 434, 436 (ICD-8 and 9), Ischemic stroke I63 (ICD-10)

and 433, 434 (ICD-8 and 9), Intracerebral Hemorrhage I61 (ICD-10) and 431 (ICD-8 and 9) (Table 2). ICD-10 is currently used, ICD-9 was used 1987-1996, and ICD-8 was used 1969-1986.

Table 2. Definitions of cardiovascular mortality with diagnose codes from ICD-10, ICD-9, and ICD-8.

	ICD-10	ICD-9	ICD-8
Cardiovascular mortality	I00-I99	390-459	390-459
Coronary heart disease	I20-I25	410-414	410-414
Stroke	I61, I63, I64	431, 433, 434, 436	431, 433, 434, 436
Ischemic stroke	I63	433, 434	433,434
Intracerebral hemorrhage	I61	431	431

International Classification of Diseases (ICD)

3.4 Inclusion of individuals

For each paper study subjects were selected by applying inclusion and exclusion criteria (Table 3 and Figure 9). Eligible individuals were those who had their school health record stored at the Archives of the City of Gothenburg and Region of Västra Götaland. At the time of writing this thesis, data collection into the BEST cohort is still ongoing. The papers included in this thesis are only based upon the data for boys since the data for girls have not yet been fully collected and validated.

Table 3. Inclusion criteria and exclusion criteria for paper I, II, III, and IV.

	Inclusion criteria	Exclusion criteria
Paper I	Childhood BMI	No PIN
Paper II	Childhood BMI Age at PHV	No PIN
Paper III	Childhood BMI Age at PHV	No PIN
Paper IV	Childhood BMI Young adult BMI	No PIN

Body Mass Index (BMI), Personal Identification Number (PIN), Peak Height Velocity (PHV)

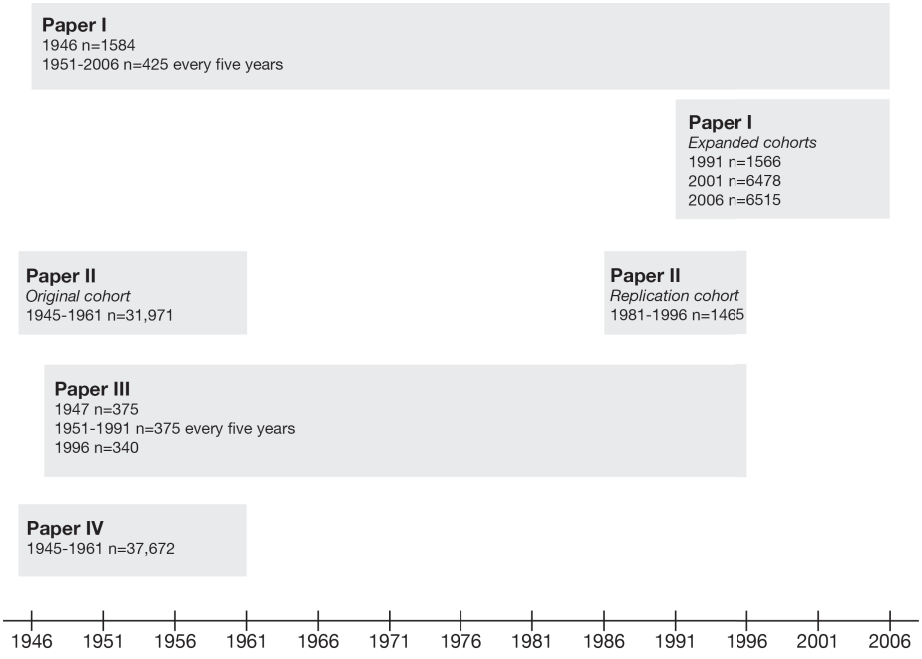


Figure 9. Schematic figure over the study subjects included in papers I, II, III, and IV.

3.5 Statistical analyses

All statistical analyses were performed using SPSS (version 22, 23, 24; IBM SPSS; Armonk; NY; USA) or R (version 3.2.3, 3.4.2; "standard", "survival", "regression modeling strategies", and "segmented" package) (129).

A p-value <0.05 was considered statistically significant.

3.5.1 Power

The statistical power of the BEST cohort has been evaluated in power analyses. The statistical power is dependent on the prevalence of the disease in focus, the cohort size, and the effect size of the association tested. The relation between prevalence, cohort size and effect size for a statistical power of 80%, is illustrated by a graph demonstrating the odds ratio (expressed per standard deviation of the exposure variable) for a significant association between the evaluated exposure variable and an outcome that can be detected with a statistical power of 80% (Figure 10).

