

Doctoral Thesis for the degree of Doctor of Philosophy, Faculty of Food Science and Nutrition

Early growth and later health

Influence of birth size and childhood growth on cardiovascular disease risk factors and mortality

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*The doctor of the future will no longer treat the human frame with drugs,
but rather will cure and prevent disease with nutrition.*
Thomas Edison (1847-1931)

Abstract

Background: Fetal and early life exposures are associated with cardiovascular disease (CVD) in later life. Furthermore, rapid growth during infancy has been implicated in increasing risk of overweight and obesity in childhood.

The aim of this dissertation was to enhance the understanding of the associations between fetal, infant, and childhood growth on later risk factors for CVD including obesity. Using a historical cohort of subjects born in the early 1900s who took part in the prospective Reykjavik Study, this dissertation evaluates the environmental influences that contribute to fetal growth and the impact of childhood growth in relation to cardiovascular risk factors and mortality in adulthood. In addition, early dietary determinants of growth during infancy and its association with infant growth and childhood body mass index (BMI) are examined in a cohort on early infant feeding practices.

Reykjavik Study: Fetal and childhood growth cohort

Information on size at birth (n=4601) and childhood growth gathered in regular intervals from ages 8 to 13 years (n=1924) were collected from the national archives for subjects born 1914-1935 who participated in the Icelandic Heart Association Reykjavik Study (1967-1991). Childhood growth was examined in relation to adult CVD risk factors and mortality with follow-up until 31 December 2009.

Infants born between 1930-1934 during the Great Depression in Iceland, which was a stressful economic period, were born lighter, had lower ponderal index and greater likelihood of obesity at adult age, odds ratio (95% confidence interval (CI)) 1.40 (1.09, 1.77) compared to infants born immediately prior to the Depression (1925-1929). In the examination of childhood growth, faster gains in BMI during ages 8 to 13 years were associated with a more adverse CVD risk profile among men and greater risk of CVD mortality among both sexes, with corresponding hazard ratios of 1.49 (95% CI 1.03, 2.15) among men, and 2.32 (95% CI 1.32, 4.08) among women. The examination among girls of timing of peak height velocity (PHV), an early marker of maturity, showed that girls who had height acceleration <11 years and between ages 11-12 years had an increased risk of CVD mortality compared to girls with PHV >12 years, hazard ratio (95% CI) 1.87 (1.07, 3.26) and 2.56 (1.52, 4.31), respectively. The associations between childhood growth and CVD mortality were stable after adjustment for mid-life (mean age 51 years) CVD risk factors and BMI.

Early nutrition and infant and childhood growth cohort

Information on early nutrition practices was collected from a cohort of Icelandic infants born in 2005 (n=154) who were prospectively followed from birth to 18 months and again at 6 years of age. Dietary data was collected from birth to 12 months of age. Anthropometric data was gathered from the maternity wards and healthcare centers participating in the study. Height and weight at 6 years of age was measured at a clinical examination at Landspítali-National University Hospital. In the examination of early feeding practices on infant growth, providing infants with solid foods already at 5 months

of age was positively associated with infant growth, particularly between 2 to 6 months of age, compared to infants who were exclusively breastfed at 5 months of age. The addition of solid foods at 5 months predicted greater BMI at 6 years, with BMI being on average 0.7 kg/m² (95% CI 0.0, 1.3) higher among infants provided solid foods compared to those exclusively breastfed at 5 months of age.

Conclusions: The Great Depression in Iceland had an unfavourable effect on fetal growth. Infants born during this period were thinner and had an increased likelihood of obesity at adult age, which is a significant contributor to CVD. Our findings that providing infants with solid foods at 5 months of age was positively associated with infant growth and BMI at 6 years suggests this is an important area where further studies are needed. Closer investigation of infant feeding practices is warranted particularly if mothers are unable to exclusively breastfeed for the recommended first 6 months of the infant's life. Educating parents to slow the introduction of complementary foods may have long-term benefits, particularly if the infant is on track to become overweight or obese and changes in infant feeding practices help normalize growth later in infancy and into childhood.

These advantages may also present later in adult life with respect to better cardiovascular health, as we found that faster gains in childhood BMI was associated with greater CVD mortality among both men and women at mid-life. Additionally, the association between early childhood height acceleration (i.e. PHV) and CVD mortality observed among females in this cohort indicates that the timing of pubertal events also needs to be considered when evaluating cardiovascular health.

The majority of preventative measures for CVD have focused primarily on interventions at adult age. The findings reported in this dissertation highlight that environmental exposures early in life can have lasting implications, increasing later risk of heart disease. It is important to place emphasis on dietary habits and lifestyle, not only in adulthood, but also during pregnancy and into childhood to ensure proper and adequate growth over the life course.

Key words: cardiovascular disease, fetal growth, infant, child, growth, mortality, obesity, cardiovascular risk factors

Ágrip

Bakgrunnur: Vísbendingar eru um að umhverfisáhrif á fósturskeiði og í barnæsku geti aukið líkur á hjarta- og æðasjúkdómum síðar á lífsleiðinni. Markmið þessarar rannsóknar var að skoða tengsl vaxtar á fósturskeiði, ungbarnaskeiði og á skólaaldri við áhættuþætti hjarta- og æðasjúkdóma síðar á lífsleiðinni. Skoðuð voru áhrif umhverfispáttá á fósturvöxt og vöxt barna á skólaaldri og tengsl vaxtar við áhættuþætti hjarta- og æðasjúkdóma hjá einstaklingum sem fæddust á öðrum og þriðja áratug tuttugustu aldar og tóku þátt í *Reykjavíkurrannsókn Hjartaverndar*. Að auki voru áhrif næringar á fyrstu mánuðum eftir fæðingu skoðuð með tilliti til þyngdar og líkamspýngdarstuðuls (LPS) að 6 ára aldri í framsýnni hóprannsókn á börnum sem fæddust á fyrsta áratug tuttugustu og fyrstu aldar.

Reykjavíkurrannsókn Hjartaverndar: Vöxtur á fósturskeiði og skólaaldri

Upplýsingum um fæðingarþyngd og -lengd ($n=4601$), ásamt hæðar- og þyngdarmælingum úr skólaheilsugæslu frá 8 til 13 ára aldurs ($n=1924$), var safnað fyrir alla einstaklinga sem fæddust í Reykjavík (1914-1935) og tóku þátt í Reykjavíkurrannsókn Hjartaverndar. Skoðuð voru tengsl vaxtar við áhættuþætti hjarta- og æðasjúkdóma, sem metnir voru við skráningu í Reykjavíkurrannsókn Hjartaverndar (1967-1991), og við dauðsföll af völdum hjarta- og æðasjúkdóma til 31. desembers 2009.

Börn sem voru fædd 1930-1934, eftir að kreppan mikla skall á árið 1930, höfðu marktækt lægri fæðingarþyngd og “ponderal index” borið saman við börn sem fædd voru áður en kreppan skall á (1925-1929). Á fullorðinsaldri, við skráningu í Reykjavíkurrannsókn, voru þeir einstaklingar sem fæddir voru eftir að kreppan skall á líklegri til að vera of feitir ($LPS \geq 30 \text{ kg/m}^2$), líkindahlutfall (odds ratio) 1.40 með 95% öryggisbil (95% CI 1.09, 1.77), borið saman við börn fædd fyrir kreppuna.

Þegar vöxtur barna á skólaaldri (8-13 ára) var skoðaður sáust tengsl milli aukningar í LPS frá 8 til 13 ára aldurs og dauðsfalla af völdum hjarta- og æðasjúkdóma; áhættuhlutfall (hazard ratio) 1.49 (95% CI 1.03, 2.15) fyrir karla og 2.32 (95% CI 1.32, 4.08) fyrir konur. Skýrari tengsl voru milli breytinga á LPS frá 8 til 13 ára og áhættuþátta hjarta- og æðasjúkdóma, þ.e. blóðþrýstings, blóðfitu og LPS, hjá karlmönnum á fullorðinsaldri, en hjá konum. Einnig voru áhrif hámarks hæðarbreytinga (Peak height velocity: PHV) stúlkna frá 8 til 13 ára aldurs skoðuð sérstaklega en PHV er metill fyrir vöxt tengdum snemmbærum kynþroska. Dauðsföll af völdum hjarta- og æðasjúkdóma voru algengari hjá þeim konum sem höfðu náð PHV fyrir 11 ára aldur og milli 11-12 ára aldurs miðað við þær sem náðu PHV við 13 ára aldur eða seinna, áhættuhlutfall: 1.87 (95% CI 1.07, 3.26) og 2.56 (95% CI 1.52, 4.31). Almennu tengsl milli vaxtar við 8-13 ára aldur og dauðsfalla af völdum hjarta- og æðasjúkdóma stöðug þótt leiðrétt væri fyrir LPS á fullorðinsaldri.

Næring á fyrstu ævimánuðum og þyngd við 6 ára aldur

Upplýsingum um mataræði á fyrstu mánuðum æviskeiðs 154 ungabarna fæddum árið 2005 var safnað og þeim fylgt eftir til 6 ára aldurs. Upplýsingar um hæð og þyngd voru einnig fengnar frá heilsugæslu. Þau börn sem voru byrjuð að fá fasta fæðu við 5 mánaða aldur voru þyngri en þau börn sem voru eingöngu á brjósti við 5 mánaða aldur og sást munur á þyngd þessara hópa strax við 2 mánaða aldur. Við 6 ára aldur voru þau börn sem byrjuð voru að fá fasta fæðu við 5 mánaða aldur með að meðaltali 0.7kg/m^2 (95% CI 0.05, 1.3) hærri LPS en þau börn sem voru eingöngu á brjósti við 5 mánaða aldur.

Ályktanir: Efnahagsþrengingar í kreppunni miklu árið 1930 í Reykjavík virðast hafa haft neikvæð áhrif á fósturvöxt sem aftur virðist hafa leitt til aukinnar áhættu á offitu á fullorðinsaldri, en offita er þekktur áhættuþáttur hjarta- og æðasjúkdóma. Varðandi fæðuveenjúr á fyrstu mánuðum ævinnar benda niðurstöður okkar til þess að neysla fastrar fæðu strax við 5 mánaða aldur hafi forspárgildi fyrir LPS við 6 ára aldur og mikilvægt sé að skoða betur áhrif fæðuvals ef ráðleggingum um eingöngu brjóstgjöf til 6 mánaða aldurs er ekki fylgt, sér í lagi m.t.t áhrifa á þyngd barna síðar meir. Þegar kemur að vexti á skólaaldri, við 8-13 ára aldur, benda niðurstöður okkar til þess að hröð aukning í LPS hafi forspárgildi fyrir dauðsföll af völdum hjarta- og æðasjúkdóma hjá bæði körlum og konum. Einnig virtist snemmbær vaxtarkippur tengdum kynþroska hjá stúlkum hafa forspárgildi fyrir dauðsföll af völdum hjarta- og æðasjúkdóma. Þar sem vaxtarmælingar eftir 13 ára aldur voru ekki skráðar var ekki unnt að skoða samsvarandi tengsl hjá körlum en niðurstöður okkar gefa samt ákveðna vísbendingu um að lífeðlisfræðilegar breytingar tengdar snemmbærum kynþroska geti hugsanlega haft áhrif á hjarta- og æðasjúkdóma síðar á lífsleiðinni.

Þegar kemur að forvörnum gegn hjarta- og æðasjúkdómum hefur mikil áhersla verið lögð á aðgerðir á fullorðinsaldri. Niðurstöður okkar benda til þess að umhverfisáhrif snemma á lífsleiðinni geti haft langvarandi áhrif og aukið líkur á hjarta- og æðasjúkdómum, og að mikilvægt sé að leggja áherslu á fæðuveenjúr og lífsstíl sem tryggja hæfilegar þyngdarbreytingar og vöxt strax á meðgöngu og barnsaldri en ekki einungis á fullorðinsaldri.

Lykilorð: hjarta- og æðasjúkdómar, börn, vöxtur, nýburar, dánarlíkur, offita, áhættuþættir hjarta- og æðasjúkdóma

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Abbreviations

BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CI	confidence interval
CVD	cardiovascular disease
HR	hazard ratio
OR	odds ratio
PHV	peak height velocity
SD	standard deviation
WHO	World Health Organization

List of Original Papers

This dissertation is based on the following papers, which will be referred to in the text by their respective Roman numerals:

- I. Imai CM, Halldorsson TI, Gunnarsdottir I, Gudnason V, Aspelund T, Jonsson G, Birgisdottir BE, Thorsdottir I. Effect of birth year on birth weight and obesity in adulthood: comparison between subjects born prior to and during the great depression in Iceland. *PLoS ONE*, 2013;7(9): e44551. doi:10.1371/journal.pone.0044551
- II. Imai CM, Gunnarsdottir I, Gudnason V, Aspelund T, Birgisdottir BE, Thorsdottir I, Halldorsson TI. Faster increase in body mass index from ages 8 to 13 is associated with risk factors for cardiovascular disease morbidity and mortality. *Nutr Metab Cardiovasc Dis.* (in press) doi:10.1016/j.numecd.2014.01.001
- III. Imai CM, Gunnarsdottir I, Gudnason V, Aspelund T, Birgisdottir BE, Thorsdottir I, Halldorsson TI. Early peak height velocity and cardiovascular disease mortality among Icelandic women. *Ann Med*, 2013;45(8):545-550. doi:10.3109/07853890.2013.852347
- IV. Imai CM, Gunnarsdottir I, Thorisdottir B, Halldorsson TI, Thorsdottir I. Introduction of solid foods prior to 6 months of age is associated with faster growth during infancy and increased body mass index in childhood. *Submitted*

Papers included by permission of publishing journals.

1 Introduction

The aim of this dissertation is to enhance the understanding of the associations between fetal, infant, and childhood growth on later risk factors for cardiovascular disease (CVD) including obesity. The interplay between early life events and later risk of disease is a complex web of connections. There are several critical periods of development – fetal growth, infancy, childhood, and puberty – where humans may be more vulnerable to environmental influences leading to long-term health consequences [1]. Using a historical cohort of subjects born in the early 1900s [2], this dissertation evaluates the environmental influences that contribute to fetal growth and the impact of childhood growth in relation to cardiovascular risk factors and mortality in adulthood. In addition, early dietary determinants of growth during infancy and its association with childhood body mass index (BMI) are examined in a small but relatively detailed cohort on early infant feeding practices.

In most industrialized countries, there have been decreased trends in CVD mortality [3]. However, CVD continues to be the number one cause of death globally [4]. As such, there is a need to look beyond the traditional risk factors that contribute to development of CVD to better target prevention measures.

It is becoming evident that the risk of chronic disease is set early in life [5]. David Barker and colleagues were one of the first to describe the association between low birth weight and higher death rates from ischaemic heart disease in adulthood [6]. In the twenty-five years since the “Barker hypothesis” was developed, numerous studies have linked low birth weight not only to CVD but also hypertension, type 2 diabetes, and even mental health disorders at adult age [7-10]. These findings stem primarily from observational cohorts and early studies have been unable to account for important covariates, such as CVD risk factors and adult body size which contribute to disease risk.

The causes of low birth weight are multifactorial and can include maternal malnutrition, hypoxia, and stress. Furthermore, adverse health outcomes have been observed in people exposed to famine in utero, as well as in individuals whose mothers were adequately nourished during pregnancy yet born with low birth weight [11, 12]. In Iceland, analysis of births after the 2008 national economic crisis, compared to the prior year, showed a higher number of low birth weight infants as well as a tendency toward babies who were small for gestational age [13, 14]. These results suggest a short-term increase in low birth weight infants following the stress from the economic collapse. Considering these findings, it is relevant to examine within a historical context if similar patterns can be detected and their potential long-term consequences.

While low birth weight is now recognized as a modest risk factor for CVD, it appears that the combination of being small at birth and remaining thin during infancy, followed by rapid growth later in childhood is associated with the greatest risk of coronary heart disease (CHD) in adulthood [15]. However, previous studies have mostly been registry based and lacked information on traditional CVD risk factors in adulthood which is important for mechanistic insight. There is also a need to identify whether the change in CVD risk occurs over a certain phase of childhood growth and to what extent it is independent of adult BMI [16] which is a well-established contributor to CVD.

The influence of early nutrition on later disease risk is a continually growing area of research. Dietary habits tend to be set early in life and there is great potential of preventing chronic diseases by imparting healthy choices in infancy [17]. Infant feeding practices can affect not only immediate postnatal growth, but also childhood growth and beyond [18]. It

is vital to consider the life-course approach when determining how early nutrition habits and growth at different life stages accumulate to impact development of CVD.

2 Background

2.1 Cardiovascular disease

2.1.1 Incidence and mortality

CVD continues to be the leading cause of death worldwide and markers of CVD are appearing earlier among youth in most developed countries [19]. An estimated 17.3 million people died from CVD in 2008 and the number is set to reach 23.3 million by 2030 [20]. In Iceland, comprehensive prevention strategies, including tobacco control and improvements in diet, have reduced the incidence of myocardial infarction by 66% and CHD mortality rates by 80% between 1981 and 2006 among men and women aged 25 to 74 years [21]. Compared to other European countries, Iceland currently has one of the lowest potential years of life lost due to CVD, a measure of deaths occurring at a younger age which should be preventable [3]. Despite these positive changes, heart disease continues to be an ongoing public health concern and it is the main cause of mortality in Iceland accounting for 38% of all deaths in 2009 [22].

2.1.2 Traditional CVD risk factors

Hypertension, dyslipidemia, and type 2 diabetes are established contributors to the development of CVD. More recently, hyperuricemia has been shown to have a modest association with CVD incidence and mortality [23]. These conditions are associated with the accumulation of plaque in the arterial blood vessels which can lead to atherosclerosis, the most common cause of CVD. Controlling these risk factors has been shown to decrease risk of CVD [24]. Non-modifiable risk factors for CVD include familial history of CVD, age and gender. Lifestyle choices such as smoking, physical inactivity, harmful use of alcohol and unhealthy diet can also hasten the onset of CVD.