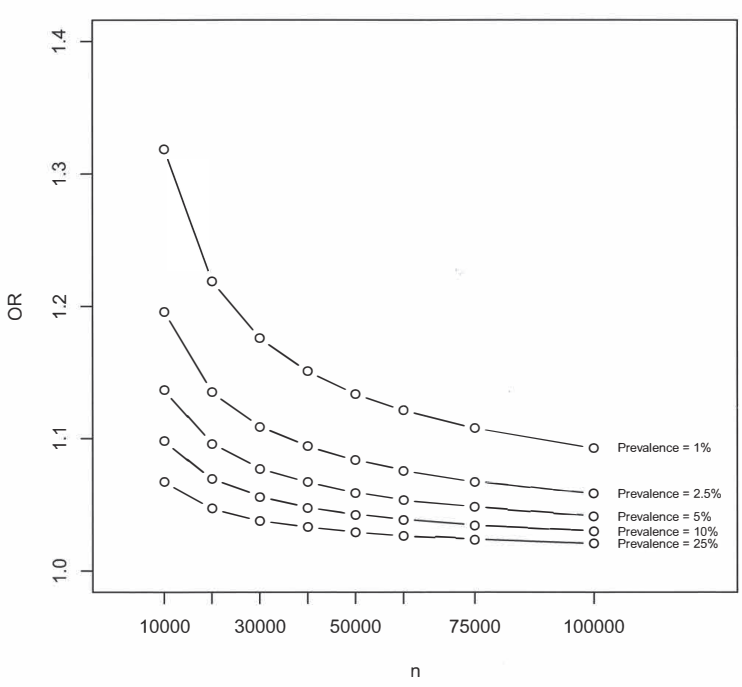


Figure 10. Power calculation of the BEST cohort. The Odds Ratio (OR) is on the y-axis, the cohort size (n) on the x-axis and the prevalence of diseases are shown by different curves in the graph.

3.5.2 Log-transformation

As expected, BMI had a skewed distribution and was therefore log-transformed for all calculations. To facilitate the interpretation, results using non-transformed BMI were reported for paper I, II and III.

3.5.3 Comparison of groups

In paper I, a two-sided Student's t-test was used to compare the continuous variable BMI between the different birth cohorts. To compare the categorical variables overweight and obesity, a chi square test was used. Since this testing involved more than one comparison, a Bonferroni correction of the p-values was used to adjust for multiple comparisons. In paper I and III, a one-way analysis of variance with Tukey's post-hoc test was used to compare the different birth cohorts with the reference birth cohort.

3.5.4 Modelling of associations and survival analysis

In order to assess the association between two variables where the outcome was continuous, the association was evaluated in a linear or non-linear model. The first step was to evaluate the association in a linear regression model and the next step to evaluate a possible non-linear model by adding a quadratic term into the linear model. In case the quadratic term in the linear regression was significant, we further explored the non-linear association – in paper II by using a piecewise linear regression and in paper IV by a restricted cubic spline approach. When the outcome was binary, as for overweight and obesity in paper I, we used logistic regression.

If follow-up time was analyzed together with a binary outcome variable, the association was modeled using a Cox proportional hazards regression (Paper IV). In this model the assumption of proportional hazards must be fulfilled as assessed by visual inspection of Schoenfeld residual plots and a goodness-of-fit test. A Kaplan-Meier survival plot describes the survival of different groups during follow-up and a log-rank test tests the difference between these groups. This method was used in paper IV, as were cumulative incidence plots to assess a potential competing risk.

The associations were also assessed for potential interactions between the exposure variables by addition of an interaction term in the models.

3.6 Summary of the work process

The four papers included in this thesis emanate from the population-based BEST cohort. The work process for the different studies can be summarized (Figure 11):

- Data collection and validation
- Linkage with the Cause of Death register and registers from Statistics Sweden
- Estimation of variables: childhood BMI, BMI change during puberty, young adult BMI, age at PHV, and mortality
- Statistical analyses of changes over time and associations between exposure variables and outcome variables

The study has been approved by the local ethics committee at the University of Gothenburg (D-nr 013-10 and several amendments).

Figure 11. Summary of the work process for the studies done using the BEST cohort.

4 RESULTS

4.1 Paper I: The childhood BMI, overweight, and obesity trend among Swedish boys

In paper I we investigated the trend for childhood BMI, overweight, and obesity over a large time span (birth years 1946-2006), using 13 birth cohorts with a total of 6684 8-year-old boys. Over the 60-year study period mean childhood BMI remained stable during the first decades, after which a statistically significant increase was found from birth cohort 1971, compared with the reference birth cohort 1946. Of note, a peak in birth cohort 1991, followed by a change in the increasing trend was detected (Figure 12). The prevalence of overweight and obesity displayed a similar pattern.

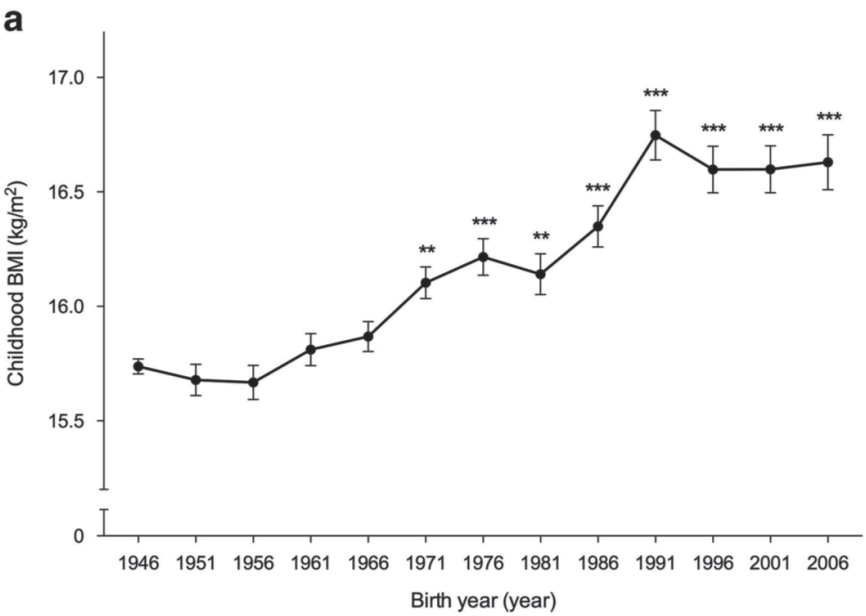


*Figure 12. Mean childhood BMI at 8 years of age for boys included in the BEST cohort born during 1946-2006. Values are presented as mean±SEM. Statistically significant differences compared with birth cohort 1946 are indicated: **p<0.01, ***p<0.001. BMI Epidemiology Study (BEST), Body Mass Index (BMI).*

For further analyses of the change in the increasing trend, the youngest birth cohorts (1991-2006) were expanded by retrieving digital school health records (total n=14,559). Bonferroni corrected p-values from t-tests and chi-square tests revealed that after birth cohort 1991 there was a statistically significant

decrease in childhood BMI, prevalence of overweight, and prevalence of obesity.