2.1.3 Sex-specific differences in CVD

Clinical symptoms of CVD manifest later in life for women compared to men, and women are predominantly more likely to delay seeking care [25]. There is a greater tendency for women to present with stroke as a first sign of CVD compared to myocardial infarction among men [26]. Jonsdottir et al. reported that the relative risk of myocardial infarction is higher among Icelandic women who smoke, have diabetes, elevated triglycerides and left ventricular hypertrophy [27]. The authors note, however, that the differences in these risk factors were not sufficient to explain the variances in occurrence of myocardial infarction between men and women. It is becoming necessary for current risk assessment models used to identify patients with high risk of CVD to look beyond traditional CVD risk factors so prevention measures can be better targeted.

2.1.4 Impact of obesity on CVD

Obesity is defined as having a BMI ≥ 30 kg/m² and is well accepted as an independent risk factor for CVD [28]. In addition to the aforementioned CVD risk factors, obesity is also associated with sleep apnea, osteoarthritis, as well as certain cancers [29, 30]. Childhood

obesity is a global problem affecting both developed and developing countries [31]. Greater adiposity has been linked to elevated C-reactive protein, a marker of inflammation, in children as young as three years of age [32]. Recent reports suggest rates of childhood obesity may be stabilizing or even decreasing in some European countries [33], including among Icelandic children 6 years of age [34]. However, it remains a legitimate concern as highlighted by data from two cross-sectional population-based samples of 14-15 year old Icelandic adolescents, which showed an increase in both mean BMI and prevalence of overweight and obesity between 2000 and 2009 [35]. This was partially attributed to the availability of modern products high in fat and sugar, particularly in urban areas. Despite these findings, there is limited but encouraging evidence that children who carry excess weight but return to a healthy adult weight may have a slightly better CVD risk profile compared to those who become or remain obese as adults [36]. As childhood and adolescent BMI tracks into adulthood, it is clear that prevention of obesity through changes in lifestyle and nutrition will help reduce CVD incidence.

2.2 Fetal growth

Fetal growth is a vital predictor of health status during infancy as well as in childhood and at adult age. Factors that cause poor fetal growth include but are not limited to maternal diet and health, placental malfunctions, and environmental influences. Being born with low birth weight not only increases risk of disease and infection in the immediate postnatal period, but also risk of chronic diseases in adulthood [5].

Size at birth is strongly related to maternal factors such as parity and the mother's own birth weight [37]. An estimated 25-50% of normal variation in birth size is driven by genetics while environmental determinants may contribute to the remaining influences [38, 39]. For example, the risk of preterm birth can be predicted by a combination of maternal genetic variations, i.e. genotypes, together with environmental exposures such as smoking [40]. Furthermore, maternal conditions such as malnutrition or infection can increase the contribution of environmental influences on birth size.

2.2.1 Developmental origins of health and disease

The developmental origins of health and disease paradigm evolved from epidemiological studies linking low birth weight due to fetal under-nutrition to adult mortality [41]. From these epidemiological observations, the fetal origins hypothesis was developed which posited that alterations in fetal nutrition during a critical period of development can result in permanent changes in the body's structure and function, which "programs" or increases susceptibility to cardiovascular and endocrine disease in adult life [42, 43].

It is now well accepted that the early life environment leads to consequences for later health. This rapidly evolving paradigm has expanded to include environmental exposures during infancy, over- and under-nutrition during pregnancy and infancy, stress during pregnancy which can have deleterious effects on fetal development, as well as the contributions of epigenetic mechanisms [41].

While U-shaped associations have been reported between birth weight and adult hypertension and type 2 diabetes [9, 44], there appears to be a more consistent inverse relationship between birth weight and CHD in adulthood [7]. Epidemiological studies on ethnically diverse populations have reproduced this relationship and small size at birth is

now considered a modest risk factor for CVD [45-49]. Among Icelandic infants, birth weight has been shown to have an inverse association with hypertension and truncal fat [50] and a U-shaped association with dysglycemia [51]. Risk of CHD among Icelandic men was inversely related to birth length, while a suggestive trend was observed for birth weight which may be explained by the relatively high birth weight among Icelandic infants [52, 53].

2.2.2 Factors influencing intrauterine growth

Fetal growth restriction can be a consequence of maternal malnutrition during gestation. When a fetus is exposed to a non-optimal environment in utero, it may not reach its growth potential and can become growth restricted. Both low maternal pre-pregnancy weight and gestational weight gain can result in low birth weight infants [54, 55]. Conversely, excessive gestational weight gain, particularly among obese mothers, increases risk of babies who are large for gestational age which also has long-term unfavourable outcomes [56].

In adequately nourished populations, placental insufficiency has been suggested to be the primary cause of reduced fetal growth [12]. Decreased umbilical blood flow can lead to placental insufficiency, disrupting the normal supply of nutrients and oxygen to the fetus [57]. This leads to infants who are small for gestational age or who have low birth weight. However, the influence of maternal diet, even among well-nourished mothers, cannot be ignored. Findings from the Southampton's Women Survey showed a high carbohydrate, low dairy protein diet during pregnancy suppressed normal placental growth [58]. Reduced placental weight and surface area has also been linked to hypertension in the offspring [59]. Thus, to some extent, low birth weight and later risk of disease such as CVD, particularly among nourished mothers, may be partially due to changes in placental function [60].

Paternal height and weight measures impact fetal growth but to a lesser extent than maternal body size [61]. Maternal smoking and alcohol intake are well established contributors to low birth weight. The negative impact of maternal infections on fetal growth is currently mainly seen in developing countries. Exposure to infections during pregnancy can reduce fetal growth and development if the disease affects the normal transfer of nutrients across the placenta [62]. Prolonged maternal stress can adversely impact the fetus by the release of stress hormones that may act by restricting placental blood flow and affecting circulation to the fetus [63, 64]. Overall, there is strong evidence that in addition to maternal nutritional status, impaired placental function via infection or stress contributes to poor intrauterine growth.

2.2.3 Effects of famine and environmental stress

Exposures to severe maternal undernutrition due to famine and stressful environments, such as war and economic crises, during pregnancy can also affect fetal and postnatal growth. Periods of famine, although very unfortunate, provide the unique opportunity to investigate involuntary caloric restriction during pregnancy and its effects on offspring. The first trimester appears to be the period during which the fetus is most vulnerable to environmental influences as cell and organ differentiation occurs during this phase. This is emphasized by findings from the Dutch Famine Birth Cohort, 1944-1945, which found greater risk of CHD, obesity, and a more atherogenic lipid profile among those exposed to

famine in early gestation [65, 66]. Although individuals who developed later CHD tended to have lower birth weight, the association with famine exposure was independent of birth weight [67]. Similar findings were reported during the more recent Chinese Great Famine, 1959-1961, where exposure during the first trimester was linked to hypertension in adulthood [68]. However, data on birth weight of the offspring was not available and it is difficult to interpret whether the outcomes were due to malnutrition, stress or other confounders.

It is important to note that poor birth outcomes have also been observed in non-famine like conditions. Periods of economic hardship have also been associated with reduced fetal growth and this has been documented both in Iceland during the 2008 economic crisis as well as in other countries [13, 14, 69-71]. Data from the National Longitudinal Survey of Youth in the United States reported that periods where unemployment rates were higher than expected or when pregnant mothers experienced an adverse employment change were associated with decreased infant birth weight [69, 70]. In Norway and Sweden, male unemployment was associated with increased risk of very low birth weight among infants born 1973-1995 [71].

Investigating whether a historically difficult period in Iceland could impact birth outcomes and adult health is pertinent and possible with data from the Reykjavik Study cohort which is analyzed in this dissertation. In the 1920s, Iceland's economy was expanding until the onset of the Great Depression which affected the country drastically due to heavy reliance on exports. The Depression hit the Western world in 1929. Historical sources identify 1930 as the year the effects of the Depression reached Iceland, forcing exports to lose up to 50% of their 1929 prices and declines in food supply data were reported for most years during the 1930s [72, 73]. This period in Iceland, while not on the same scale as a famine, was nowhere as affluent compared to the period during the 2008 economic crisis where the standard of living in Iceland was still quite high. The unique timing of the Depression in Iceland makes it possible to investigate the potential impact of sudden environmental shifts on birth size and later health where significant socioeconomic and nutritional changes were taking place under non-famine like conditions.

2.3 Growth and nutrition in infancy

Several factors in infancy have been implicated in increasing risk of diseases and predisposition to CVD. Observational studies have linked slower rate of infant growth followed by rapid childhood growth to greater risk of ischaemic heart disease at adult age [47]. A systematic review by Fisher et al. reported that larger size in infancy was associated with a reduced risk of ischaemic heart disease only among men, but an increased risk of insulin-dependent diabetes in both sexes [74]. Low infant weight gain has been associated with higher concentrations of low density lipoprotein cholesterol levels in adulthood [75] and infants who were born thin appear to be at greater risk. It has been speculated that the normal function of the liver, where low density lipoprotein cholesterol levels are regulated, may have been impaired in utero and these changes are further influenced by poor infant growth [47, 76]. Furthermore, low infant weight gain may be a reflection of growth faltering, which itself may program later risk of disease [77]. These associations are based largely on historical cohort studies that have limited information on infant feeding practices which can also contribute to infant growth and later health outcomes.

In more recent cohort studies, it has been rapid infant growth that has been associated with adverse health outcomes such as childhood and adolescent obesity [78-80]. Furthermore, the differences in weight gain during early infancy are at least partly explained by means of infant feeding [81, 82]. The factors contributing to differences in infant growth are further discussed.

2.3.1 Breastfeeding

The first year of life is a critical period for good nutrition. The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life with continued breastfeeding along with complementary foods up to two years of age or beyond [83, 84]. In 2003, the Icelandic infant nutrition recommendations were revised with an added emphasis on exclusive breastfeeding for 6 months and increasing total breastfeeding duration [85]. The prevalence of breastfeeding in Iceland is fairly high. From 2004-2008, 92% of Icelandic infants were exclusively breastfed at 2 months of age, although this number drops to 8% at 6 months. Prevalence of partial breastfeeding is higher, with 74% of mothers continuing to breastfeed at 6 months and 27% at 12 months [86]. Research has shown that interventions such as peer support to initiate and continue breastfeeding are not as successful in high income countries and there is a need to evaluate policies so effective ways to promote breastfeeding can be identified [81, 87].

Breast milk can provide infants the nutrients needed for healthy development. The immediate benefits of breastfeeding include decreased risk of gastrointestinal infections, acute otitis media, respiratory infections, and sudden infant death syndrome [88]. Potential long-term advantages of breastfeeding include protection against overweight or obesity, better cognitive development, and reduction in type 2 diabetes, hypertension and hyperlipidemia [89-92]. In addition, exclusive breastfeeding until 4 months of age may slow weight gain in infancy without affecting normal growth and may be a possible mechanism behind the protective effects of breastfeeding [81].

There has been conflicting evidence about breastfeeding practices and cardiovascular health in childhood and adulthood. Duration of breastfeeding was positively related to high density lipoprotein cholesterol levels in Icelandic girls at 6 years of age [93]. In a study from the Netherlands, exclusive breastfeeding from 3-6 months was associated with greater carotid-intima media thickness, but not carotid stiffness, at 5 years of age [94], which could be related to the elevated serum cholesterol levels observed in exclusively breastfed infants [95]. In the Young Finns Study, men who were breastfed exhibited better brachial endothelial function compared to men who were formula-fed [96]. Furthermore, findings from the Nurses' Health Study reported a tendency towards reduced risk of ischaemic heart disease among women who were breastfed [97]. However, a recent analysis found that duration of breastfeeding did not have an impact on cardiovascular risk factors at age 32 years [98]. There is also limited evidence that breastfeeding is associated with CVD mortality [99].

2.3.2 Protein intake during infancy

In the early 20th century, consumption of milk and dairy products was high in Iceland and was a significant source of protein. The first study on Icelandic nutrition and diet reported that on average, the intake of milk and dairy products was 1000 ml/day [100, 101]. Findings from a Danish cohort reported that maternal milk intake of ≥ 150 ml/day was

associated with greater birth size, suggesting that it may have a growth-promoting effect [102]. Furthermore, when these offspring were followed up at 20 years of age, they had a tendency to have greater height and insulin-like growth factor 1 levels. This suggests a programming effect of milk consumption in utero. However, there is also evidence that postnatal infant feeding practices may have similar effects. For example, the relatively higher protein content of infant formula compared to breast milk may have a stimulating effect on insulin-like growth factor 1, which can accelerate growth [103]. This is an important effect due to the potential adverse consequences of faster infant growth on later health outcomes.

Before the theory was established that greater protein intake could hasten infant growth and increase risk of overweight, it was common to provide cow's milk to infants during the weaning process. Prior to 2003, the recommended practice in Iceland was to introduce cow's milk to infants at 6 months along with breastfeeding and after breastfeeding was discontinued [53]. Unlike breast milk, cow's milk is not nutritionally appropriate for children younger than age 12 months as it contains excessive levels of protein for human infant requirements and insufficient iron levels [104]. Among Icelandic infants, excess cow's milk intake (>500 ml/day) in children less than one year was shown to negatively affect iron status [105]. High consumption of cow's milk leads to a larger proportion of the infant's total energy intake coming from protein. There is evidence now that greater protein intake is linked to faster growth in infancy and higher childhood BMI [106-108].

In 2003, Icelandic infant nutrition recommendations were revised and the use of iron-fortified formula was recommended in the weaning period and after discontinuation of breastfeeding from 6 months to 2 years [85]. The recommendations further advised avoiding use of cow's milk and dairy products throughout the first year. Positive effects on infant nutrition status were observed after the implementation of these revised recommendations. In particular, infants were consuming less cow's milk, and thus less protein, and had improvements in iron status [109].

2.3.3 Timing of complementary feeding

The timing of when to introduce complementary foods may vary depending on the nutritional needs of the infant as well as the circumstances of the caregiver. Most often, by the age of 6 months, breast milk alone may not be enough to meet the energy and nutrient requirements of the infant. However, WHO recommendations advise that breastfeeding should not be decreased when starting on solids as infants are at greater risk of poor energy intake and growth faltering during this period [83].

The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition concluded that the gradual expansion of the infant's diet to include fluids and solid foods, other than breast milk or infant formula, should not begin before 17 weeks but not later than 26 weeks of age [110]. Furthermore, although exclusive breastfeeding for 6 months is a desirable goal, there is evidence that most mothers in European countries begin to introduce solid foods prior to 6 months of age [111]. This may have potential public health consequences since there is some evidence that introducing solid foods before 4 months of age can lead to rapid weight gain in infancy and greater likelihood of early childhood overweight and obesity [112-114].

There are several factors that contribute to variations in infant feeding practices. Strong predictors of early solid food introduction include young maternal age, maternal smoking, low maternal education, and short duration (<4 weeks) of breastfeeding [115]. In

addition, obese mothers are less likely to initiate breastfeeding and if they do, plan to breastfeed for a shorter period than normal weight women [116]. However, there is also evidence that excessive gestational weight gain even among normal-weight women is associated with failure to initiate or sustain breastfeeding [117]. The earlier cessation of breastfeeding together with the introduction of solid foods may also be related to maternal concerns that breast milk alone is not adequate to meet the child's nutritional needs [118].

The acceptance and tolerance of complementary foods also appears to be connected with the timing and type of foods introduced. Breastfed infants are more likely to accept new food textures due to earlier exposure to new flavours in their mothers' milk [119, 120]. The benefit of slowly introducing solid foods is evident when considering food sensitivities. A nested case-control study reported that continuing breastfeeding and delaying the introduction of solids until 17 weeks of age may protect against development of food allergies [121]. However, introducing fruits and vegetables early during infancy allows time for their acceptance and is a good indicator of later frequent consumption [122].

2.3.4 Infant growth and cardiovascular risk factors

Growth during infancy is an important determinant of later cardiovascular health. Babies born with low birth weight often display "catch-up" growth during the first few months of life [123]. These infants tend to be longer and thinner at birth (i.e. lower ponderal index) and have reduced adiposity, but as children may become fatter (greater BMI and body fat percentage) with more central fat distribution [124]. It has been proposed that the body composition of infants born thin and with low birth weight have relatively low muscle mass, i.e. thin-fat phenotype, and if they become overweight, will exhibit a disproportionally high fat to lean mass ratio [125] and thus have greater CVD risk. However, findings from the Pune Maternal Nutrition Study found that regardless of whether Indian infants were born "thin-fat", it was the faster tempo of growth up to 6 years of age that was associated with greater CVD risk [126, 127].

Both postnatal under- and over-nutrition may program later disease risk. Older epidemiological studies have reported that lower weight gain during infancy and small body size at one year were predictors of CHD in adulthood, especially among infants who had poor fetal growth [47, 128, 129]. This is most likely due to the effects of fetal undernutrition continuing to impact infant size and growth. Conversely, in contemporary cohorts, rapid growth during the first year of life, primarily due to excess nutrition, has been associated with increased risk of overweight and obesity in childhood [79, 80, 106, 130]. In this setting, CVD risk may be increased if inappropriate weight gain continues into adulthood [131].

Infant growth patterns are becoming better understood and the timing and tempo of growth rate appears to be important variables. A Finnish cohort reported growth velocity over the first 2 years of life to be positively associated with blood pressure, BMI, and waist circumference in adulthood [132]. Data from a Dutch cohort identified a more specific time period; where faster weight gain in the first 3 months of life was associated with more central adiposity and reduced insulin sensitivity in early adulthood [133].

Growing evidence suggests that different growth velocities in infancy are associated with adverse health outcomes; however, there is insufficient data to indicate that infant growth should be altered in any way to prevent adult disease [74]. Therefore, it is becoming imperative to consider infant feeding practices, such as duration of breastfeeding

and timing of solid food introduction, which can be adapted to support normal infant growth while decreasing later disease risk.

2.4 Childhood growth

2.4.1 Factors influencing childhood growth

Growth during childhood is regulated by both genetic and environmental influences [134]. Growth hormone and insulin-like growth factor 1 are hormones required for linear childhood growth [135]. Adequate nutrition during childhood is also a strong predictor of normal growth. Stunting is the most prevalent form of childhood undernutrition worldwide [136] and is seen primarily in developing countries due to food insecurity, infections, and lack of access to medical care [137]. In industrialized countries, children are at lower risk of communicable diseases and are more susceptible to nutrition-related chronic diseases such as obesity and diabetes [138].

The degree and timing of weight gain in infancy is an important variable in predicting childhood weight status [139]. Furthermore, nutritional factors such as dietary composition, in particular greater animal protein intake is associated with increased levels of serum insulin-like growth factor 1 which can accelerate early growth [103, 140]. There is also probable evidence that intake of animal protein during childhood is associated with earlier entrance into puberty [140].

2.4.2 Childhood growth and CVD

The effects of excess weight gain in early childhood are cumulative, and greater childhood BMI is associated with adult CHD risk [141]. Findings from a large registry based Danish cohort reported an increased risk of a fatal CHD event in adulthood with greater childhood BMI at each age between 7 to 13 years [142]. Analyses of the same registry based Danish cohort and a Finnish cohort found that both birth weight and BMI at seven years were independently associated with CHD in adulthood [143]. Furthermore, analysis of height in Danish children found an inverse association between height z-score at 7 years of age and CHD risk, which was not modified by birth weight [144]. The older registry based cohorts provide valuable information on growth and later disease risk, but have limited information on cardiovascular biomarkers or body size at adult age.

There is a need to determine whether there are changes in CVD risk over a certain period of childhood growth as opposed to BMI at one time point, while accounting for CVD risk factors and body size at adult age. Examining the rate of BMI gain over multiple points during childhood may help identify growth patterns that effect later disease risk. Childhood growth in relation to adult CVD risk has not been investigated in the Icelandic population and there is a need to address this gap in the literature.

2.4.3 Growth as a marker for puberty

There is a great deal of variability in the timing of growth and onset of puberty. When exact stages of pubertal growth are not available, many studies rely on recalled data (i.e. adolescent height, age at menarche) often collected decades after the pubertal events. In the

early 20th century, the recording of height and weight measures of children was an important public health initiative to monitor the nutritional status and health of the population where both severe infections and food shortage due to economic factors were more common. In the present day environment where children are exposed to a nutrient abundant setting, monitoring of childhood growth more often captures overnutrition. From both a historical and current perspective, growth measures can provide data on timing of pubertal events and is a suitable method for tracking variations in growth. Height velocity increases during puberty and can be used as a marker for early development [145, 146].

2.4.4 Factors contributing to early puberty

Timing and duration of puberty has a critical impact on growth. Pubertal growth is triggered by sex hormones, primarily by secretion of gonadotropin releasing hormone [147]. There is a strong genetic component to the timing of puberty, however, environmental exposures such as endocrine disruptors may also modify pubertal onset [148, 149]. Peripheral signals involved in puberty such as leptin, insulin and insulin-like growth factor 1, which are hormones related to body fat, have also been suggested to influence onset of puberty [150, 151].

Physical characteristics such as greater childhood BMI may lead to earlier entrance into puberty. In a recent longitudinal study, greater prepubertal body composition in German children had a weak association with initiation of pubertal growth spurt, but was suggested to influence the pace at which puberty advances, leading to earlier attainment of pubertal stages [152]. However, secular trends in timing of puberty have been observed independent of prepubertal BMI levels. In Denmark, a trend in earlier sexual maturation was reported from 1930 to 1969 [146]. Both heavier boys and girls in this Danish cohort entered puberty earlier, but the decreasing age at puberty was observed irrespective of childhood BMI [145].

Sex also plays a role and girls enter puberty, on average, two years earlier than boys [153]. In successive cohorts of Icelandic women born 1900 to 1950, mean age at menarche declined from 14.9 to 13.5 years with no apparent decline thereafter [154]. The standard of living drastically changed in Iceland in the early 20th century and the falling age at menarche is likely related to nutritional and economic improvements during this period.

2.4.5 Early puberty and cardiovascular risk factors

A recent systematic review reported that earlier pubertal timing was a predictor of higher adult BMI and greater risk of obesity [155]. In the Northern Finland Birth Cohort, earlier pubertal development, estimated by height growth, was shown to be independently associated with adult metabolic syndrome-related derangements in both men and women [156].

Among girls, there is indication that early menarche may be associated with risk factors for CVD such as elevated plasma lipids and greater adult BMI [157, 158]. More recently, inverse relationships with menarcheal age and adult CVD mortality have also been reported [159, 160]. However, previous studies investigating puberty in girls and later disease risk have largely relied on retrospective self-reported age at menarche [156-161]. Although recalled age at menarche has been somewhat validated, the reliability varies depending on the subject's age at recall and education level [162]. The use of height

measures, when available, can be useful for estimating pubertal timing in girls as peak height velocity (PHV) is a pubertal event achieved prior to menarche [163, 164]. While prepubertal BMI has been investigated with respect to CHD risk [142], the association between timing of height acceleration itself and CVD risk is not well established [144] and needs to be better elucidated. Growing evidence indicates that early puberty may cause long-term physical and metabolic changes that cannot be reversed and is a period of growth that needs to be further investigated with respect to adult disease risk.

3 Aims

The principal aim of this dissertation was to increase knowledge of the connections between fetal, infant, and childhood growth on later risk of CVD including obesity.

The specific aims were to evaluate the following:

1. The influence of environmental factors on birth weight and later obesity and CVD risk factors.
2. The contribution of childhood growth, both height and weight, on adult CVD risk factors and mortality.
3. The connection between peak height velocity and adult CVD risk factors and mortality.
4. The relationship between infant feeding practices and infant and childhood growth up to 6 years of age.

4 Methods

4.1 The Reykjavik Study

The Reykjavik Study is a prospective population-based cohort study initiated by the Icelandic Heart Association in 1967 to assess and manage cardiovascular diseases in Iceland [165]. Men and women born 1907-1935 (N=30,795) living in the Reykjavik area were considered eligible for recruitment, which was approximately 35% of this age-specific population in Iceland at the time. Thereof, 27,281 individuals were randomly selected through the National Register and invited by letter or telephone to the Heart Preventive Clinic in Reykjavik, of which 19,381 individuals attended equaling a 71% response rate [2, 166].

4.2 Study population

4.2.1 Birth weight and childhood growth cohort

The birth weight and childhood growth cohort (Papers I-III) was a sub-cohort from the randomly selected participants of the Reykjavik Study [2]. Eligible subjects for the birth cohort were born in the greater Reykjavik area from 1914-1935, singleton birth, aged 33-65 years, living in Reykjavik in 1967, and had complete information on size at birth from historical midwives' birth records (N=4601). Birth records were available for all birth years. However, annual recording of childhood height and weight measures began in two main schools in Reykjavik in 1929 for children 8 years of age and older (birth year ≥ 1921). Of the 4601 total individuals included in the birth sub-cohort, 782 were born prior to 1921. Furthermore, clinical growth records were unavailable for 1699 individuals, as they did not attend the schools where growth measures were recorded or the records were poorly archived. Thus, the childhood growth cohort consisted of 2120 individuals (1085 boys and 1035 girls) with birth measures who had attended school in Reykjavik where height and weight measurements from 8 to 13 years were recorded.

For paper I, to evaluate the impact of the Great Depression which began affecting Iceland in 1930, five year periods were combined (1925–1929 and 1930–1934) prior to and after the onset of the Depression as a proxy for environmental exposure. These two birth year groups, totaling 2750 participants or 60% of the source population with birth measures, covers a span of ten years allowing the analysis of the immediate consequences of the Great Depression.

For papers II and III, individuals with childhood growth measures from ages 8 to 13 years were considered. Of the 2120 individuals included in the childhood growth cohort, for 196 individuals, it was not possible to determine BMI-velocity between ages 8 and 13 years due to missing height or weight values at 8 or 13 years. Therefore, for paper II, a total of 1924 individuals were used in the analysis.

In paper III, peak height velocity (PHV), an early marker of maturity, was used to determine whether timing of height acceleration was associated with CVD mortality. Girls enter puberty, on average, two years earlier than boys [153]. For this reason, the 1085 boys were excluded from this analysis as growth measures beyond 13 years of age would have been needed to estimate timing of PHV. The remaining sample size of 1035 women was

further narrowed by 62 as PHV could not be accurately identified due to missing values on growth at critical time points, leaving 973 women available for analysis.

In table 1, we compare the birth and adult cardiovascular risk variables of the 1924 individuals with birth and childhood growth measures to the 2677 individuals who were not included in this current study. While there were differences between crude measures for several variables, these associations were attenuated after adjustment for birth year. All analyses for papers II and III are adjusted for birth year.

Table 1. Differences between subjects with and without growth measures in the Reykjavik Study.

	With only birth measures (n=2677)	With birth and growth measures (n=1924)	Crude <i>p</i> value	Birth year adjusted <i>p</i> value
Variable	Mean (SD)	Mean (SD)		
Birth year	1925 (6)	1928 (4)	<0.001	-
Age (years)	52.9 (5.9)	50.6 (5.7)	<0.001	-
Males (%)	50.4	50.8		
Birth weight (kg)	3.75 (0.59)	3.74 (0.55)	0.89	0.82
BMI (kg/m ²)	25.7 (3.8)	25.7 (3.9)	0.96	0.93
Cholesterol (mmol/L)	6.4 (1.1)	6.3 (1.1)	0.03	0.85
Triglycerides (mmol/L)	1.2 (0.7)	1.3 (0.8)	0.03	0.09
Uric acid (μmol/L)	5.0 (1.2)	5.0 (1.2)	0.99	0.10
Systolic BP (mm Hg)	136 (22)	134 (20)	0.001	0.54
Diastolic BP (mm Hg)	86 (12)	86 (11)	0.03	0.53
Fasting glucose (mmol/L)	4.5 (0.8)	4.5 (0.8)	0.54	0.19

4.3 Data collection

4.3.1 Collection of birth and childhood growth data (Papers I-III)

Birth weight was recorded to the nearest ± 50 g and birth length from crown to heel (in centimeters) was obtained from midwives' birth records stored in the National Archives of Iceland. Ponderal index was calculated as birth weight (g)/birth length (cm)³ x 100. Ponderal index below 2.60 g/cm³ was used as a cut-off to describe thinness at birth [47, 167]. Childhood growth measures from ages 8 to 13 were recorded in school health records at regular yearly intervals beginning in 1929 at two main schools in Reykjavik. At each yearly exam, the child's height and weight were recorded along with the year and month of measurement. The mean height of subjects who had information available on childhood growth was compared to reference data for all public schools in Reykjavik. At age 10 years, the average height difference from the reference values was 0.4 cm (men) and 0.1 cm (women), suggesting the growth data in this cohort is a fair representation of school children in Iceland [168].

4.3.1.1 Timing of peak height velocity (Paper III)

From the growth measures which were collected yearly from ages 8 to 13 in two main Reykjavik schools, height velocity was defined as differences in heights between two consecutive annual measurements divided by the time between the two measurements ($\Delta\text{cm}/\Delta\text{time}$). The height velocity curves from 8 to 13 years were visually assessed for location of PHV for each participant by two independent reviewers. The maximum height acceleration after 8 years of age was identified as PHV [146] if it was followed by a clear increase in height velocity from the previous year (if after age 8) followed by at least a 1 cm decrease the next year with a consistent decrease in height velocity at the subsequent years. If the greatest height acceleration occurred at the measurement taken between 12 to 13 years or there was no marked increase in height velocity, the women were categorized as having late PHV (n=476). PHV prior to age 12 was identifiable in 497 women (51%), of which one-half (n=248) had clearly reached PHV prior to 11 years of age (early) and the second half (n=249) between ages 11 to 12 (middle), leading to the categories used in our analyses. Figures 1A-3B show the growth patterns of select individuals in each PHV category.

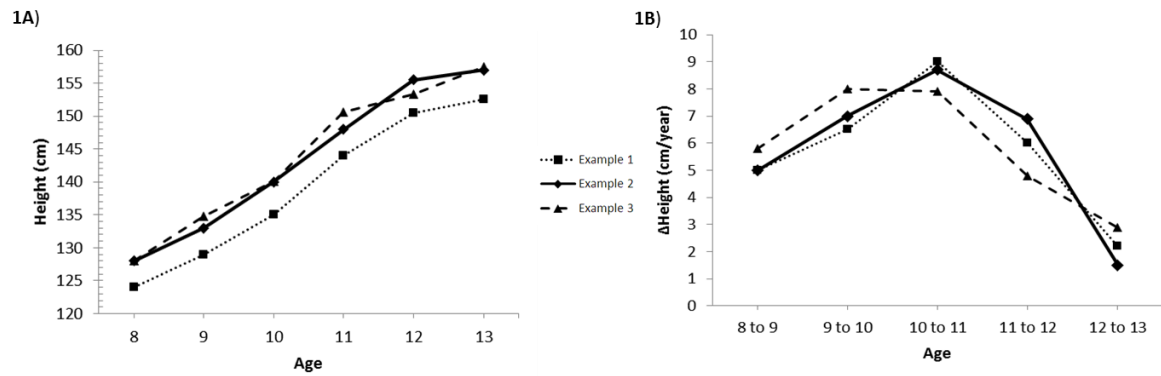


Figure 1A and 1B. Yearly height (A) and height velocity (B), cm/year, from ages 8 to 13 of three participants with peak height velocity *prior to 11 years*.

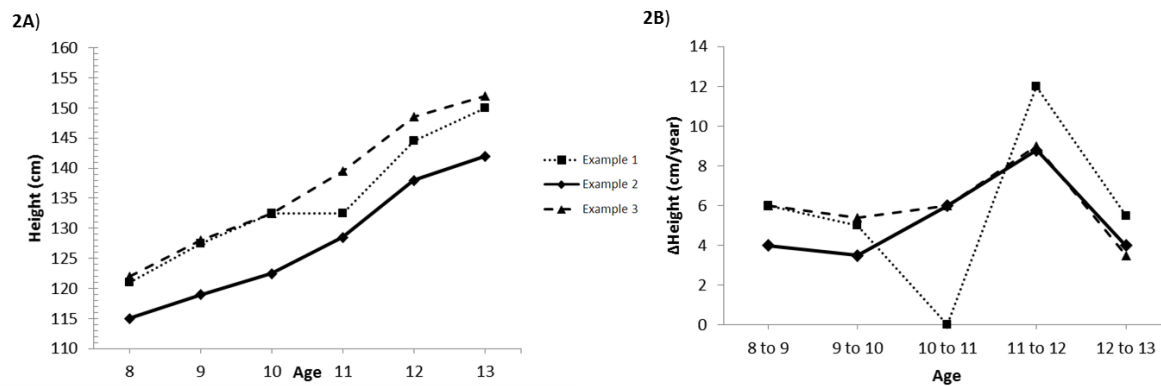


Figure 2A and 2B. Yearly height (A) and height velocity (B), cm/year, from ages 8 to 13 of three participants with peak height velocity *between 11-12 years*.

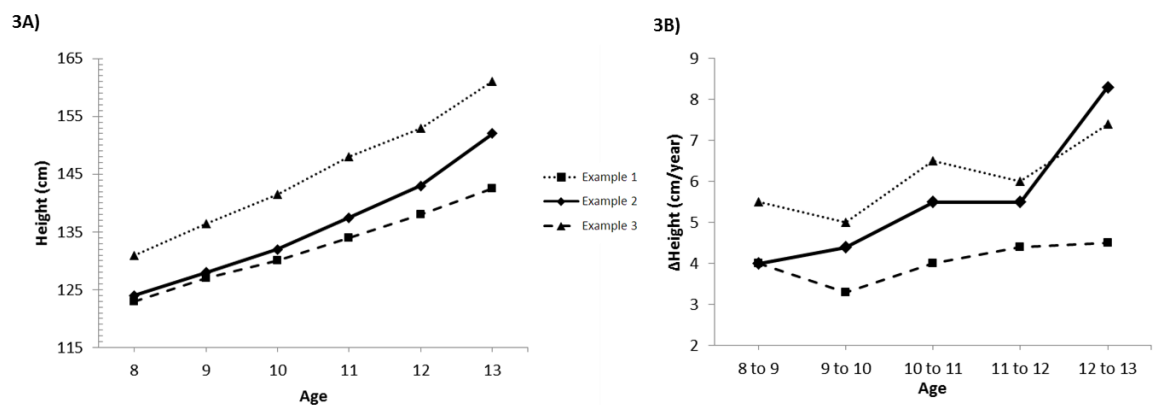


Figure 3A and 3B. Yearly height (A) and height velocity (B), cm/year, from ages 8 to 13 of three participants with peak height velocity *after 12 years of age*.

4.3.2 Collection of adult data

Participants who agreed to take part in the Reykjavik Study were asked to arrive at the Icelandic Heart Association Heart Preventive Clinic in a fasting state to complete a medical examination, blood sample collection, and lifestyle questionnaire. Each participant's height was recorded to the nearest 0.5 cm and weight to the nearest 100 g, without shoes and in light undergarments. Subjects were instructed to consume no food or drink after 10 pm the night prior to the exam. Blood samples were drawn after the overnight fast and total cholesterol, triglycerides, glucose, and uric acid levels were analyzed. Quality control of the lipid and glucose measurements was employed throughout the recruitment period [27]. Blood pressure was measured after a five minute rest to the nearest 2 mm Hg with a mercury sphygmomanometer Erkameter wall-model (Erka, Germany) using a cuff size of 12x23 cm. The same cuff was used throughout the study. Skinfold measures were collected at two areas, triceps and subscapular, with calibrated calipers to the nearest 1.0 mm [50]. Information on medical history, family history, use of medication, and smoking habits was assessed by a standardized health questionnaire completed by the participant and verified by study personnel.

4.3.3 Outcome ascertainment

Paper I - Obesity was defined as BMI ≥ 30 kg/m². Impaired fasting glucose was classified as fasting glucose ≥ 6.1 mmol/L and/or diagnosis or confirmation of type 2 diabetes. Dyslipidemia was defined as triglycerides >1.7 mmol/L and total cholesterol >6.2 mmol/L.