During the large time-span this study covers the population in Gothenburg has experienced demographic changes due to migration. As a result, the study population has not had the same demographic structure over the years. To account for this, we performed a sub-analysis including only boys born in Sweden with parents born in Sweden. In this sub-analysis the increase of mean childhood BMI, prevalence of overweight, and prevalence of obesity up until birth cohort 1991 was maintained indicating that the increase was not due to demographic changes of the study population. The decrease after birth cohort 1991 was also maintained in this sub-analysis.

Results paper I

1. Childhood BMI, prevalence of overweight, and prevalence of obesity have increased substantially since birth cohort 1946

2. Childhood BMI, prevalence of overweight, and prevalence of obesity have decreased slightly since the peak in birth cohort 1991

4.2 Paper II: Association between childhood BMI and pubertal timing in boys

In paper II we evaluated the association between childhood BMI and pubertal timing in boys. In the original cohort of 31,971 boys born 1945-1961, the mean and Standard Deviation (SD) childhood BMI was 15.74 (1.41) kg/m^2 and the mean (SD) age at PHV was 14.06 (1.11) years. We repeated the analyses performed in the original cohort in a cohort of boys born later (1981-1996) exposed to the childhood obesity epidemic. In this replication cohort of 1465 boys, the mean (SD) childhood BMI was 16.47 (2.06) kg/m^2 and the mean (SD) age at PHV was 13.71 (1.08) years.

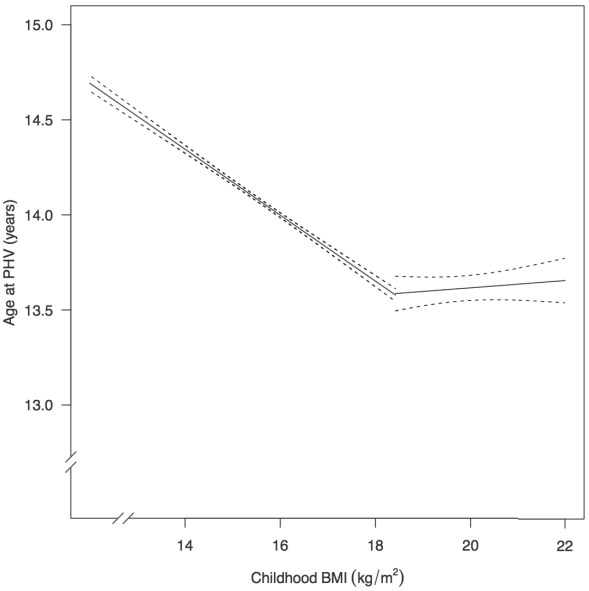


Figure 13. Association between childhood BMI and age at PHV in the original cohort (n=31,971; 95% CI represented by dotted lines). Via a piecewise linear regression model, we identified a threshold at 18.42 kg/m^2 (95%CI 17.97; 18.87) for the association between childhood BMI and age at PHV, when evaluated in the original cohort adjusting for birth year and country of birth. Body Mass Index (BMI), Peak Height Velocity (PHV), Confidence Interval (CI).

We observed an inverse association between childhood BMI and age at PHV using a linear regression model. The association was non-linear as assessed by inclusion of a quadratic childhood BMI term in the model ($p<0.001$ for non-linearity). This non-linear association was further investigated in a piecewise linear regression model and a threshold at 18.42 kg/m^2 was detected. Below, but not above this threshold, an inverse association between childhood BMI and age at PHV was observed (Figure 13). The identified threshold was close

to two commonly used cut-offs for overweight in boys at 8 years of age (44, 45). In further analyses we used these cut-offs for overweight and examined the association in normal weight and overweight boys with nearly unchanged results compared to when using the identified threshold (Table 4). Repeating the analyses in a replication cohort of boys born later produced similar results (Table 4).

Table 4. Association between childhood BMI and age at PHV according to BMI status.

	Childhood BMI *(kg/m²)*	
	Below threshold	***Above threshold***
Original cohort *(years/BMI unit)*	-0.17 (-0.18;-0.16)	0.02 (-0.03;0.06)
Replication cohort *(years/BMI unit)*	-0.16 (-0.21;-0.11)	-0.03 (-0.11;0.05)
	Normal weight	***Overweight***
Original cohort *(years/BMI unit)*	-0.17 (-0.19;-0.16)	0.01 (-0.03;0.05)
Replication cohort *(years/BMI unit)*	-0.16 (-0.22;-0.11)	-0.02 (-0.10;0.05)

The threshold was identified in a piecewise linear regression model (18.42 kg/m²), the cut-off for normal weight and overweight for 8-year-old boys (17.93 kg/m²) was categorized according to the definition from CDC. Beta-values from linear regression analyses are given in years/BMI unit with 95% CIs in parenthesis. Body Mass Index (BMI), Peak Height Velocity (PHV), Centers for Disease Control and prevention (CDC), Confidence interval (CI).