Papers II and III - The data and cause of all deaths in the cohort were obtained through linkages with the Icelandic Statistical Bureau. Death from any cause and fatal cardiovascular deaths, differentiated by CHD and non-CHD deaths, that occurred from the time of study recruitment to 31 December 2009 were considered. Death from CHD (as presented in the Papers section of this dissertation, Paper II, Table 3 and Paper III, Table 3) was ascertained if death certificates included the following International Classification of Disease: code 420 (1967-1970, 7th revision), codes 410-413 (1971-1980, 8th revision), codes 410-414 (1981-2009, 9th revision), and codes I20-I25 (2010, 10th revision). Death certificates were reviewed and coded by an official government pathologist [52].

4.4 Early nutrition and infant and childhood growth (Paper IV)

In 2005, a prospective infant cohort study was initiated to collect important data on the growth and nutrition habits of Icelandic infants. A random sample of 250 Icelandic infants born in 2005 was collected by Statistics Iceland. The inclusion criteria for the infant study were Icelandic parents, singleton birth with a gestational length of 37-41 weeks, birth weight within the 10th-90th percentiles, no birth defects or congenital long-term disease, and the mother had early and regular antenatal care. Children who were still participating at 12 months of age, n=219, were invited to the follow-up study at 6 years of age. Eligible subjects for this study had anthropometric measures at birth, completed a dietary record at 5 months of age, and had weight and height measurements at 6 years of age (n=154). Our analyses are mainly based on the infant feeding practice at the age of 5 months, where we

compare the growth in infancy between those children who were exclusively breastfed at this time point to those who were either formula fed or had been introduced to solid food at the age of 5 months. The 5 month dietary registration was used for our primary analysis because it was the earliest detailed food registration available in the present study.

Infant and childhood growth data collection

Birth information on weight and length was gathered from the maternity wards. Infant anthropometric measurements were gathered from healthcare centers monthly from 1-6 months, then singularly at 9, 12, and 18 months. As close to the child's sixth birthday as possible (mean 73.4 ± 3.2 months), weight (Marel M series 1100, Iceland; ± 0.1 kg) and height (Ulmer stadiometer, Prof. Heinze, Busse design Ulm; ± 0.5 cm) were measured in a clinical examination at the Landspítali-National University Hospital. BMI was calculated as weight (kg)/ height (m^2). When the infant was 12 months of age, the parent or caregiver was asked to complete a questionnaire regarding information on age, education, and physical characteristics including self-reported height and weight of both parents.

Dietary assessment

Information on breastfeeding was gathered monthly during the first 12 months. The parents or caregivers completed a 24-hour food record monthly from 5-8 months and 10-11 months using common household measures, such as cups and spoons. At 9 and 12 months of age, weighed food records were kept for three consecutive days on accurate scales (PHILIPS HR 2385, Austria; PHILIPS HR 2385, Hungary; ± 1 g accuracy). Average daily consumption of energy and the contribution of energy providing nutrients at the age of 9 and 12 months were estimated using ICEFOOD, a software program used by The Icelandic Nutrition Council. Special infant products, such as cereals and purées, were added to the database and nutrient losses due to food preparation were taken into account in the calculations.

4.5 Statistical analyses

Statistical analyses for all manuscripts were carried out using SPSS version 20.0 (IBM Corp., NY, USA). The level of significance was set at $P < 0.05$, two-sided.

4.5.1 Paper I – Environmental effects on birth and later health

Linear regression analysis was performed for each outcome variable separately by sex with birth year exposure to the Depression being a predictor. Adjustments were made for maternal age and parity. Participant age at study recruitment was included when analyzing outcome variables collected in adulthood.

Logistic regression was used to estimate odds ratio (OR) of obesity, impaired fasting glucose, and dyslipidemia with adjustments for the same covariates used in linear regression. Analyses were performed for both sexes combined with adjustment for sex and separately for women and men.

4.5.2 Paper II – Childhood BMI-velocity and CVD mortality

Cox-proportional hazards regression was used to evaluate the association between childhood BMI-velocity, BMI z-scores and CVD mortality. BMI-velocity was quantified as the mean change in BMI per year for the period between 8 and 13 years. BMI z-scores were calculated separately by sex based on the internal distribution in our cohort. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated. The underlying timescale was time from study recruitment to fatal CVD event or until 31 December 2009, whichever came first. Childhood BMI-velocity ($\Delta\text{kg/m}^2$ per year) was classified into tertiles and entered as a categorical variable to determine the relationship with fatal CVD events in adulthood. All analyses were adjusted for birth year (dichotomous 3-year intervals from 1921 to 1935), maternal parity, birth weight, BMI at age 8, and age at recruitment. We further adjusted for known CVD risk factors at mid-life (mean age: 50.6 years, SD 5.8), including total cholesterol, systolic blood pressure, previous and current smoking (dichotomous) and history of familial hypertension (dichotomous). To account for the proportion of obese participants, adjustments for mid-life BMI were categorized as follows: BMI <25, 25-29.9 or ≥ 30 .

4.5.3 Paper III – Peak height velocity and CVD mortality

Cox-regression analyses were used to estimate HR and 95% CI for the association between age at PHV and CVD mortality. The underlying time scale was time from study recruitment to fatal CVD event or until 31 December 2009, whichever came first. PHV was categorized as early (prior to age 11), middle (between ages 11 to 12), and late (after age 12). PHV after 12 years of age was used as the reference category.

Age-adjusted analyses were performed along with further variable adjustments for birth year (dichotomous 3-year intervals from 1921 to 1935), maternal parity, previous and current smoking (dichotomous), age at clinical examination, total cholesterol, systolic blood pressure, familial hypertension and additionally for birth weight, BMI at age 8 and mid-life adult BMI.

4.5.4 Paper IV – Early nutrition and infant and childhood growth

Mean and standard deviation (SD) or proportion was used to describe infant and maternal characteristics. Linear regression analysis was used to determine the association between infant feeding practice at 5 months of age and BMI at 6 years with adjustments for sex, birth weight, and maternal education level categorized as completion of elementary school, high school or vocational school, or university. Changes in growth were calculated from crude differences in measurements at the two different time points.

4.5.5 Data validity and approvals

This dissertation includes studies based on the Reykjavik Study (papers I-III) and the early nutrition cohort (paper IV) which are both population-based cohort studies. Possible biases associated with cohort studies are discussed hereafter.

Selection bias

Selection bias occurs if the relationship between the exposure and disease is different for individuals who participate compared to those who are eligible but do not participate. Both the Reykjavik Study and early nutrition cohort were prospective studies, thus, the individual's consent to participate would not necessarily be motivated by the outcome being measured, which was not known at the time of recruitment for either study.

Information bias

Information bias is a possibility, more so in the early nutrition cohort. Information bias occurs when the data is misclassified with respect to the exposure, covariates or outcome variables. Adult variables from the Reykjavik Study were collected from clinical examinations and report of body size was not self-reported, thus decreasing the possibility that the prevalence of obesity in the subjects would be misclassified. Furthermore, cause of death was determined through death certificates which were coded by a government pathologist and there is some but limited possibility that this endpoint would be categorized incorrectly. In the early nutrition cohort, the use of 24-hour food records may not accurately reflect the infant's usual intake and if the infant's diet was incorrectly recorded, it can lead to over- or under-reporting of dietary intake. To decrease this possibility, the parents or caregivers were shown by trained researchers how to properly record dietary intake and although still possible, the effect of this bias should be minimal.

Confounding

Confounding, as in all observational studies, is a major concern. With respect to the papers in this dissertation, confounding is a particular concern for paper I, where we perform an ecological comparison using birth year as an exposure. Lack of more detailed information about the individual subject's exposure to the Depression does leave considerable room for confounding, as other factors which also changed from 1925 to 1934 may have influenced our results. Confounding is also possible in papers II and III with the analysis of childhood growth, but detailed information at follow-up is a strength of the analysis. In paper IV, more detailed information on maternal characteristics, such as barriers to breastfeeding and possible reasons behind initiating formula or solid food introduction, would have been beneficial. A strength of the two cohorts is the relative homogeneity of the study population. For the early nutrition cohort, the infants of Icelandic parents were recruited into the study. In the Reykjavik Study, the subjects were born in Reykjavik from 1907-1935. There was limited immigration into Iceland during this period and it is very unlikely that race would be a confounding factor.

Approvals

Informed consent was obtained from all participants in the Reykjavik Study and the study received approval from the Icelandic National Bioethics Committee and the Data Protection Commission.

The early nutrition cohort with follow-up at 6 years was approved by the Icelandic Data Protection Authority (S5099/2011), Local Ethical Committee at Landspítali- National University Hospital (1104Ref.16 2011) and the Bioethics committee (VSNb2011010008/037). Informed written consent was obtained from all parents.

5 Results

The main findings from the four papers are hereby discussed. Detailed descriptions of results are found in papers I-IV.

5.1 Environmental effects on birth and later health (Paper I)

In this analysis, the impact of the Great Depression on size at birth and adult obesity, impaired fasting glucose, and dyslipidemia was investigated in 2750 Icelanders.

When comparing infants born prior to the Depression (1925-1929) to those born during the Great Depression (1930-1934), a decrease in birth weight of 97 g for boys and 70 g for girls was observed (Table 2). There was also a concurrent decrease in ponderal index from 2.66 for males and 2.65 for females to 2.50 g/cm³ for both sexes ($p < 0.01$ for both) (Figure 4). Furthermore, among Depression-born infants there was a marked increase in proportion with ponderal index below 2.60 g/cm³, a cut-off used for describing thinness in infants [47, 167].

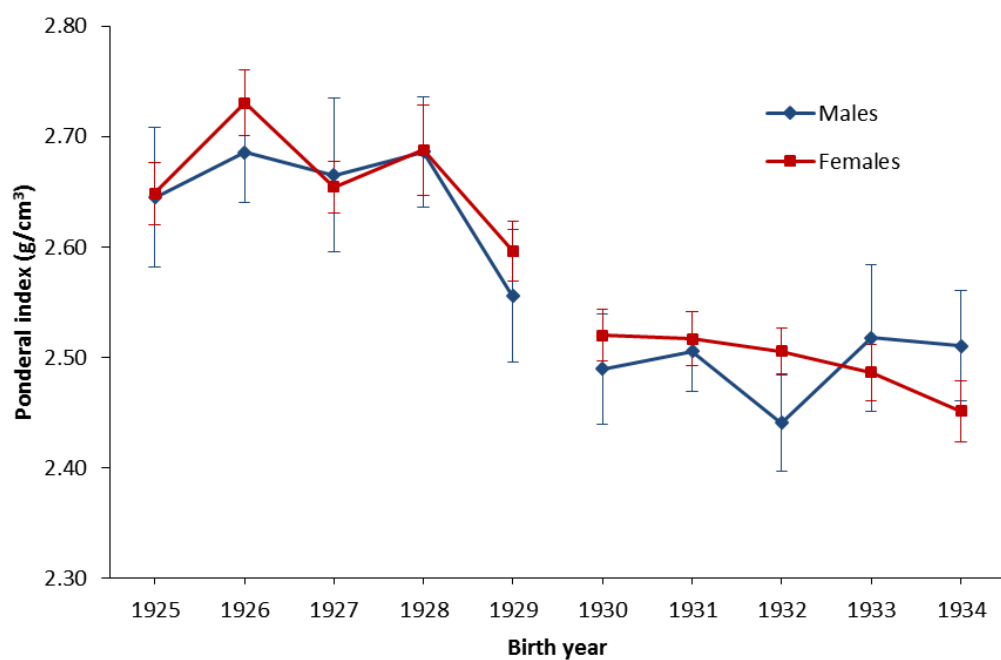


Figure 4. Yearly ponderal index for males (diamond) and females (square) born between 1925 and 1934.

Mean BMI from ages 8 to 13 years stratified by birth year exposure to the Depression are shown in Figure 5A for boys and 5B for girls. When plotted against the WHO BMI-for-age standards, mean BMI from ages 8 to 13 years of children born before the Depression (but growing during the Depression) followed approximately along the 25th percentile while the BMI of Depression-born children, who were primarily growing up after the Depression, was closer to the 50th percentile. Growth differences based on birth year exposure were more pronounced among girls than boys.

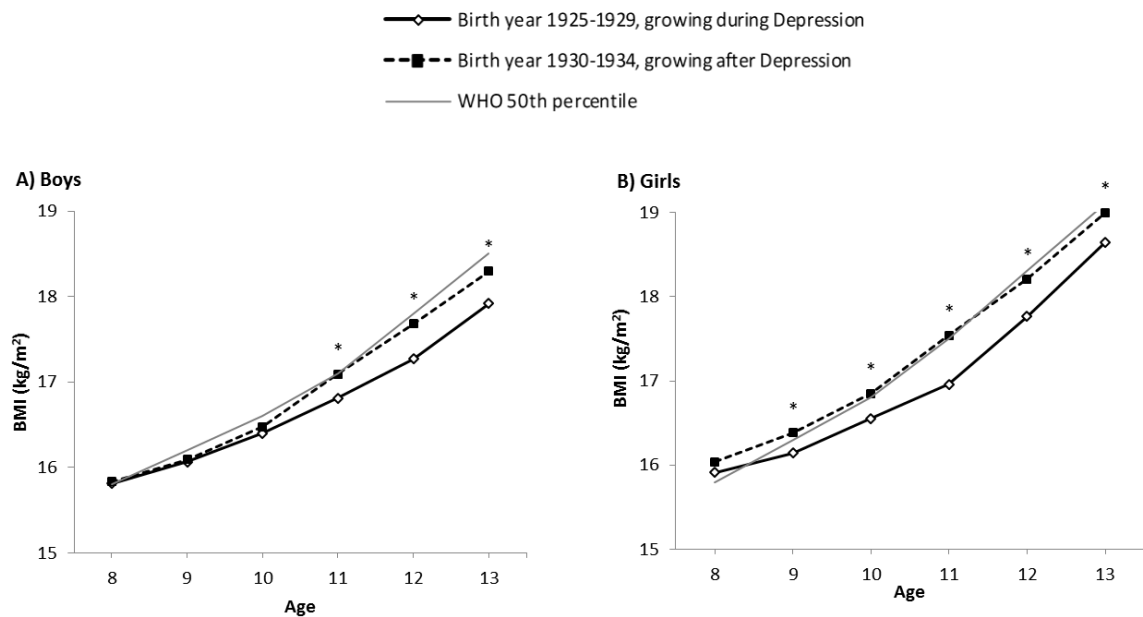


Figure 5A and 5B. Mean BMI from 8 to 13 years comparing those born pre-Depression (1925-1929) and growing during the Depression to those born during the Depression (1930-1934) and growing after the Depression for (A) boys and (B) girls.

*Significantly different from those born pre-Depression.

Table 2. Subject characteristics at birth and in adulthood comparing those born Pre-Depression and during the Depression.

	Men			Women		
	Pre-Depression	Depression	Δ (95% CI)	Pre-Depression	Depression	Δ (95% CI)
<i>At birth*</i>	N=685	N=738		N=687	N=640	
Birth weight (g)	3880 (593)	3751 (531)	-97 (-156, -39)	3732 (555)	3638 (544)	-70 (-129, -11)
Ponderal index ^a (g/cm ³)	2.66 (0.34)	2.50 (0.30)	-0.15 (-0.18, -0.12)	2.65 (0.34)	2.50 (0.27)	-0.15 (-0.18, -0.11)
Ponderal index <2.6 g/cm ³ (%)	45.7	66.9		42.4	64.3	
<i>In adulthood†</i>						
BMI (kg/m ²)	26.1 (3.5)	26.1 (3.6)	0.2 (-0.2, 0.7)	25.0 (3.9)	25.5 (4.1)	0.6 (0.2, 1.1)
Triceps skinfold (mm)	10.7 (7.2)	11.8 (8.0)	-0.2 (-1.1, 0.7)	20.6 (10.1)	22.2 (10.1)	1.9 (0.8, 3.0)
Subscapular skinfold (mm)	16.8 (7.6)	17.1 (8.0)	1.3 (0.4, 2.2)	18.8 (10.0)	21.5 (10.8)	3.3 (2.2, 4.4)
<i>Biomarkers</i>						
Total cholesterol (mmol/L)	6.3 (1.0)	6.2 (1.0)	-0.16 (-0.3, -0.04)	6.4 (1.1)	6.2 (1.1)	-0.15 (-0.3, -0.04)
Triglycerides (mmol/L)	1.4 (0.9)	1.5 (0.8)	0.1 (0.01, 0.2)	1.1 (0.5)	1.1 (0.5)	0.06 (0.01, 0.1)
Fasting glucose (mmol/L)	4.6 (0.9)	4.5 (0.7)	-0.06 (-0.2, 0.03)	4.3 (0.6)	4.5 (0.9)	0.16 (0.07, 0.23)

* Birth characteristics adjusted for maternal age and parity.
^a Ponderal index = birth weight (g)/birth length (cm)³ x 100
[†] Adjusted for maternal age, maternal parity, and participant age at recruitment.

In adulthood, women born during the Depression had higher mean BMI ($\Delta 0.6$ kg/m², 95% CI 0.2, 1.1) and fasting blood glucose ($\Delta 0.16$ mmol/L, 95% CI 0.07, 0.23) (Table 2). Their body composition measures were also more unfavourable with greater triceps and subscapular skinfolds. Men born during the Depression had larger subscapular skinfold measures, yet differences in BMI compared to men born before the Depression were not significant. Total cholesterol levels were slightly decreased among Depression-born men and women, though this may be attributed to the introduction of low-fat milk in 1982 (prior to 1982 only full fat milk was available in Iceland) as well as low-fat butter and margarine [169].

Despite the availability of less saturated fat foods, those born during the Depression had slightly elevated triglyceride levels and were still more likely to be obese at mid-life, adjusted odds ratio, 1.40 (1.09, 1.77) (Table 3). Separate analysis by sex showed that likelihood of obesity was only significant among women, OR 1.43 (95% CI 1.01, 2.02). Non-significant but slightly elevated risk was observed for females with respect to impaired fasting glucose and dyslipidemia based on birth year exposure to the Depression.

Table 3. Adjusted odds ratios for obesity, impaired fasting glucose, and dyslipidemia at study recruitment in Adulthood comparing men and women born during the Great Depression to Pre-Depression. Reference group: participants born Pre-Depression (1925-1929).

Variable	All ^a		Males ^b		Females ^b	
	OR	95% CI	OR	95% CI	OR	95% CI
Obesity (BMI ≥ 30 kg/m ²)	1.40	1.09, 1.77	1.27	0.89, 1.81	1.43	1.01, 2.02
Impaired fasting glucose ^c	0.98	0.62, 1.57	0.75	0.40, 1.41	1.41	0.69, 2.90
Dyslipidemia ^d	0.95	0.73, 1.24	0.72	0.51, 1.03	1.22	0.80, 1.86

^a. Adjusted for maternal age, maternal parity, participant age at examination and sex

^b. Adjusted for maternal age, maternal parity, and participant age at examination

^c. Impaired fasting glucose: fasting glucose ≥ 6.1 mmol/L and/or diagnosis or confirmation of type 2 diabetes.

^d. Dyslipidemia: total cholesterol > 6.2 mmol/L and triglycerides > 1.7 mmol/L.

5.1.1 Appropriateness of analyzing birth year exposure to the Great Depression

The following information details the trends in birth weight and ponderal index by birth year for participants who were excluded from this current analysis.

Birth weight, ponderal index and odds ratio for obesity in adulthood compared in 5-year periods from 1915 to 1934 are summarized in Table 4. Although there are fluctuations in birth weight over the years, there is a clearer pattern when evaluating ponderal index. More specifically, there is a clear drop in ponderal index during 1930-1934 compared to Icelanders born prior to the Depression in 1925-1929, as well as between the years 1920-1924 likely due to the effects of the Spanish flu. Lower ponderal index suggests that infants are becoming more disproportionate or thinner.

In addition to the decreased likelihood of obesity observed in individuals born 1925-1929 compared to those born 1930-1934, a similar decrease in odds ratios was observed in

the other periods (1915-1919 and 1920-1924) where participants also had significantly higher ponderal index than those born 1930-1934.

The Depression began in Iceland in 1930 and the prolonged effects on both the economy and basic living standards of Icelanders born and growing up during this period are well documented [72, 73]. Therefore, it was appropriate to focus on the well-defined period just prior to and immediately after the onset of the Great Depression in Iceland to investigate the potential influence of this unique environment on birth size.

Table 4. Birth characteristics of men and women combined (crude mean and SD) and odds ratio for obesity comparing participants born before the Great Depression in 5-year increments to those born during the Depression (1930-1934).

	Birth year group (males and females combined)			
	1915-1919 N=699	1920-1924 N=1083	1925-1929 N=1372	1930-1934 N=1378
Birth characteristics				
Birth weight (g)	3769 (580)	3738 (599)	3806 (579)	3698 (540)
Birth length (cm)	51.5 (2.2)	52.2 (2.5)	52.3 (2.43)	52.9 (2.4)
Ponderal index (g/cm ³)	2.74 (0.35)	2.62 (0.34)	2.66 (0.33)	2.50 (0.29)
Characteristics at recruitment				
Body mass index (kg/m ²)*	-1.0 (-1.4, -0.5)	-0.6 (-0.9, -0.2)	-0.5 (-0.8, -0.2)	Referent [^]
Obesity odds ratio*	0.72 (0.49, 1.05)	0.66 (0.49, 0.88)	0.72 (0.57, 0.92)	Referent

*Adjusted for age at examination and sex

[^]Relative difference in body mass index compared to birth year group 1930-1934 where crude mean body mass index = 25.8 kg/m²

5.2 Childhood BMI-velocity and CVD mortality – men and women (Paper II)

In this study, the influence of childhood growth was examined with respect to CVD mortality. Faster gains in BMI from ages 8 to 13 years were associated with a greater risk of CVD mortality. Adjustments for birth weight and BMI at age 8, to partially account for growth prior to age 8, did not significantly alter the risk estimates. Furthermore, the effects were observed in both sexes, but were stronger in women and appeared to be somewhat independent of adult BMI.

The mean BMI of the subjects during childhood and at adult age are presented in Table 5.

Table 5. BMI during childhood and at mid-life, mean and SD, by sex.

	Men (N=979)		Women (N=945)	
	Mean	SD	Mean	SD
During childhood				
BMI age 8 (kg/m ²)	15.8	(1.2)	15.9	(1.4)
BMI age 13 (kg/m ²)	18.0	(1.9)	18.8	(2.3)
BMI-velocity 8-13 years (Δ kg/m ² per y)	0.4	(0.3)	0.6	(0.3)
In adulthood				
Age (years)	49.9	(5.4)	51.4	(6.0)
BMI (kg/m ²)	26.2	(3.7)	25.1	(4.1)

During a mean follow-up period of 25.9 years (SD 8.4), there were 202 CVD deaths among men and 90 CVD deaths among women. Risk estimates for the highest versus lowest tertile of BMI-velocity from ages 8 to 13 corresponded to HR 1.49 (95% CI 1.03, 2.15) among men and 2.32 (95% CI 1.32, 4.08) among women (Table 6). Based on a linear test for trend, the association with childhood BMI-velocity and CVD mortality among men was attenuated after adjustment for mid-life BMI ($P=0.005$ versus 0.062), but was more stable among females ($P=0.005$ versus 0.007). Men who grew fastest during childhood had greater BMI and skinfold measures, as well as higher blood pressure, uric acid and triglycerides levels at adult age (see Paper II, Table 3). Women with the fastest childhood growth had greater adult BMI and skinfold measures, while only triglycerides levels were significantly higher compared to women who grew slower during childhood.

We performed a secondary analyses examining childhood BMI at a specific age (BMI z-scores) and CVD mortality. We observed a gradual rise in risk of CVD mortality with increasing age from 8 to 13 years. Among boys, the HR for CVD mortality per 1-unit increase in BMI z-score went from 1.00 (95% CI 0.86, 1.16) at 8 years to 1.19 (0.96, 1.49) at 13 years of age and among girls, the HR went from 0.92 (0.72, 1.18) at 8 years to 1.55 (1.13, 2.13) at 13 years of age; estimates were very similar for CHD mortality (data not shown). Our risk estimates were comparable to those previously reported in Danish school

children of the same age born between 1930-1976 [142]. In contrast to the registry based Danish cohort, we further report in our study important covariates including BMI in adulthood, as well as CVD risk factors in midlife and are able to adjust our analyses for these variables.

Table 6. Hazard ratios and 95% confidence intervals for the association between BMI-velocity from 8 to 13 years and fatal CVD events.

Fatal CVD Events			
BMI-velocity ¹	Tertile 1 (n=327)	Tertile 2 (n=326)	Tertile 3 (n=326)
Boys	Hazard ratios (95% CI)		
Mean age at CVD event, years (cases)	70.7 (n=53)	72.3 (n=67)	68.2 (n=82)
Adjusted model 1 [†]	1.0	1.31 (0.91, 1.89)	1.70 (1.19, 2.43)
Adjusted model 2 [‡]	1.0	1.28 (0.89, 1.85)	1.49 (1.03, 2.15)
Girls	Tertile 1 (n=315)	Tertile 2 (n=315)	Tertile 3 (n=315)
Mean age at CVD event, years (cases)	76.5 (n=21)	75.6 (n=31)	72.8 (n=38)
Adjusted model 1 [†]	1.0	1.51 (0.85, 2.67)	2.38 (1.36, 4.16)
Adjusted model 2 [‡]	1.0	1.43 (0.80, 2.54)	2.32 (1.32, 4.08)

¹BMI-velocity 8 to 13 ($\Delta\text{kg/m}^2$ per year), mean (SD):

Boys, T1: 0.20 (0.10), T2: 0.40 (0.05), T3: 0.73 (0.23)

Girls: T1: 0.27 (0.12), T2: 0.54 (0.07), T3: 0.93 (0.26)

[†]Adjusted for birth year, maternal parity, birth weight, BMI at age 8, adult age at examination, current and previous smoking, total cholesterol, systolic blood pressure and familial hypertension

[‡]Adjusted for co-variates in model 1 with further adjustment for mid-life BMI at recruitment

5.3 Peak height velocity and CVD mortality in women (Paper III)

This study was a follow-up to paper II to determine whether the larger risk estimates observed among girls with greater childhood BMI-velocity and CVD mortality was due to timing of pubertal growth.

The median height of the girls by timing of PHV are shown in Table 7. Among the girls in the late PHV group, there was a trend toward increasing height-velocity, median 7.2 cm/yr between the ages of 12 and 13 years, but the velocity was lower in comparison to girls with earlier PHV, as expected. This also indicates a combination of girls who had reached PHV between the ages of 12 and 13 along with those who had not.

Table 7. Median values for height and height velocities from ages 8 to 13 by timing of peak height velocity.

	Timing of peak height velocity					
	Early		Middle		Late	
	< 11 years (N=248)		11 to 12 years (N=249)		> 12 years (N=476)	
Age (y)	Height (cm)	Height velocity (cm/y)	Height (cm)	Height velocity (cm/y)	Height (cm)	Height velocity (cm/y)
8 ^a	129.0		127.1		126.5	
9	134.7	5.5	132.2	5.1	131.0	5.0
10	140.0	6.3	138.0	5.2	135.6	5.0
11	148.0	7.9	143.5	5.5	141.0	5.3
12	154.0	5.5	143.5	8.2	141.0	5.5
13	158.0	4.2	152.0	5.1	147.0	7.2 ^b
			157.0		154.0	

^aBMI at age 8, mean (SD): PHV <11 years 16.1 (1.4), 11-12 years 16.0 (1.3), >12 years 15.7 (1.4)

^bLate PHV category (>12 years) reflects the median height velocity of girls with PHV identified between ages 12 to 13 and those who had not reached PHV.

To determine whether there was a secular trend in timing of PHV, we analyzed the proportion of girls with PHV prior to age 12 by dividing birth year into 3-year intervals. As shown in Table 8, there were some indications of a higher proportion of girls having PHV prior to 12 years of age in 1933-1935 compared to the previous years, but no clear linear trend was observed from 1921-1935.

When comparing girls with PHV after versus prior to age 12, the age-adjusted HR for CVD mortality goes from 1.77 (95% CI 1.14, 2.75) to 1.88 (95% CI 1.21, 2.93) after further adjusting only for birth year. We adjusted for birth year in our analyses due to this secular trend and the association does not appear to be confounded by birth year.

Table 8. Proportion (%) of girls with peak height velocity prior to age 12 by birth year.

	Birth year groups				
	1921-1923	1924-1926	1927-1929	1930-1932	1933-1935
PHV prior to age 12	51.9%	37.6%	49.0%	53.9%	62.8%

In our complete analyses of timing of PHV and CVD mortality (Table 9), we observed that girls who reached PHV <11 years (early) or between 11-12 years (middle) had greater CVD mortality risk compared to girls with PHV after age 12 (late) with corresponding HRs of 1.87 (95% CI 1.07, 3.26) and 2.56 (95% CI 1.52, 4.31), respectively. These associations remained stable after adjustment for both childhood BMI at age 8 and mid-life adult BMI. There were no marked differences between CVD risk factors based on timing of PHV (see Paper III, Table 2). However, we note these markers were measured at only one time point at mid-life and it is possible that they contributed to the differences in risk, but were not captured at the time of study recruitment.

Table 9. Hazard ratios and 95% confidence intervals for CVD mortality by category of peak height velocity.

Fatal CVD events			Age-adjusted	Adjusted Model 1*	Adjusted Model 2**
PHV category	Cases/N	Mean age (SD) at CVD event	<i>Hazard ratios (95% confidence intervals)</i>		
Late (>12 yr)	32/476	76.3 (8.5)	1.0	1.0	1.0
Middle (11-12 yr)	31/249	75.0 (9.2)	1.89 (1.15–3.09)	2.58 (1.53–4.33)	2.56 (1.52–4.31)
Early (<11 yr)	23/248	72.0 (10.0)	1.64 (0.96–2.81)	1.87 (1.07–3.26)	1.87 (1.07–3.26)

*Adjusted for birth year, maternal parity, smoking (current and previous), age at clinical examination, total cholesterol, systolic blood pressure, familial hypertension, birth weight and BMI at age 8

**Adjusted for the same co-variables as in Model 1, and additionally for adult BMI

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; PHV, peak height velocity

5.4 Early nutrition and infant and childhood growth (Paper IV)

In this study, early infant feeding practices in relation to infant growth and childhood BMI at 6 years were examined. We found that infants who were formula fed and who had been provided solid foods at 5 months grew faster after birth, particularly between 2 and 6 months of age, compared to exclusively breastfed infants.

Table 10 shows the participants' characteristics comparing infants at 5 months of age who were exclusively breastfed (n=62), exclusively formula fed (n=12), or who were provided solid foods (n=80). The total duration of breastfeeding was shortest among infants who were formula fed at the age of 5 months but had not been introduced to solid foods.

There were no differences in birth weight among the infants based on infant feeding practice at 5 months. However, infants who had been provided solid foods at 5 months grew faster after birth compared to exclusively breastfed infants (Table 11). Furthermore, the addition of solid foods at 5 months predicted greater BMI at 6 years, with BMI being on average 0.7 kg/m² (95% CI 0.05, 1.3) higher among infants provided solid foods compared to those exclusively breastfed at 5 months of age. The association with formula feeding at 5 months of age and BMI at 6 years was non-significant, although the small number of infants getting formula limits the ability to detect a difference.

Table 10. Participant characteristics and dietary variables by infant feeding practice at 5 months of age.

	Infant feeding practice at 5 months		
	Exclusively breastfed (n=62)	Exclusively formula fed (n=12)	Solid foods (n=80)
	Mean±SD	Mean±SD	Mean±SD
<i>Maternal characteristics</i>			
Age (years)	31.5±4.5	30.4±4.0	30.7±4.8
BMI (kg/m ²)	23.7±3.2	29.2±9.1	25.3±4.8*
University education [n (%)]	31 (50)	8 (67)	34 (43)
<i>Infant characteristics</i>			
Exclusive breastfeeding (months)	5.0±0.9	1.7±1.7*	2.6±1.7*
Breastfeeding duration (months)	9.5±1.9	3.9±2.7*	6.9±3.2*

*Significantly different from exclusively breastfed infants

Table 11. Changes in weight from birth to 18 months by infant feeding practice comparing infants on formula or solid foods, Δ (95% CI), to exclusively breastfed infants.

	Exclusively breastfed (n=62) Mean \pm SD	Exclusively formula fed (n=12) Δ (95% CI) ^a	Solid foods (n=80) Δ (95% CI) ^a
Changes in weight (g)			
Birth to 2 months	1859 \pm 500	-188 (-531, 155)	79 (-91, 250)
2 to 6 months	2208 \pm 480	512 (147, 877)*	336 (101, 571)*
6 to 12 months	1775 \pm 550	172 (-193, 538)	170 (-30, 368)
12 to 18 months	1649 \pm 563	-56 (-450, 338)	-152 (-352, 47)

^aMean difference in weight compared to exclusively breastfed infants.

*Significantly different from exclusively breastfed infants.

6 Discussion

6.1 Environmental effects on birth and later health (Paper I)

The results from this study show that changes from a relatively stable economic environment to one of increased adversity during the Great Depression led to infants who were born lighter and more likely to be obese at adult age. In a separate analysis by sex, only the Depression-born women in our cohort exhibited slightly elevated fasting glucose and greater odds of adult obesity. There were non-significant associations with other CVD risk factors such as dyslipidemia and impaired fasting glucose. Data from the Dutch Famine showed that compared to men, famine exposed women had an elevated BMI and waist circumference at 50 years of age and suggested that women tend to store fat intra-abdominally [170, 171] which can affect glucose tolerance. The conditions during the Depression in Iceland were likely very difficult, but not as severe as a famine. However, these findings support our conclusion that women appear to be more sensitive to environmental stressors in utero. This is also in line with previous findings from this cohort which showed stronger inverse relationships between birth weight and truncal fat [50] and glucose intolerance [51] among women at adult age.

During the 1920s and 1930s, Iceland was undergoing both economic and nutritional changes and it is interesting to observe how unexpected environmental changes impacted birth size in this natural setting. Based on the early programming hypothesis, these Depression-born infants exposed to an undernourished intrauterine environment may have experienced physical changes in the womb that increased their susceptibility to later disease. These infants were also born relatively thinner based on their ponderal index measures. Whether this thinness was a reflection of decreased muscle versus fat mass is not known in this cohort, but as muscle is an important site for glucose homeostasis this could be a possible explanation for the slightly higher glucose levels observed among the women.

Previous epidemiological studies have reported that environmental insults during the first trimester of pregnancy may have the most deleterious effects in terms of increased risk of adult disease [13, 65, 69]. In this current analysis, we could not differentiate between mothers based on trimester exposure and rather used birth year as a proxy for environmental exposures as the Depression continued to affect Iceland for most years in the 1930s [72, 73]. The mothers who were pregnant during the Depression would have been exposed to an adverse environment for the majority, if not all, of their gestation. Gestational weight gain in the first and third trimester is positively associated with ponderal index of infants at birth [172]. The markedly lower ponderal index measures among Depression-born infants suggests that restriction in weight in utero may have occurred to protect essential organs and normal brain development. This would imply that increasing gestational weight gain during the third trimester of pregnancy may help to prevent the birth of thin infants [172]. Data on gestational weight gain would have been beneficial, but was not available in this cohort.

We further observed marked differences in childhood growth during this period. It is possible that a combination of an adverse prenatal environment coupled with the increased efforts by parents of Depression-born infants to encourage weight gain may have contributed to the different growth patterns compared to those born before the Depression. Furthermore, the 1940s was a period of improved economic wealth in Iceland, and Depression-born children were growing up in a more plentiful environment compared to

those born prior to but growing during the Depression. Although there is a strong genetic component to postnatal growth, our findings emphasize that both size at birth and childhood growth during this period was influenced by environmental factors.

6.2 Childhood BMI-velocity and CVD mortality (Paper II)

In this study, faster gains in childhood BMI from ages 8 to 13 years was strongly associated with greater CVD mortality. When looking at BMI at each age from 8 to 13 years, we observed similar CVD mortality risk estimates to those reported by Baker et al. from a large cohort of Danish subjects born 1930-1976 [142]. Given the different populations studied and the time period in which they were growing, this association may be causal or alternatively share the same confounders. However, the added strength of our study is the possibility to examine CVD risk factors at adult age in conjunction with childhood growth. The same directionality of our findings with the Danish cohort along with the consideration of adult CVD risk factors, lends support to the potential mechanism being the biological effects of childhood BMI, though it is necessary for these findings to be replicated in more diverse populations.