Results paper II

1. There is a non-linear association between childhood BMI and pubertal timing among boys

2. There is an inverse association between childhood BMI and pubertal timing below, but not above a threshold at 18.42 kg/m², and in normal weight but not in overweight boys

4.3 Paper III: A secular trend for earlier pubertal timing in boys

In paper III we investigated the change in age at PHV, an assessment of pubertal timing, over a large time span. We used 11 birth cohorts: 1947 and every five years between 1951 and 1996, with a total of 4090 boys. Compared to reference birth cohort 1947 there was a significant decrease in age at PHV from birth cohort 1976 and onwards (Figure 14).

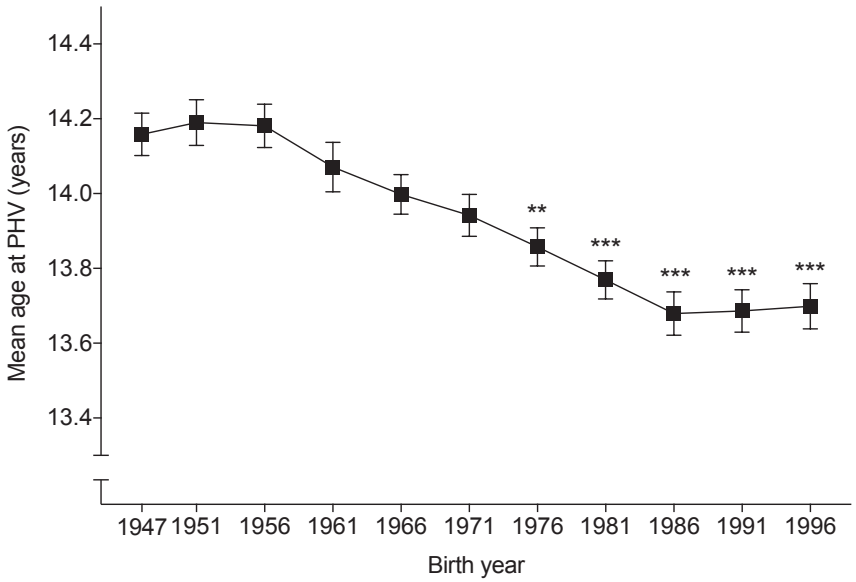


*Figure 14. Mean age at PHV for boys included in the BEST cohort born 1947-1996 (p for trend<10^{-26}). Values are presented as mean±SEM, statistically significant differences versus birth cohort 1947 are indicated **p<0.01, ***p<0.001. Body Mass Index (BMI), Peak Height Velocity (PHV), BMI Epidemiology Study (BEST), Standard Error of the Mean (SEM).*

A linear regression analysis revealed that age at PHV was 1.5 months earlier per decade increase in birth year during the study period (β: -0.12, 95% Confidence Interval [CI]: -0.14;-0.10). Given the inverse association between childhood BMI and pubertal timing (Paper II) and the increase of childhood BMI during the same study period (Paper I), we investigated the secular trend independent of childhood BMI showing that age at PHV was 1.2 months earlier per decade increase in birth year (β: -0.10, 95%CI: -0.12;-0.07).

During the large time-span this study covers, the population in Gothenburg has experienced demographic changes. As a result, the study population has not had the same demographic structure over the years. Accounting for this, we performed a sub-analysis using a subgroup including only boys born in Sweden with parents born in Sweden. In this sub-analysis, the secular trend of an earlier age at PHV persisted. This indicates that the observed secular trend was not due to demographic changes of the study population.

Results paper III

1. There is a secular trend for earlier pubertal timing among boys
2. This trend is to some extent, but not completely, explained by the increasing childhood BMI during the study period
3. The secular trend of earlier pubertal timing was not caused by demographic changes in the population

4.4 Paper IV: The association between BMI change during puberty and cardiovascular mortality

In paper IV, 37,672 men born 1945-1961 were followed for a mean of 37.8 years from 20 years of age. During the study period 710 deaths due to cardiovascular disease occurred. We observed that childhood BMI and BMI change during puberty were only marginally correlated (r=0.06) indicating that these two parameters provide non-overlapping information.