The interesting aspect of our cohort is that the children were growing during a period prior to the obesity epidemic. Based on the International Obesity Task Force BMI-for-age cut-offs for overweight [173], less than 5% of the children would be classified as overweight or obese at any age between 8 to 13 years. This is in sharp contrast to Icelandic school health registry data from 2009 which estimated 21% of 9-year-old Icelandic children to be overweight, primarily as a result of increasing energy intake and decreasing physical activity [174]. Furthermore, while children with more rapid childhood growth in this cohort had greater adult BMI, we found similar risk estimates for CVD mortality when excluding obese adults from the analysis. Thus, the association between faster childhood growth and CVD mortality persisted even among individuals who had normal weight in childhood and were non-obese at adult age.

It appears that the potential mechanisms involved in this association in our cohort differed by gender. Previous studies have implicated greater prepubertal BMI and body fat percentage during early childhood in progressing pubertal development among girls [175, 176]. Conversely, greater childhood fat mass among boys was not linked to earlier onset of puberty based on Tanner genitalia stage [177]. The faster childhood growth among the males in our cohort may reflect a greater proportion of fat gain and earlier exposure to cardiometabolic risk factors which can track into adulthood, thereby increasing CVD risk [178]. This is supported by a previous analysis, which showed that faster gains in BMI from 8 to 13 years were positively associated with adult hypertension in males, but not females [179]. Among the females in our cohort, the majority had likely begun puberty by 13 years of age. The greater CVD mortality risk may be a consequence of greater gains in BMI coupled with early pubertal development, which can accelerate timing of menarche and early menarche itself has been associated with CVD mortality [159, 160]. Data from a large multi-country study has shown that genetics appear to have a stronger influence on weight and BMI from birth to 19 years among boys, while environmental influences seem to play a more important role among girls [134]. This may partially account for the differences in the risk estimates we observed between the males and females.

There are studies to support that childhood growth can be useful in estimating risk of adverse health outcomes [141]. Findings from the Fels Longitudinal Study showed that BMI at ages 13 and 18 years have good predictive values in terms of obesity at age 35 years [180]. The period of growth from ages 8 to 13 years that we have in our cohort is unique in terms of capturing prepubertal changes in childhood growth and the faster tempo of BMI gain predicted greater risk of fatal CVD events. Compared to childhood BMI at one time point, the rate of BMI gain takes into consideration how the child is growing, and although it provides only some information on body fatness [181] it can be easily recorded and monitored. Childhood growth is a very complex period and the sex-specific differences in terms of timing of growth acceleration needs to be better investigated.

6.3 Peak height velocity and CVD mortality (Paper III)

In this study, earlier timing of PHV in girls was associated with greater risk of CVD mortality. Previous studies have not investigated timing of PHV in relation to CVD mortality, however, our findings are in line with studies which examined age at menarche and CVD. In the European EPIC-Norfolk study of Caucasian women, early menarche (prior to age 12) was associated with increased risk of CVD mortality, HR 1.28 (95% CI 1.02, 1.24) [159]. In a cohort of Singaporean Chinese women, early menarche was also found to be associated with CVD mortality, HR 1.17 (1.07, 1.27) [160]. The early maturing girls in these cohorts were likely experiencing PHV prior to age 12, which would be similar to the girls in our cohort who experienced early PHV (<11 years). However, we note that the greater risk estimates in our cohort and lack of a dose response effect between girls with PHV <11 years and between 11 to 12 years may be due to the modest number of CVD cases (total n=86). Furthermore, the broad confidence intervals in our analyses suggests our findings may be compatible with a smaller hazard ratio.

Associations between early age at menarche and adult CVD risk factors have been inconsistent. Some observational studies report elevated blood pressure, glucose, insulin and BMI levels among women with early age at menarche [157, 182, 183]. Other studies have reported no associations with early menarche and hypertension [184] or adiposity measures such as waist circumference [184, 185]. In our cohort, we did not find marked differences by timing of PHV and CVD risk factors. Adjustments for select CVD risk factors and mid-life BMI did not appear to influence the association between PHV and CVD mortality; however, their contributions to the outcome we observed were likely not fully accounted for since they were measured at only one time point.

Very few studies investigating pubertal timing among girls have been able to simultaneously report childhood growth data along with mid-life risk factors with follow-up until death. Similar analyses were not possible for the males in our cohort as boys enter puberty, on average, two years after girls and growth measures beyond age 13 would be needed to better estimate timing of height acceleration (i.e. PHV).

While greater childhood BMI has been associated with later disease risk, the effect of pubertal height acceleration has been less clear. In a separate analysis, we adjusted for BMI-velocity from ages 8 to 13 years as a continuous covariate in the Cox-regression analysis of PHV (after versus prior to age 12) and CVD mortality. For PHV, this mutual adjustment yielded a HR of 1.91 (95% CI 1.20, 3.04) versus 2.21 (95% CI 1.39, 3.50) without adjustment for childhood BMI-velocity. The corresponding HR for childhood BMI-velocity and CVD mortality in the same analyses was 1.95 (0.98, 3.87). Among the

women in this cohort, faster height velocity appears to have a somewhat independent influence on adult CVD mortality, apart from childhood BMI-velocity.

Due to the birth year span of the subjects in this analysis, there were differences in growth during this time period and relatively large changes in height and pubertal onset. Tryggvadottir et al. reported a secular trend in age at menarche where it declined from 14.9 to 13.5 years among women born between 1900 to 1950, with an apparent halt thereafter [154]. Information on age at menarche was not collected in the Reykjavik Study. The historical school health records, which included the childhood growth measures, only sporadically included age at menarche as reported by the subject and when available, this age was documented. The majority of subjects did not have data on age at menarche recorded and once the girls left school at approximately age 14, this information was not available.

Thus, information on age at menarche was available for 97 of the 973 girls, or 10% of the cohort. We observed a correlation between earlier PHV and lower age at menarche ($P=0.001$). This available data is likely not missing at random but suggests that PHV is, as expected, a predictor of age at menarche in our study population.

Our findings highlight that the timing of height acceleration itself is a possible early marker for CVD risk among girls and further studies are needed to determine whether these findings can be replicated. In healthy girls, PHV occurs prior to the age at menarche [163, 164] and may be an appropriate indicator for early pubertal timing. The age at menarche in the latter 20th and early 21st century has not dropped as notably compared to the previous century [186]. It is possible that in modern-day girls, age at menarche is a later marker of puberty and not adequate to catch girls undergoing early maturity.

6.4 Early nutrition and infant and childhood growth (Paper IV)

In this current analysis, we found that infants who had started solid foods at 5 months of age had faster growth up to 12 months of age, especially between the ages of 2 to 6 months. Furthermore, the addition of solid foods at 5 months of age predicted greater BMI at the age of 6 years. Our findings are in line with existing evidence that show exclusively breastfed infants grow slower compared to infants who have started complementary feeding [81, 187].

It is possible that a combination of factors may be influencing the differences in infant growth. Greater protein intake from complementary feeding is associated with faster weight gain and higher adiposity in infancy [188]. In addition, low maternal control in infant feeding has also been associated with higher toddler energy intake [189]. Interestingly, a recent randomized controlled trial found no difference in growth, energy intake or risk of becoming overweight at 18 or 29-38 months of age between infants exclusively breastfed for 4 versus 6 months [190-192]. However, the lack of differences early in infancy may be due to the relatively low amount of energy from complementary foods [191]. In other studies, exposing infants to solid foods prior to the age of 4 months was associated with being overweight or obese in early childhood [113], although the risk appears to be greater among children who are no longer breastfed.

In this cohort, 70% of infants in the solid food group were exclusively breastfed at 2 months of age. Mothers may introduce solid foods earlier if they find their infant appears

hungry or worry that breast milk is inadequate for their infant's needs [118]. Infants who had already started on solid foods at 5 months of age had a tendency towards faster growth from birth to 2 months and growth differences became significant between 2 to 6 months compared to exclusively breastfed infants. Our findings might indicate a separate possible explanation, that mothers began introducing food earlier to the children as a result of rapid growth, as opposed to the growth being a secondary effect of the early solid food introduction. Information on reasons affecting the duration of breastfeeding would have been beneficial, particularly among the infants who had been provided solid foods at 5 months to better understand the observed association with BMI at 6 years. Further studies with more detailed information on infant feeding practices and statistical power are needed to determine whether rapid infant growth leads to children demanding more feedings or whether the introduction of solid foods is the main contributor.

6.5 General discussion and public health perspectives

When analyzing the impact of fetal and childhood growth on CVD risk factors and mortality over the life course, there are likely several mechanisms simultaneously contributing to the outcomes we observe. While environmental stressors such as economic conditions are difficult to control, our finding that faster growth during childhood was associated with greater CVD mortality risk, even among normal weight children, highlights that this may be a period where public health monitoring may provide long-term benefits.

In paper II, we found that men with the fastest childhood growth between ages 8 to 13 years had higher blood pressure (both systolic and diastolic), triglycerides, fasting glucose and uric acid levels compared to men who grew slower during childhood, while among women the differences in these biomarkers were more mild. This may possibly be due to estrogen levels in pre-menopausal women which have been shown to protect against CVD by inhibiting the development of atherosclerosis [193]. However, there were differences in CVD mortality risk in which the women in our cohort with the fastest childhood growth had markedly higher risk estimates than men. Furthermore, in paper III, the association with timing of PHV and CVD mortality among the women in this cohort also emphasizes that early height acceleration, an early marker of maturity, may be an important determinant for later cardiovascular health, although it could not be determined if this was the same case for the men in our cohort. There is a need to identify non-traditional CVD risk factors, particularly among women, as there is evidence that women are less likely to be properly treated for CVD compared to men [194].

Although mechanisms are hard to establish with observational studies, there is suggestive evidence that the inverse association between earlier pubertal timing and cardiovascular mortality is partially independent of childhood BMI [155]. The findings among the women in our cohort also suggest that faster pubertal height acceleration from ages 8 to 13 years may have distinct influences on later risk of disease, apart from childhood BMI-velocity. Investigating these longitudinal changes in health provides insight into how CVD develops and underlines areas where public health interventions can have the most impact.

In the current environment where childhood obesity continues to be an ongoing concern, there is a need to monitor children with excessive growth so prevention measures can be implemented in a timely manner. Lifestyle changes encouraging appropriate weight

attainment and maintenance during childhood should be emphasized so progression of CVD can be slowed or halted.

There is no doubt that early nutrition is important in preventing future adverse health outcomes [195]. Our findings in the small but detailed early nutrition cohort in paper IV stress that the timing of complementary feeding impacts the growth rate during infancy and may be associated with greater childhood BMI. The 2003 revisions to the Icelandic infant nutrition recommendations resulted in lower protein intakes among infants on a population level [34]. However, the amount of protein intake of the infants in this current cohort may still be associated with faster growth and greater BMI in early childhood. It is difficult to conduct controlled studies in such a young population and the optimal intake of protein for healthy infants continues to be a source of discussion. Better breastfeeding promotion strategies are still necessary and the appropriate composition of infant formulas needs to be determined [196]. Few studies are able to account for the contribution of infant feeding practices beyond the first year of life in relation to childhood overweight or obesity. Detailed data on dietary intake in infancy provides the opportunity to identify factors influencing the growth rate and identify areas where revisions in nutrition recommendations may be most valuable.

7 Strengths and limitations

The rich longitudinal data available from the Reykjavik Study cohort allowed us to analyze the impact of early growth on adult health outcomes. Previous studies investigating these associations had limited or no data on covariates during childhood and at adult age. The strength of this cohort compared to other studies lies in the relatively detailed data on anthropometry and CVD risk factors at mid-life, well before the occurrence of fatal CVD events. The important contribution of the early nutrition cohort from paper IV provides insight into the early determinants of infant growth and its association with childhood BMI.

In paper I, we investigated the impact of the Great Depression on fetal growth in a unique setting in Iceland where famine was not an issue, yet adequate access to proper nutrition was challenging. There are various factors including nutrition, stress, and infections that can affect fetal growth and there were confounding factors that we were unable to account for in our analysis. However, previous studies investigating economic crises have primarily reported on birth size and did not have follow-up data at adult age which we had in our cohort. Adverse economic situations are still occurring today and our findings suggest its negative effects on size at birth may have long-term consequences.

In the analysis on childhood growth in papers II and III, adjustment for adult BMI has been suggested to be misleading as it is in the causal pathway, however, it is necessary to consider it as a mediator when analyzing adult mortality and also allows our findings to narrow the focus on postnatal growth patterns.

For the analysis of height velocity among girls, we recognize that data on age at menarche would have been beneficial. The original Reykjavik Study was designed to primarily assess risk factors for CVD in the Icelandic population and thus questions regarding onset of menarche were not included in the study questionnaire. However, as mentioned in the discussion section of paper III, for the small portion of girls for whom age at menarche was recorded on the school health records, there was a correlation between early PHV and lower age at menarche. It would be important for future studies to compare PHV alongside menarche data to determine if it is independently associated with CVD mortality in women.

In the early nutrition cohort in paper IV, the sample size is a possible limitation, however, the thorough data on dietary intake and growth variables provides valuable information. Furthermore, the number is sufficient to analyze differences in infant growth and detect modest associations between introduction of solid foods at 5 months and BMI at 6 years of age. When evaluating complementary feeding, the period continues until 2 years of age and it would have been valuable to have dietary records beyond 12 months of age as it is necessary to also assess dietary composition later in infancy.

It would have been beneficial to have information on early feeding practices in the Reykjavik Study. We acknowledge that infant feeding practices in the early 20th century would have been very different from current day practices. Given the growing interest in life course epidemiology, collection of detailed information on growth, diet, and lifestyle from birth to old age can be anticipated in the future to provide better insight into the associations between genetic and environmental exposures and diseases [197, 198].

8 Conclusions and future directions

This dissertation expands the knowledge on environmental influences on birth size, the impact of early nutrition on infant growth, and the effects of childhood growth on adult health outcomes.

Our findings show that unfavourable intrauterine conditions, such as exposure during pregnancy to psychobiological stress and mild undernutrition during a period of economic hardship was associated with infants who were born thinner and, at least among females, had increased odds of obesity at adult age. While the external environment cannot be completely controlled, in modern day societies where there is now an abundance of food, there is the possibility of improving health outcomes by identifying areas where dietary choices could be enhanced. Our findings that providing infants with solid foods even from 5 months of age was positively associated with infant growth and BMI at 6 years suggests this is an important area where prevention could take place and further research is needed. Faster growth during infancy is associated with risk of overweight and obesity in childhood [78-80]. Increasing access to lactation consultants and educating mothers to slow the introduction of complementary foods may have long-term benefits, particularly if the infant is on track to become or already is overweight or obese. These improvements in infant feeding practices may help to normalize growth later in infancy and into childhood. In addition, there is a need for better understanding of the appropriate composition of infant formula and timing of introduction of solid foods particularly if mothers are unable to breastfeed exclusively for 6 months.

The advantages of proper infant feeding practices may also present later in adult life with respect to better cardiovascular health. In our analysis of childhood growth, we found that faster gains in childhood BMI among normal weight children was associated with a poorer CVD risk profile among men and greater CVD mortality among both men and women at mid-life. Additionally, the association between earlier childhood pubertal height acceleration (i.e. PHV) and CVD mortality, which we observed among the females in this cohort, suggests that the timing of growth and pubertal development also needs to be considered. There is a need for future studies to simultaneously investigate the impact of PHV and age at menarche among girls to determine whether they are independently related to CVD risk.

Although some individuals may have a genetic predisposition to obesity and CVD, a growing area of interest has been the study of epigenetics, which suggests disease risk can also be transmitted by non-genomic means. The process is complex, but it appears that the period of early development can be shaped by the external environment through modification of gene expression without altering DNA sequences [199, 200]. Godfrey et al. showed that maternal diet during pregnancy causes DNA methylation marks in the umbilical cord and had significant associations with the child's adiposity at age 9 years [201]. These findings show that it is vital to nurture the child in a healthy environment while in the womb as it can have far-reaching effects.

The majority of preventative measures for CVD have focused primarily on interventions at adult age. The findings reported in this dissertation highlight that environmental exposures early in life can have lasting implications, increasing later risk of heart disease. Early life conditions, such as low birth weight and infant feeding practices, should be considered in risk models for CVD. It is important to place emphasis on dietary habits and lifestyle, not only in adulthood, but also during pregnancy, infancy, and into childhood to ensure proper and adequate growth over the life course.

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Papers I-IV

PAPER I

Effect of Birth Year on Birth Weight and Obesity in Adulthood: Comparison between Subjects Born Prior to and during the Great Depression in Iceland

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Abstract

Background: Many epidemiological studies have linked small size at birth to adverse adult health outcomes but the relative influence of environmental exposures is less well established.

Methods: The authors investigated the impact of prenatal environmental exposure by comparing 2750 participants born before (1925–1929) and during (1930–1934) the Great Depression in Reykjavik, Iceland. Calendar year served as proxy for environmental effects. Anthropometric measurements at birth and school-age (8–13 years) were collected from national registries. Participants were medically examined as adults (33–65 years).

Results: Mean birth weight, adjusted for maternal age and parity, decreased by 97 g (95% confidence interval (CI): 39, 156) for men and 70 g (95% CI: 11, 129) for women from 1925 to 1934; growth at school-age was significantly reduced for participants growing during the Depression. As adults, women prenatally exposed to the Depression had higher body mass index ($\Delta 0.6$ kg/m², 95% CI: 0.2, 1.1), higher fasting blood glucose levels ($\Delta 0.16$ mmol/L, 95% CI: 0.07, 0.23) and greater odds of being obese 1.43 (95% CI: 1.01, 2.02) compared to unexposed counterparts. Non-significant associations were observed in men.

Conclusion: Reduction in birth weight due to rapid shifts in the economic environment appears to have a modest but significant association with later obesity for women while male offspring appear to be less affected by these conditions.