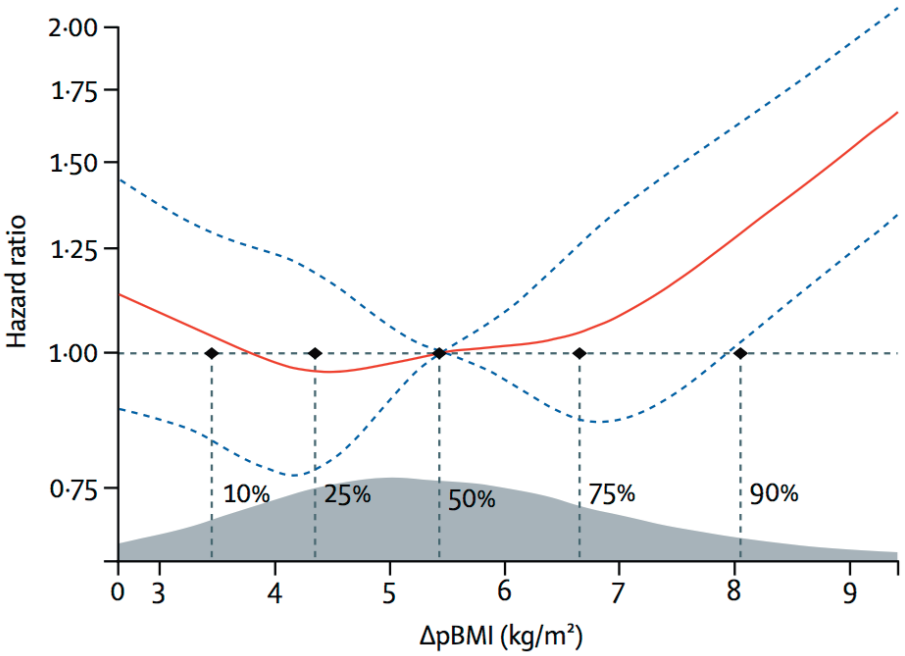


Figure 15. Smoothed plots of HR for cardiovascular mortality according to ΔpBMI in 37,672 Swedish men followed for a mean of 37.8 years after age 20 years. Cox regression analysis with a restricted cubic spline-approach for a flexible non-linear assessment of the HR for cardiovascular mortality after age 20 years in relation to ΔpBMI (p<0.0001). Five knots were placed at the ΔpBMI percentiles 10, 25, 50, 75, and 90 (vertical black lines). The model was adjusted for birth year and country of birth. Data are presented as HR (red line) and the 95% CI (blue dotted line). The distribution of participants according to ΔpBMI is shown in gray in the lower part of the figure. The horizontal dashed line corresponds to the reference (median ΔpBMI=5.44 kg/m²) HR of 1.0 (no excess rate of events). Hazard Ratio (HR), BMI change during puberty (ΔpBMI), Body Mass Index (BMI), Confidence Interval (CI).

When assessed separately, both childhood BMI and BMI change during puberty were associated with an increased risk of cardiovascular mortality

(Figure 15). However, in a combined analysis, only BMI change during puberty was independently associated with cardiovascular mortality (Hazard Ratio [HR] per SD increase: 1.21, 95%CI: 1.13;1.30). The association between BMI change during puberty and cardiovascular mortality was non-linear as assessed by inclusion of a quadratic BMI term in the model. For individuals whose BMI change during puberty increased more than 6.7 kg/m^2, corresponding to the highest quartile, there was a 22% increased risk of cardiovascular mortality per additional increase in BMI units (HR: 1.22 per unit increase above 6.7kg/m^2 in BMI change, 95%CI: 1.15;1.29). There was no association for quartiles 1-3.

Results paper IV

1. There is only a marginal correlation between childhood BMI and BMI change during puberty, indicating that these two parameters provide non-overlapping information
2. BMI change during puberty, but not childhood BMI, is independently associated with an increased risk of cardiovascular mortality
3. Individuals in the highest quartile of BMI change during puberty had a 22% increased risk per additional BMI unit increase

5 GENERAL DISCUSSION

5.1 Methodological considerations

5.1.1 Study design

The papers included in this thesis contribute to the understanding of how childhood BMI and pubertal timing have changed over time and to an expanded understanding of the developmental origins of cardiovascular mortality. Through observation of patterns in a large data set, this thesis expands the knowledge of childhood BMI and puberty in boys. In all research, the possibility of errors is present, both systematic and random. The maximal reduction in these errors is desirable when planning a scientific study. In evidence-based medicine, the highest level of evidence is attributable to a randomized controlled trial where the exposure is randomly assigned to the study participants. In this thesis, a randomization of the exposures is not ethically or practically possible, but efforts have been made to reduce potential systematic and random errors. The validity of a study can be assessed by the systematic errors and the precision by the random errors.

5.1.2 External validity

External validity is the possibility to draw general conclusions from the study results to a general population and can be assessed by the representativeness of the study population. Sweden has had compulsory school attendance since 1936, starting from the year the child turned seven. School health care has been an integrated part of school with low degree of dropout. From the early 1950s, 98.5% of all children were followed by school health care (119), thus, virtually all children in Sweden have been included in health care activities, and have had their height and weight measured and recorded regularly. The school health records from all schools in Gothenburg have been stored in the central archive. The data collection started with digitalization of these records before the height and weight data could be transcribed into a database. This indicates that our recruitment base (records in the archive) is population-based. To evaluate the representativeness of our cohort, we used young adult BMI from the conscription register and tested if those included and those not included in the study were different with regard to young adult BMI (Paper IV). Young adult BMI at conscription was similar for the two groups (BMI mean [SD]; Included subjects 21.13 [2.53] kg/m^2; Not included subjects 21.15 [2.57]; non-significant using t-test), suggesting that the cohort is representative for Gothenburg, the second largest city in Sweden. All subjects could not be included in the trend studies (Paper I, III) since we did not have the possibility

or resources to collect all data for subjects born 1945 to 2006. We therefore chose the approach to include approximately 400 consecutively selected subjects born every five years. Consecutive inclusion represents a method to reduce selection bias. To avoid a potential seasonal effect, we consecutively included subjects born from the 1st of January every year and onwards.

In conclusion, efforts have been made to reduce selection bias and the representativeness and external validity of the BEST cohort is good.

5.1.3 Internal validity

For internal validity, it is necessary to assess whether the effect on the outcome is attributable to the exposure and not to other factors as selection bias, information bias, or confounding variables. Since the BEST cohort includes all children who finished school in Gothenburg and school in Sweden is, and has been, mandatory during the recruitment period, the risk for selection bias is low. The measurements of height and weight in school health care and at conscription have followed a national standardized program. The classification of the causes of death for cardiovascular disease in the national Swedish Causes of Death register is reported to be of good accuracy (123) Therefore, both exposures and outcomes have low risk of information bias. In addition to exposures and outcomes with good validity, we also adjust for potential confounders. One of the most important confounders in paper I is demographic changes in the population over time. To address this, we performed a sub-analysis using a subpopulation born in Sweden and with parents born in Sweden. Our findings were robust in this sub-analysis and we were able to conclude that the findings were not confounded by demographic changes in the population. In paper II we used a replication cohort consisting of boys born later and were able to repeat our findings. In paper III a potential confounder was demographic changes in the population over time but also the coinciding increase of childhood BMI. We concluded that there still were a secular trend for earlier pubertal timing after accounting for these potential confounders. In paper IV we adjusted for birthweight, country of birth, and birth year but were not able to adjust for other possible confounders like socioeconomic factors and BMI at middle age.

5.1.4 Precision

The precision of a study can be assessed by the random errors of the estimates from the study and is dependent on the sample size. The statistical power of a study is also dependent on the sample size and in the BEST cohort it has been evaluated (Figure 10). Large sample sizes reduce the variability of the studied parameters and suggest that the study has good precision and reproducibility.

5.2 Major findings and discussion

5.2.1 Paper I: The childhood BMI, overweight, and obesity trend among Swedish boys

We have followed the rise and decline of childhood mean BMI, prevalence of overweight, and prevalence of obesity for boys from birth year 1946 until present day. We reported a significant increase in mean childhood BMI from birth year 1971 and onwards, with a peak in birth year 1991. Interestingly, there was a decrease in mean childhood BMI, prevalence of overweight, and prevalence of obesity after the peak in birth year 1991. Our results regarding the increase in childhood BMI are consistent with results from other cohort studies (130, 131). A stabilization of the childhood obesity epidemic has previously been suggested by short-term studies from both Swedish and international populations (52, 53, 132). Earlier studies have indicated an increase in childhood BMI and a recent stabilization using shorter time-spans, fewer birth cohorts, and without adjustment for demographic changes. The increase in childhood BMI is the consequence of many contributing factors which might include changes in dietary habits toward more energy-dense food, decrease in physical activity, and increase in sedentary behavior. One of the greatest health care challenges facing society is to stop the trend of increasing BMI and if possible, revert it. Therefore, the most interesting and encouraging finding is the recent decrease in childhood BMI in Sweden, which indicates that trends in overweight and obesity in the young are modifiable. The reasons behind this are unknown but would be important to understand. An increasing public awareness of the risks of overweight and obesity might have contributed to the observed change of trend. The sub-population of boys born in Sweden with parents born in Sweden appeared to have a more pronounced decrease after the peak in childhood BMI in 1991 and this might be related to the socioeconomical differences between these groups. Socioeconomic factors are involved in the risk for overweight and obesity and have also been shown to be the main reason behind ethnical differences in childhood BMI (52, 133).

Table 5. Strengths and limitations in paper I.

Paper I	
Strengths	***Comment***
Long time span (60 years), 13 birth cohorts, and a large number of included participants	Possibility to distinguish between temporary changes and persistent trends
Sub-analysis using children born in Sweden and with parents born in Sweden, accounting for demographic changes in the population (possible confounder)	Maintained result
Limitations	***Comment***
No data on girls	Future studies in girls are needed to get a complete picture of the childhood obesity epidemic in Sweden

5.2.2 Paper II: Association between childhood BMI and pubertal timing in boys

For girls, an inverse association between childhood BMI or obesity and pubertal timing has been established (75-77). For boys the results are conflicting with both positive and negative associations (134, 135). In paper II, we show that prepubertal BMI is inversely associated with age at pubertal timing in normal weight but not in overweight boys. We used two cohorts of boys born before and during the obesity epidemic. In normal-weight boys, age at PHV is approximately two months earlier for every unit increase in prepubertal childhood BMI. Given the earlier pubertal timing in overweight compared with normal weight girls, it has been assumed that fat mass drives the observed association between childhood BMI and pubertal timing in girls. A proposed mechanism for this is that the adipocyte-derived hormone leptin, reflective of the amount of fat mass, is involved (78). Among normal weight boys, BMI is reported to mainly be an indicator of lean mass while it is an indicator of fat mass in overweight boys (136). Therefore, it is possible that lean mass rather than fat mass drives the inverse association between childhood BMI and pubertal timing in normal weight boys. However, another hypothesis is that the influence of fat mass for initiation of puberty becomes saturated above a certain BMI threshold corresponding to overweight in boys.

Table 6. Strengths and limitations in paper II.

Paper II	
Strengths	
Large population-based cohort of boys	
Replication of findings in a cohort of boys born later	
Limitations	***Comment***
Unable to distinguish between fat mass and lean mass	
No data on girls	Girls more well-studied

5.2.3 Paper III: A secular trend for earlier pubertal timing in boys

We present evidence for a secular trend for earlier pubertal timing in boys using age at PHV as an objective assessment of pubertal timing in 11 birth cohorts born 1947 to 1996. We used this data set to strengthen the long-suspected but poorly documented belief that puberty timing in boys – similar to the well-documented trend in girls – shows a trend toward younger age over the past 50 years. Efforts have been made over the years to study this but there is no clear picture (80, 81). Possible causes behind the observed secular trend for earlier pubertal timing might be the coinciding increased childhood BMI or demographic changes over time in the study population. However, when adjusting for childhood BMI or stratifying for country of birth our finding of a secular trend for earlier pubertal timing remained. The causes behind this trend are unknown, although other studies have speculated that the secular trend of pubertal timing might be a result of the increased use of endocrine-disrupting chemicals, but further studies are needed to evaluate this (137, 138).

Table 7. Strengths and limitations in paper III.