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Introduction

There is strong evidence suggesting risk of some metabolic disorders is set early in life and that these risks can be further heightened by factors in the external environment [1,2]. An estimated 25–50% of normal variation in birth size is driven by genetics while environmental determinants contribute to the remaining influences [3,4]. Exposure to an adverse intrauterine environment (i.e. undernutrition during pregnancy) has been proposed to lead not only to infants with low birth weight but also decreased muscle mass, with a disproportionately high fat to lean mass ratio [5].

The association between low birth weight and adverse adult health outcomes has been observed in well-nourished as well as famine exposed populations [6–9]. The Dutch famine studies showed that extreme fetal undernutrition is linked to glucose intolerance, cardiovascular disease, and mental health disorders in adulthood [10–12]. The first weeks of pregnancy (e.g. first

trimester) appear to be the most vulnerable period and those exposed to famine during this time have consistently poorer health outcomes compared to unexposed individuals [12]. These findings have been reproduced in animal studies where mimicking prenatal malnutrition led to obesity and insulin resistance in offspring [13,14]. However, it remains unclear from both animal and human studies the degree to which such perinatal conditions have a lasting effect.

The environment that humans live in can change swiftly and birth weight provides a snapshot of the intrauterine environment. Stressful events have been linked to adverse birth outcomes, particularly low birth weight. In the 1920s Iceland's economy was expanding until the onset of the Depression which affected the country drastically due to heavy reliance on exports [15]. The Great Depression hit the Western world in 1929. However, historical sources identify 1930 as the year the effects of the Depression reached Iceland forcing exports to lose up to 50% of

their 1929 prices [15,16] and declines in food supply data were reported for most years in the 1930s [17].

The rapid occurrence of the Depression in Iceland makes this time period relevant for investigating sudden environmental shifts and their impact on birth size under non-famine like conditions. The aim of this study was to identify the association between size at birth and later health prior to and after the onset of the Great Depression in a cohort of 2750 Icelanders born between 1925 and 1934. We hypothesized that those exposed to the Depression in utero would be of lower birth weight and have greater risk of disorders associated with smaller size at birth, specifically obesity and cardiometabolic risk factors.

Materials and Methods

Ethics Statement

The study protocol was granted approval by the Icelandic National Bioethics Committee and the Data Protection Commission and written informed consent was obtained from all study participants.

Study Population

The source population for this study consists of 4601 subjects, aged 33–65 years, who were born in Reykjavik 1914–1935 and resided there when recruited into the longitudinal Reykjavik Study at the Icelandic Heart Association from 1967–1991. Methods regarding data collection have previously been described [18]. In brief, participants were randomly selected from the population register and invited to attend a clinical examination where anthropometrics, fasting blood samples, and medical history were collected by research personnel. Results on infant growth and later health have been previously published in this population [19–22], but environmental influences on birth weight have not been investigated.

To evaluate the impact of the Great Depression which began affecting Iceland in 1930, five year periods were combined (1925–1929 and 1930–1934) prior to and after the onset of the Depression as a proxy for environmental exposure. These two birth year groups, totaling 2750 participants or 60% of the source population, covers a span of ten years allowing the analysis of the immediate consequences of the Great Depression; subjects born greater than ten years apart will reflect other factors such as economic recovery, public health initiatives, medical advances as opposed to the specific influence of the Depression.

Exposure assessments

Information on infant sex, birth weight to the nearest 50 grams (g), and birth length from crown to heel in centimeters (cm) was gathered from midwives' birth records in the National Archives of Iceland. Mean birth weight in Iceland is one of the highest in the world [23]. In the complete cohort, there was a low prevalence (2.9%) of infants weighing below 2.5 kilograms (kg). Therefore, lower birth weight was defined as infants weighing less than 3.0 kg (prevalence 4.9%). Ponderal index was calculated as birth weight in g/birth length in $\text{cm}^3 \times 100$. Yearly growth measures from ages 8 to 13 years including weight, height, and date of measurement were recorded by a school nurse and gathered from school health records.

Data on childhood growth was available for a total of 1500 individuals, representing 55% of participants in the current study. Despite this attrition, previous analysis has shown that anthropometric measures at birth and adult age do not differ markedly between subjects with and without growth data [24]. Furthermore, the mean height of subjects who had information on childhood

growth available was compared to reference data for all public schools in Reykjavik [25]. At age 10 years, the average height difference from the reference values was 0.4 cm (men) and 0.1 cm (women) (data not shown) suggesting our growth data is a fair representation of school children in Iceland during this time. To determine differences in childhood growth patterns based on prenatal exposure to the Depression, mean BMI values from ages 8 to 13 for each birth year group were plotted against the current World Health Organization (WHO) BMI-for-age standards [26] for males and females separately.

Outcome assessment

The longitudinal Reykjavik Study collected information on health and anthropometric measures at adult age. Body weight was measured to the nearest 100 g without shoes and height was measured with accuracy of ± 0.5 cm. Subcutaneous skinfolds were measured with calibrated calipers to the nearest 1.0 mm [22]. Obesity was defined as body mass index (BMI) $\geq 30 \text{ kg/m}^2$. Fasting blood glucose, serum triglycerides, and total cholesterol were measured after an overnight fast [27]. Impaired fasting glucose [28] was classified as fasting glucose $\geq 6.1 \text{ mmol/L}$ and/or diagnosis or confirmation of type 2 diabetes. Dyslipidemia was defined as triglycerides $> 1.7 \text{ mmol/L}$ and total cholesterol $> 6.2 \text{ mmol/L}$.

Statistical analyses

The mean and standard error of the mean for birth weight and ponderal index were calculated separately by sex and yearly from 1925 to 1934. The mean and standard deviation (SD) or percentages were used to describe the cohort.

Linear regression analysis was performed for each outcome variable with birth year exposure to the Depression being a predictor and separately by sex. Maternal age and parity were included in all linear regression models as independent covariates. Participant age at examination was also included as a covariate when analyzing outcome variables collected in adulthood.

Logistic regression was used to estimate odds ratio (OR) of obesity, impaired fasting glucose, and dyslipidemia with the same covariates used in the linear regression models. Outcomes were adjusted for sex (when appropriate) however, women and men were also analyzed separately. Analyses included in the tables were not adjusted for participant's current or previous smoking habits as it post-dates birth year, however, data on percentage of current and previous smokers was obtained and is reported for descriptive purposes. Further modeling was performed to test for a recruitment effect by narrowing of study recruitment years and age range of participants, as well as addition of smoking variables although this data is not presented. All statistical analyses were carried out using SPSS 20.0 (IBM, New York). Significance was defined as $P < 0.05$, 2 sided.

Results

Figure 1 shows the mean birth weight by year of birth stratified by sex and Table 1, Panel A summarizes the birth characteristics. From birth year group born pre-Depression (1925–1929) to the group born during the Great Depression (1930–1934), there was a mean adjusted decrease in birth weight of 97 g for men and 70 g for women. Concurrently, ponderal index (Figure 2) decreased from 2.66 for males and 2.65 for females to 2.50 g/cm^3 for both sexes.

Lower birth weight ($< 3.0 \text{ kg}$) was more common in infants with birth year exposure to the Depression compared to unexposed participants (Table 1, Panel A). Although differences were non-

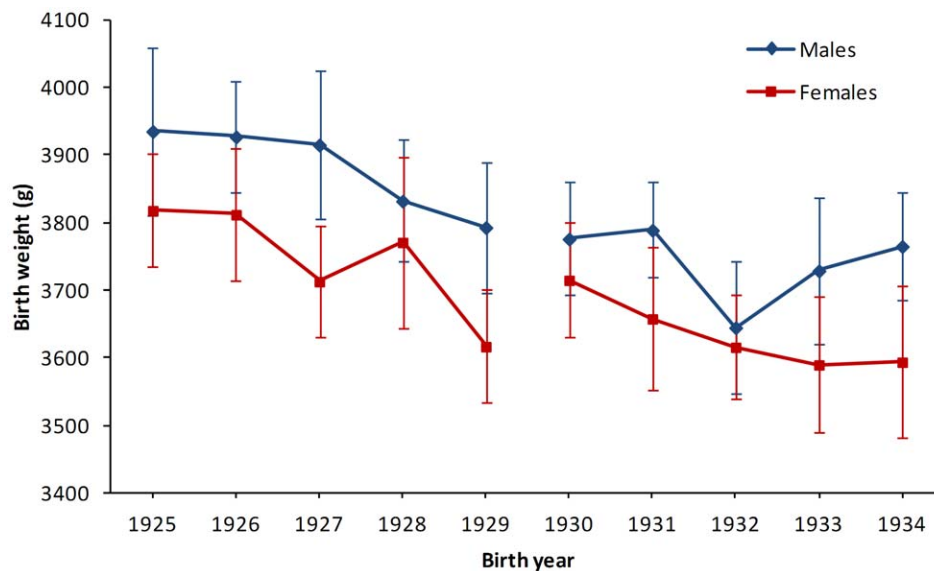


Figure 1. Yearly birth weight for men (diamond) and women (square) born between 1925 and 1934. Footer: Data are shown as means and the bars denote the standard error of the means. Birth weight is further stratified by sex and grouped by birth during pre-Depression (1925–1929) and during the Depression (1930–1934).
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significant prevalence of lower birth weight rose from 3.9% to 4.6% and 4.4% to 7% for men and women, respectively. Among Depression-born infants there was a marked increase in ponderal index below 2.60 g/cm^3 , a cut-off used for describing thinness in infants [9,29].

Childhood growth characteristics (Table 1, Panel B) show that at 10 years of age both boys and girls with birth year exposure to the Depression were greater in weight and height than their unexposed counterparts. However, only Depression-born girls had significantly higher BMI compared to girls born before the Depression. At study recruitment (Table 1, Panel C), women with birth year exposure to the Depression had higher mean BMI (adjusted $\Delta 0.6 \text{ kg/m}^2$, 95% CI 0.2, 1.1) and fasting blood glucose (adjusted $\Delta 0.16 \text{ mmol/L}$, 95% CI 0.07, 0.23) compared to women born pre-Depression. Triceps and subscapular skinfold measures were also significantly higher among Depression-born women. Men born during the Depression had greater subscapular skinfold measures, yet, differences in BMI among men were negligible. Total cholesterol levels were slightly decreased among Depression-born men and women while triglycerides levels were slightly higher.

Mean BMI from ages 8 to 13 stratified by birth year exposure to the Depression are shown in Figure 3A for males and 3B for females. When plotted against the WHO BMI-for-age standards, mean BMI from ages 8 to 13 of children born before the Depression followed approximately along the 25th percentile while the BMI of Depression-born children was closer to the 50th percentile. Growth differences based on birth year exposure were more pronounced among girls from ages 9 to 13 than among boys.

In Table 2, adjusted odds ratios for obesity, impaired fasting glucose, and dyslipidemia are presented with comparisons between birth year exposure to the Depression. In multivariate analysis, the combined OR for obesity was 1.40 (95% CI 1.09, 1.77). In separate analysis by sex, the odds of obesity remained significant only among women OR 1.43 (95% CI 1.01, 2.02) exposed prenatally to the Depression. Comparison of odds of impaired fasting glucose was not significant among women or among men. There was no marked difference in corresponding OR for

dyslipidemia for either sex based on birth year exposure to the Depression. Addition of current smoking and previous smoking as categorical variables to the regression models did not change the results (data not shown).

In order to further examine whether participant age influenced outcomes due to a recruitment effect we narrowed the age range of participants from 33–65 years to 45–55 years as well as recruitment year from 1967–1991 to 1974–1983. Adjusted combined OR for obesity remained significant 1.59 (95% CI 1.12, 2.26), with a stronger association among women 2.16 (95% CI 1.25, 3.76) while the combined odds ratios for impaired fasting glucose and dyslipidemia remained non-significant (data not shown).

Discussion

In this Icelandic cohort we investigated the impact of the Great Depression on size at birth and adult obesity, impaired fasting glucose, and dyslipidemia in 2750 Icelanders living in Reykjavik. We found that infants with birth year exposure to the Depression were of lower birth weight. In adulthood, Depression-born females had a higher mean BMI and greater odds of being obese but this effect was not observed in males.

With regards to size at birth, there was a significant decline in birth weight and ponderal index from 1925 to 1934 indicative of infants being born thinner as the economic impact of Depression progressed. Although birth weight still remained relatively high, the decreasing ponderal index is suggestive of increasing fetal undernutrition along with economic and environmental influences as there is a distinct and consistent drop after the initial onset of the Depression in Iceland in 1930.

The 1920s and 1930s in Iceland were characterized by increased migration into Reykjavik and changes in birth weight may also be a consequence of overcrowding which can negatively affect birth size [30]. The current cohort includes only city residents and may capture some of this early migratory trend into Reykjavik. The flux in birth weight may be reflective of the shifting urban population however, as all participants were born and living

Table 1. Characteristics at Birth, Age 10 Years, and at Study Recruitment, Mean (SD) or Percentage, for Men and Women Born Pre- or During the Great Depression.

	Men				Women			
	Pre-Depression	Depression	Δ	95% CI	Pre-Depression	Depression	Δ	95% CI
Panel A: Birth characteristics*								
	N = 685	N = 738			N = 687	N = 640		
Birth weight (g)	3880 (593)	3751 (531)	−97	−156, −39	3732 (555)	3638 (544)	−70	−129, −11
Ponderal index ^a (g/cm ³)	2.66 (0.34)	2.50 (0.30)	−0.15	−0.18, −0.12	2.65 (0.34)	2.50 (0.27)	−0.15	−0.18, −0.11
BW<3.0kg (%)	3.9	4.6	0.7	—	4.4	7.0	2.6	—
Ponderal index <2.6 g/cm ³ (%)	45.7	66.9	21.2	—	42.4	64.3	21.9	—
Panel B: Growth characteristics at age 10*								
	N = 390	N = 401			N = 392	N = 317		
Height (cm)	137.7 (5.2)	140.0 (5.9)	1.7	1.0, 2.5	137.1 (5.2)	139.1 (5.9)	1.8	1.0, 2.6
Weight (kg)	31.2 (3.8)	32.3 (4.6)	1.0	0.4, 1.5	31.2 (4.4)	32.7 (5.2)	1.4	0.7, 2.1
BMI (kg/m ²)	16.4 (1.3)	16.5 (1.5)	0.1	−0.1, 0.3	16.6 (1.6)	16.9 (1.9)	0.3	0.02, 0.5
Panel C: Characteristics at study recruitment								
	N = 685	N = 738			N = 687	N = 640		
Age	51.5 (4.6)	46.5 (5.1)	−5.0	—	52.3 (5.3)	51.0 (5.1)	−1.3	—
Current smoker (%)	57.1	62.0	4.9	—	41.9	44.6	2.7	—
Previous smoker (%)	21.2	21.6	0.4	—	14.0	20.1	6.1	—
Impaired fasting glucose (%)	4.9	2.5	−2.4	—	2.2	2.8	0.6	—
Dyslipidemia (%)	14.0	12.1	−1.9	—	6.8	7.7	0.9	—
Type 2 diabetes (%)	4.8	2.4	−2.4	—	2.0	2.5	0.5	—
Obese (% BMI ≥30 kg/m ²)	11.9	13.6	1.7	—	10.3	13.0	2.7	—
Anthropometrics†								
Height (cm)	178.3 (6.0)	179.6 (6.3)	0.6	−0.2, 1.3	164.8 (5.1)	165.6 (5.4)	0.5	−0.04, 1.1
Weight (kg)	83.1 (12.3)	84.2 (13.0)	1.2	−0.3, 2.7	68.0 (11.2)	70.0 (11.6)	2.2	0.9, 3.4
BMI (kg/m ²)	26.1 (3.5)	26.1 (3.6)	0.2	−0.2, 0.7	25.0 (3.9)	25.5 (4.1)	0.6	0.2, 1.1
Triceps skinfold (mm)	10.7 (7.2)	11.8 (8.0)	−0.2	−1.1, 0.7	20.6 (10.1)	22.2 (10.1)	1.9	0.8, 3.0
Subscapular skinfold (mm)	16.8 (7.6)	17.1 (8.0)	1.3	0.4, 2.2	18.8 (10.0)	21.5 (10.8)	3.3	2.2, 4.4
Biomarkers†								
Total cholesterol (mmol/L)	6.3 (1.0)	6.2 (1.0)	−0.16	−0.3, −0.04	6.4 (1.1)	6.2 (1.1)	−0.15	−0.3, −0.04
Triglycerides (mmol/L)	1.4 (0.9)	1.5 (0.8)	0.1	0.0, 0.2	1.1 (0.5)	1.1 (0.5)	0.06	0.0, 0.1
Fasting glucose (mmol/L)	4.6 (0.9)	4.5 (0.7)	−0.06	−0.2, 0.03	4.3 (0.6)	4.5 (0.9)	0.16	0.07, 0.23

Adjusted differences (Δ) and 95% Confidence Intervals are presented.

*Birth and growth characteristics at age 10 years adjusted for maternal age and parity.

†Adult anthropometrics and biomarkers adjusted for maternal age, maternal parity, and participant age at recruitment.

BW: birth weight; BMI: body mass index

^aPonderal index BW(g)/BL(cm)³×100.

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in Reykjavik when recruited into the study, it is unlikely that this had a substantial influence on the outcomes measured.

In addition to birth size, studies have suggested accelerated postnatal growth in thin infants may contribute to later disease [31]. Between the ages 8 to 13 years, both Depression-born boys and girls were taller and heavier than their counterparts born prior to the Depression, despite having a similar BMI at age 8. As the children grew older, those with birth year exposure to the Depression had faster BMI gain which may have placed them at greater risk of developing obesity in adulthood.