Paper III	
Strengths	***Comment***
Long time span (50 years), 11 birth cohorts, and a large number of included participants	Possibility to distinguish between temporary changes and persistent trends
Sub-analysis using children born in Sweden and with parents born in Sweden, accounting for demographic changes in the population (possible confounder)	Maintained result
Accounting for coinciding increase in childhood BMI	Maintained result
Limitations	***Comment***
No information on psychosocial or socioeconomic factors as potential confounders	
No data on secondary sex characteristics, and therefore rely on the notion that the interval between onset of puberty and PHV has not changed	

5.2.4 Paper IV: The association between BMI change during puberty and cardiovascular mortality

This was the first paper published on the BEST cohort. We made the novel observation that the correlation between childhood BMI and BMI change during puberty was only marginal (r=0.06). The low correlation between childhood BMI and BMI change during puberty indicates that the determinants of childhood BMI and BMI change during puberty are most likely to a large extent separate. These two distinct developmental BMI parameters therefore have the potential to contribute non-overlapping information as risk markers for adult diseases. We demonstrate that BMI change during puberty, but not childhood BMI, is independently associated with the risk of adult cardiovascular mortality. The association between BMI change during puberty and cardiovascular mortality was non-linear, with a 22% increased risk of cardiovascular mortality per additional BMI unit for participants in the highest quartile. Childhood and adolescence are two important periods thought to influence adult health and disease (139, 140). Many studies have assessed the

risks for these two periods separately, but only a limited number have investigated the relative contributions to adult cardiovascular mortality and no previous study has investigated the independent role of BMI change during puberty (99-104, 106). In combined models only BMI change during puberty was associated with increased risk for cardiovascular mortality, independent of both childhood BMI and young adult BMI. This finding indicates that puberty is an important period for future cardiovascular mortality risk but the mechanisms underlying this are unknown. The GOOD study has previously been used to show that BMI increase during childhood is especially associated with the amount of subcutaneous fat in young adult age, while BMI increase during puberty is also associated with the amount of visceral fat. This finding provides a hypothesis to the mechanisms behind the observed finding in this study. Since visceral fat is associated with cardiometabolic dysfunction and cardiovascular diseases, visceral fat might be a link between BMI change during puberty and cardiovascular mortality. One may speculate that a high BMI change during puberty increases the amount of visceral fat that in turn increases the risk for cardiovascular mortality.

Table 8. Strengths and limitations in paper IV.

Paper IV	
Strengths	***Comment***
Large population-based cohort with near complete participation in free school health care	
Long, near-complete follow-up thanks to Swedish registers	
Adjustment for birth weight	
Limitations	***Comment***
No information on socioeconomic factors during childhood	
No information on other risk factors (smoking, exercise, BMI in middle age)	
No data on women	Conscription register with data on young adult height and weight only for men
The cohort born as early as 1945-1961 predates the obesity epidemic, the association might be different post-obesity epidemic	Long follow-up is needed and this is not possible for a cohort born later yet

5.3 Limitations and strengths

The limitations and strengths specific to the different papers have been addressed in connection to the discussion of each separate paper (Table 5, 6, 7, and 8). Data included in the BEST cohort are secondary data, i.e. data collected by someone else for other purposes than research, in contrast to primary data which is data collected by the researcher for the purpose of the study. In the BEST cohort, exposure data are collected from school health care where the purpose is to follow children's overall health and the outcome and confounder data are collected from Swedish registers. The advantages of secondary data are that the collection of data is less time-consuming and costly since the data already exist. The studies performed using the BEST cohort would not be feasible using primary data since the studies cover an extended period of up to 70 years. When using secondary data, it is often easier to collect a larger representative sample size, with reduced bias since the non-response is low. Two main disadvantages using secondary data are that the data might not include other variables or confounders of interest and that the quality of the methods is not controlled by the researcher (20).

5.4 Ethical considerations

An ethical analysis is needed when dealing with research regarding humans (and animals). In regard to the studies included in this thesis, the potential risks for the participants are mainly integrity issues. Subjects included in the BEST cohort are not put at physical risk by the study activities. We have collected data of height and weight; in the association study (Paper IV) historic height and weight data of subjects well into their middle age, but in the trend studies, also contemporary data for the youngest birth cohorts. The Regional ethical review board in Gothenburg approved our study and waived the need for consent, indicating that their assessment was that the benefits of the data being collected and studies being performed outweighed the potential risks related to integrity intrusion. After data collection of height and weight, we linked this information with demographic information and causes of death from Swedish registers held by national authorities (National Board of Health and Welfare, Statistics Sweden). After linkage, data were returned to us with the personal identities coded and we can no longer identify any subjects. The benefits of the studies from the BEST cohort are that we can follow a large population from birth until death and elucidate the risks of a high BMI during different time periods. It is a population-based cohort with a near-complete data set with very little selection bias. The BMI Epidemiology Study has emerged from a unique material that provides the opportunity to explore knowledge gaps that would be difficult to study in other settings or study designs.

5.5 Gender aspects

The studies included in this thesis are all focused on boys and not girls which is a fundamental limitation. The reason for this is that we started to collect data for men, since one of our aims was to study puberty, and puberty in boys is not as well-studied as in girls. Unfortunately, one aspect makes studies on girls more difficult. In Sweden, all men have been measured for height and weight during military conscription. However, girls have not had a mandatory measurement of height and weight in late adolescence. A measurement of BMI after puberty is crucial for development of the exposure parameter BMI change during puberty that has the most impact on cardiovascular mortality in boys. One late height measurement is also required for the pubertal assessment, age at PHV, or at least one measurement of final height. However, data collection is ongoing and results for girls will be presented in the near future.

5.6 Implications and clinical significance

The studies included in this thesis have described the childhood obesity epidemic in boys in Sweden: the rise, the peak, and the decline. We have also presented evidence of a secular trend for earlier pubertal timing among boys and an inverse association between childhood BMI and pubertal timing among normal weight but not overweight boys. These studies have addressed some of the prominent knowledge gaps regarding puberty for boys. Lastly, we have demonstrated an independent association between BMI change during puberty and the risk for cardiovascular mortality. The knowledge gained from this thesis fits well into the scientific discussion on these topics and expands the field further. The large BEST cohort is a unique resource to further increase knowledge gained from smaller cohorts or cross-sectional studies.

From a clinical perspective, the results have the potential to transmit directly into patient benefit through adjustments of the school health care program for improved identification of individuals at risk. Our findings suggest that BMI should be monitored closely during the pubertal years. The relative contribution of distinct periods during childhood to adult cardiovascular mortality risk represents a knowledge gap, and the findings could also be of importance for decision-makers, when preventive measures targeted against childhood and adolescent obesity are planned and implemented.

6 CONCLUDING REMARKS

Although puberty in boys has long been an under-investigated area of research, some important findings have begun to emerge. The studies included in this thesis will contribute to the extension of knowledge regarding this area of research. In this thesis, we have shown that:

- Childhood BMI and the prevalence of overweight and obesity among 8-year-old boys have overall increased substantially since birth year 1946 until birth year 2006. However, after a substantial increase in BMI starting in the 1970s and with a peak at birth year 1991, we observed a moderate but significant decrease (Paper I).
- Childhood BMI is inversely associated with pubertal timing in normal weight but not in overweight boys (Paper II).
- Age at PHV displayed a clear secular trend towards earlier puberty among boys. Since the 1940s until birth year 1996, the pubertal growth spurt was 1.5 months earlier per decade, and this trend could only slightly be explained by the coinciding increase in childhood BMI (Paper III).
- Childhood BMI and BMI change during puberty is only marginally correlated (r=0.06) indicating that these developmental parameters may provide non-overlapping information for prediction of risk of adult disease (Paper IV).
- BMI change during puberty, but not childhood BMI, was independently associated with the risk of adult cardiovascular mortality. The association between BMI change during puberty and cardiovascular mortality was non-linear, with 22% increased risk of cardiovascular mortality per additional increase in BMI units for individuals in the highest quartile. (Paper IV).

In conclusion, the measurements in school health care that has been ongoing for almost 100 years and included essentially all children in Gothenburg have together with information from the military conscription register provided data on height and weight both before and after puberty in a large cohort of boys. This unique material has enabled us to identify long-term trends of childhood BMI and pubertal timing. We identified a large BMI increase during puberty as a novel independent risk marker for increased risk of adult cardiovascular mortality.

7 FUTURE PERSPECTIVES

The BEST cohort is a large population-based cohort that is the result of great efforts and hard work. However, some challenges still remain. Although the data is secondary, retrospective data, it needs to be collected from paper records and transcribed to digital format, something that is both time-consuming and expensive. The data material that the studies in this thesis are based upon are all originating from the first step of the data collection in the BEST cohort (boys born 1945-1961 and every five years until 2006). Step two of the data collection in the BEST cohort is completed but work regarding validation and register linkage still remains (boys and girls born 1920-1968). Step three of the data collection is initiated and will continue until completed (boys and girls born 1920-present).

For future studies, there are several knowledge gaps that need to be addressed, such as if BMI change during puberty remains an important determinant of cardiovascular mortality after adjustment for BMI at middle age. Another highly interesting research question that also is clinically significant is what the impact of the secular trend of earlier pubertal timing has on future public health and disease. Is pubertal timing in boys associated with adult diseases? It will also be interesting to investigate the trends for childhood BMI and pubertal timing in girls as well as to investigate potential sex-differences in the association between childhood BMI and pubertal timing. Another exciting aspect to explore is the socioeconomic perspective. Socioeconomic factors are without doubt of great importance for health and disease, and in the future we will study this in the BEST cohort.

Future studies could include linking the large BEST cohort with smaller but more data-dense cohorts, for investigation of mediators and potential mechanisms in the discovered associations. For example, linking the BEST cohort with cohorts that have body composition data for a broader understanding of the results in paper II. Furthermore, linking the BEST cohort with a cohort like the Swedish CardioPulmonary bioImage Study (SCAPIS) cohort, that have information on risk factors for cardiovascular disease such as smoking, blood pressure, serum levels of cholesterol, triglycerides, or measurements of markers of disease progress such as coronary or carotid plaques for a broader understanding of the results in paper IV. We also want to further evaluate the importance of the new interesting and distinct exposure variable BMI change during puberty for different diseases.

The benefits from the studies included in this thesis and also all the future studies originating from the BEST cohort are numerous, both clinically and in a research perspective. This research, based on the efforts made by school health care nurses over a long period of time, can be directly transferred back with new knowledge. This is one of the many beautiful things about science!

8 RELATED PUBLICATIONS NOT INCLUDED IN THE THESIS

1. Ohlsson C, Bygdell M, Sonden A, Jern C, Rosengren A, Kindblom JM. **BMI increase through puberty and adolescence is associated with risk of adult stroke**. Neurology. 2017;89(4):363-369.

2. Kindblom JM, Bygdell M, Sonden A, Celind J, Rosengren A, Ohlsson C. **BMI change during puberty and the risk of heart failure**.
   Journal of Internal Medicine. 2018;283(6):558-567.

3. Ohlsson C, Bygdell M, Nethander M, Rosengren A, Kindblom JM. **BMI change during puberty is an important determinant of adult type 2 diabetes risk in men**.
   The Journal of Clinical Endocrinology & Metabolism. 2018.

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