It should be noted that the environment during which these participants were growing was quite different. Children born

between 1925 and 1929 were growing-up during the Depression where access to food was more difficult and costly [17], while those born during the Depression (1930–1934) were on average growing in a more plentiful environment. Historically, this was primarily due to improvements in the fortunes of the Icelandic economy during the Second World War. Iceland experienced higher economic growth than most European countries from increased exports and greater demand created by the British and later American occupation forces in the country. The simultaneous economic change was likely linked with increased efforts by parents of Depression-born children to encourage weight gain. The relative contribution of an adverse prenatal environment

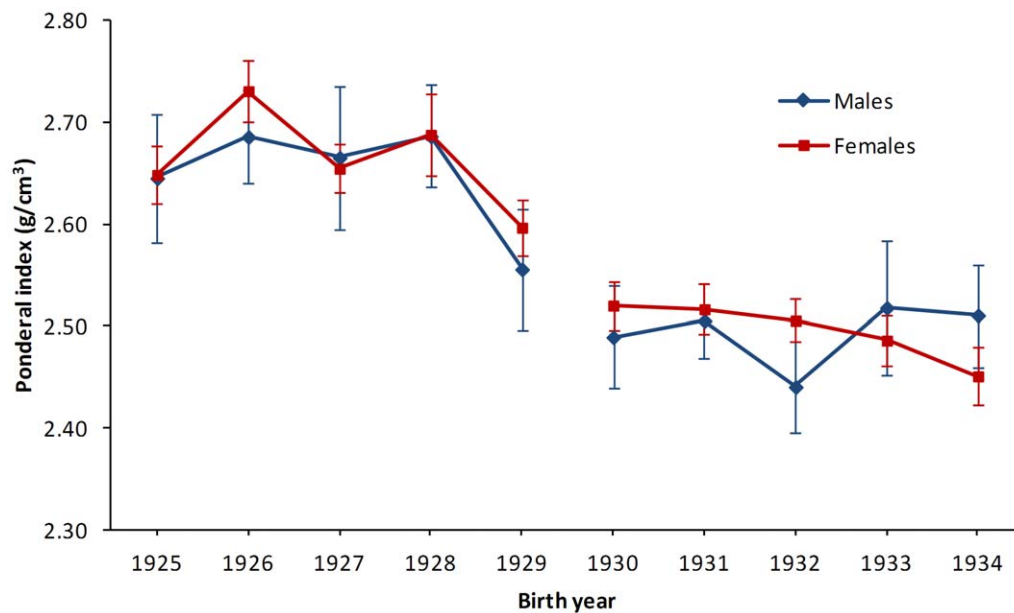


Figure 2. Yearly ponderal index^a for men (diamond) and women (square) born between 1925 and 1934. Footer: Data are shown as means and the bars denote the standard error of the means. Ponderal index is further stratified by sex and grouped by birth during pre-Depression (1925–1929) and during the Depression (1930–1934). ^a Ponderal index = (birth weight in g/birth length in cm³*100). doi:10.1371/journal.pone.0044551.g002

during the Depression coupled with an improved situation may have contributed to the differences in childhood growth patterns.

In adulthood, we observed higher fasting glucose levels and increased obesity only among women with birth year exposure to the Depression. We observed virtually no difference in BMI or odds of obesity among men. There is evidence that increasing glucose intolerance and obesity can coincide with transition to an environment of better nutrition [32]. However, both sexes experienced comparable environmental exposures suggesting the gender differences did not arise from the same etiologies. Previous analysis in this cohort also found sex-specific differences, with

stronger inverse associations between birth weight and truncal fat [22] and glucose intolerance [19] among women at adult age. Similar findings were published on the Hertfordshire cohort, where fasting glucose measures were more strongly inversely associated with birth weight in women [33].

We did not find the odds of impaired fasting glucose or dyslipidemia for either sex to be linked to birth year exposure to the Depression. The relatively high birth weight in Icelandic infants has been proposed to have a protective effect with respect to diseases (i.e. coronary heart disease, hypertension) related to small size at birth [19]. In several epidemiological studies higher

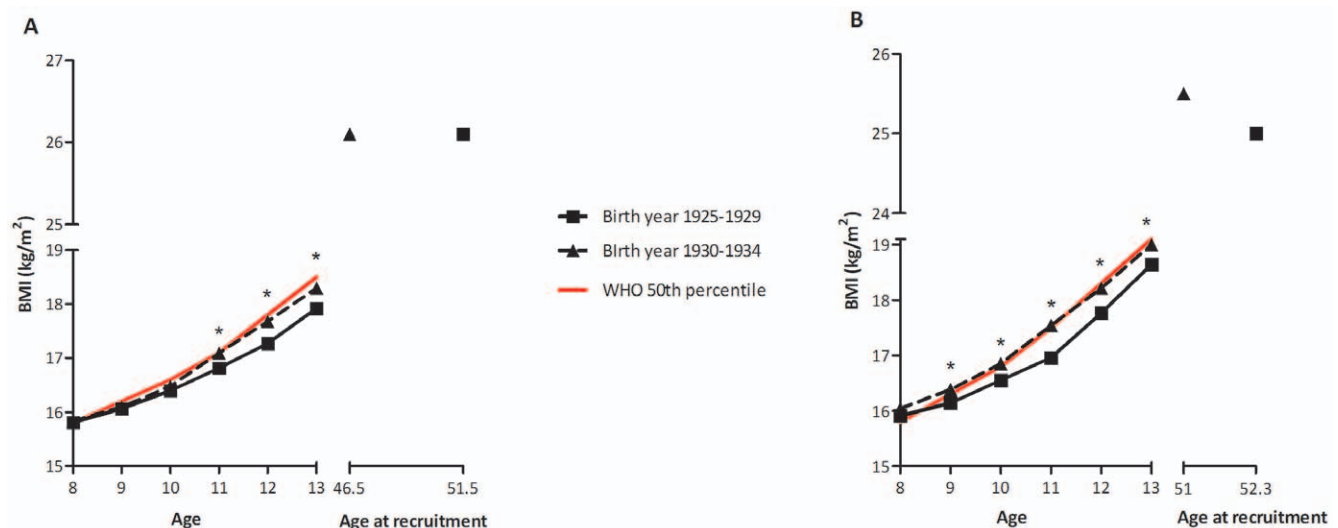


Figure 3. Mean BMI from 8–13 years by birth year group for A) men and B) women. Footer: Subjects born pre-Depression (1925–1929) were growing up during the Depression; while subjects born during the Depression (1930–1934) were growing up after the Depression and had faster BMI gain, growing closer to the 50th percentile for the WHO BMI-for-age standards compared to their counterparts born earlier. *Significant difference between BMI values of participants born pre-Depression vs. during the Depression, adjusted for maternal age and parity ($P < 0.05$). doi:10.1371/journal.pone.0044551.g003

Table 2. Adjusted Odds Ratios for Obesity, Impaired Fasting Glucose, and Dyslipidemia at Study Recruitment in Adulthood Comparing Men and Women Born During the Great Depression to Pre-Depression.

Variable	All ^a		Males ^b		Females ^b	
	OR	95% CI	OR	95% CI	OR	95% CI
Obesity (BMI ≥ 30 kg/m ²)	1.40	1.09, 1.77	1.27	0.89, 1.81	1.43	1.01, 2.02
Impaired fasting glucose ^c	0.98	0.62, 1.57	0.75	0.40, 1.41	1.41	0.69, 2.90
Dyslipidemia ^d	0.95	0.73, 1.24	0.72	0.51, 1.03	1.22	0.80, 1.86

Reference group: participants born Pre-Depression (1925–1929).

BMI: body mass index; OR: odds ratio, CI: confidence interval

^aAdjusted for maternal age, maternal parity, participant age at examination and sex.^bAdjusted for maternal age, maternal parity, and participant age at examination.^cImpaired fasting glucose: fasting glucose ≥ 6.1 mmol/L and/or diagnosis or confirmation of type 2 diabetes.^dDyslipidemia: total cholesterol > 6.2 mmol/L and triglycerides > 1.7 mmol/L.

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birth weight was associated with increased lean body mass but not adiposity later in life [34–36]. The difference in birth weight between infants born prior to versus during the Depression may reflect a lower proportion of lean body mass in the Depression-born infants. As muscle is an important site for glucose homeostasis, this could lead to abnormalities in blood glucose [37] which may explain the slightly higher fasting glucose levels we observed in adult women.

While the findings of greater cardiometabolic risk factors primarily among women corroborate those from other epidemiological cohorts [33,38], there are several possible limitations. Data on maternal birth or adult size, gestational age or placental function was not available for analysis and their effect on birth size in this cohort is unknown. However, adjustments for strong predictors of birth weight (i.e. maternal age and parity) performed in this current study did not affect the outcomes.

Although the participants were recruited at various ages (33–65 years), our findings do not appear to be influenced by a recruitment effect. In addition, Vilbergsson et al [26] previously reported that prevalence of non-insulin dependent diabetes in this cohort was consistent between the recruitment years 1967–1991. Thus, with regards to impaired fasting glucose this allows for a fair comparison between the subjects based on calendar year exposure to the Great Depression. The relationship between birth size and adult hypertension has been previously discussed in this cohort

[24]. We observed minor decreases in systolic and diastolic blood pressure which coincided with increasing use of anti-hypertensive medication for those recruited later in the cohort. Adjusting for this trend would not be possible without adjusting for calendar year therefore blood pressure was not included in this current analysis.

This cohort represents a population that was undergoing both economic and environmental changes during development in utero and early childhood [16]. The individual impact of the Depression cannot be fully quantified, yet on a cohort level the distinct increase in infants with low ponderal index suggests the environmental strain was considerable enough to affect maternal health condition and birth size of offspring. Ultimate height and weight is a result of the interaction between many factors, but it appears that even restricted but non-famine like conditions experienced in utero (based on birth year) can modestly influence risk of later obesity while other cardiometabolic risk factors (i.e. triglycerides, cholesterol, and glucose levels) seem to be less sensitive to these conditions. We also found that women were more susceptible to these conditions, which is in line with previous findings from this cohort.

Conclusion

In this current paper, we have presented that birth year exposure to the Great Depression was associated with offspring who were lighter at birth and Depression-born women had a greater likelihood of being obese in adulthood. With the increasing frequency of obesity-related diseases, the ability to identify environmental triggers that may negatively impact birth size in offspring and continue to affect weight status in later life is becoming critical. Pregnancy during periods of economic downfalls may inadvertently stress the fetus initiating a pathway favoring obesity development. Our findings contribute to the existing literature on environmental influences on size at birth and disease in middle-aged adults and emphasize the importance of accounting for both prenatal and postnatal environmental influences when exploring these associations.

Author Contributions

Conceived and designed the experiments: CMI TIH. Performed the experiments: CMI TIH. Analyzed the data: CMI TIH. Wrote the paper: CMI TIH IG VG TA GJ BEB IT. Contributed historical insight and interpretation of the time period in the current study and assisted in manuscript revision: GJ. Designed the original Icelandic Heart Association cohort study and provided critical revision of final manuscript: VG TA. Collected birth and growth data and provided critical revision of final manuscript: BEB IG IT.

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PAPER II

Faster increase in body mass index between ages 8 to 13 is associated with risk factors for cardiovascular morbidity and mortality

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Abstract

Background and Aims Excess childhood weight is associated with cardiovascular disease (CVD) in adulthood. Whether this is mediated through adult body mass index (BMI) and associated risk factors such as metabolic derangements remains unclear. The aim was to examine whether childhood BMI-velocity ($\Delta\text{kg/m}^2$ per year) was associated with adult CVD mortality and to examine how adult BMI and cardiometabolic risk factors contribute to the association.

Methods and Results Subjects were 1924 Icelanders born 1921-1935 and living in Reykjavik when recruited into a longitudinal study from 1967-1991. From ages 8-13 years BMI-velocity was calculated to quantify the association between childhood growth and adult CVD mortality. Deaths from recruitment to December 31, 2009 were extracted from the national register. There were 202 CVD deaths among men and 90 CVD deaths among women (mean follow-up 25.9 years). Faster BMI-velocity from ages 8–13 years was associated with CVD mortality when comparing those in the highest vs. lowest tertile with corresponding HR (95% CI): 1.49 (1.03, 2.15) among men and 2.32 (1.32, 4.08) among women after adjustment for mid-life BMI and CVD risk factors. Faster childhood BMI-velocity was associated with elevated CVD risk factors among men at mid-life but these associations were less pronounced among women.

Conclusion Faster increase in BMI from ages 8–13 years was associated with increased CVD mortality risk. Children with early growth spurts coupled with excess weight gain during this transition period from childhood into adolescence should be closely monitored to ensure better health in adulthood.

Key words: body mass index, cardiovascular disease, cohort studies, growth, mortality

Acronyms:

BMI: body mass index

CHD: coronary heart disease

CI: confidence interval

CVD: cardiovascular disease

HR: hazard ratio

SD: standard deviation

INTRODUCTION

Excess weight in early life may have health implications such as increasing risk of cardiovascular disease (CVD) later in life. While small size at birth is recognized as a modest risk factor for CVD,¹⁻⁴ it appears that the combination of being small at birth and remaining thin during infancy, followed by rapid growth later in childhood is associated with the greatest risk of coronary heart disease (CHD) in adulthood.⁵

Growing evidence, though, suggests that it is the tempo of childhood growth that increases the risk of CVD later in life regardless of size at birth. Previous studies investigating early growth and adult CVD or CHD risk have focused on the association between a 1 kg/m² or 1-SD increment change in BMI at each age during childhood.⁶⁻⁸ These findings provide some understanding into how CVD risk develops. However, there is a need to ascertain whether there are changes in risk over a certain phase of growth compared to BMI at a specific age during childhood. This period has been difficult to establish partially due to the close interaction between BMI, both during child- and adulthood, and CVD risk.^{9,10} Mechanistic insight has also been a challenge and there is a need to consider cardiometabolic risk factors simultaneously with childhood growth to determine their contributions to adult disease risk. Thus, in order to quantify whether childhood growth influences later CVD risk, both adult BMI and conventional CVD risk factors need to be taken into consideration. Furthermore, examining the rate of BMI gain over multiple time points during childhood may provide better understanding of when CVD risk changes.

To our knowledge, previous studies have not investigated the association between childhood BMI-velocity and CVD mortality while adjusting for the effect of adult body size which is a well

established contributor to CVD. In this current analysis, our objective was to determine whether the tempo of childhood growth is associated with CVD mortality while accounting for adult body size. Moreover, the aim was to explore whether any associations between childhood growth and CVD death persist while also considering traditional adult CVD risk factors.

METHODS

Study Population

The longitudinal Reykjavik Study was initiated by the Icelandic Heart Association in 1967.¹¹ The source data consisted of 4601 singletons born in Reykjavik between 1914 and 1935 and who resided there when recruited into the study from 1967 to 1991. Growth measures from ages 8 to 13 years were recorded at regular intervals from 1929 (birth year ≥ 1921) in two main schools in Reykjavik. The 782 subjects born prior to 1921, as well as the 1699 subjects for whom clinical records were unavailable were excluded from this analysis. Furthermore, 196 subjects had multiple missing growth measures leaving a total of 1924 subjects in our current analyses. At recruitment into the Reykjavik Study (1967-1991) we found small differences between subjects included in our analyses (n=1924) vs. those who were not (n=2677) with respect to characteristics such as (included vs. not included) birth weight (3743 vs. 3745 g), adult BMI (25.7 vs. 25.7 kg/m²), triglycerides (1.3 vs. 1.2 mmol/L) and total cholesterol (6.3 vs. 6.4 mmol/L). It has also previously been shown that the childhood growth measures in this cohort are a fair representation of school children in Iceland during this period when compared to reference data from public schools in Reykjavik.¹²

Informed consent was obtained from all participants. The collection of birth and childhood growth measures was approved and released by the Icelandic National Bioethics Committee and the Data Protection Commission.

Anthropometric measures at birth and during growth

At birth, weight was recorded to the nearest ± 50 g and birth length from crown to heel (in centimeters) was obtained from midwives' birth records. Childhood growth measures were collected from school health records stored in the National Archives of Iceland.

At each yearly exam from ages 8 to 13 years, the child's height, weight and date of measurement was recorded by school health professionals. BMI-velocity was quantified as the mean change in BMI per year for the period between 8 and 13 years.

Collection of adult data

Methods on data collection from the Reykjavik Study have been previously described.¹³

Participants were asked to arrive at the Icelandic Heart Association Heart Preventive Clinic in a fasting state to complete a medical examination, blood sample collection, and lifestyle questionnaire. Each participant's height was recorded to the nearest 0.5 cm and weight to the nearest 100 g, without shoes and in light undergarments. Blood samples were drawn after an overnight fast and total cholesterol, triglycerides, glucose and uric acid levels were analyzed. Quality control of the lipid and glucose measurements was employed throughout the recruitment period.¹⁴ Blood pressure was measured after a five minute rest to the nearest 2 mm Hg with a mercury sphygmomanometer Erkameter wall-model (Erka, Germany) using a cuff size of 12x23

cm. The same cuff was used throughout the study. Skinfolts were measured at two sites, subscapular and triceps, with calibrated calipers to the nearest 1.0 mm.¹⁵

Fatal CVD and CHD events

Information on cause of death was collected from files at the Statistical Bureau of Iceland from the time of recruitment (beginning in 1967) to December 31, 2009. Mortality due to CVD was determined if death certificates included the following International Classification of Diseases: code 420 (1967-1970, 7th revision), codes 410-413 (1971-1980, 8th revision), and codes 410-414 (1981-2009, 9th revision).

Statistical analysis

The mean and standard deviation (SD) or proportions were used to describe participant characteristics. The child's BMI z-scores were calculated separately by sex based on the internal distribution in our cohort. Trend tests for adult characteristics and CVD risk factors were calculated using t-tests for continuous variables and Chi-square tests for categorical variables.

Cox-proportional hazards regression was used to evaluate the association between childhood BMI-velocity, BMI z-scores and CVD mortality. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated. Childhood BMI-velocity ($\Delta\text{kg/m}^2$ per year) was classified into tertiles and entered as a categorical variable to determine the relationship with fatal CVD and CHD events in adulthood. All analyses were adjusted for birth year (categorical variable using 3-year intervals from 1921 to 1935), maternal parity, birth weight, BMI at age 8, and age at recruitment. We further adjusted for known CVD risk factors at mid-life (mean age: 50.6 years, SD 5.8) including total cholesterol, systolic blood pressure, previous and current smoking (dichotomous) and history of familial hypertension (dichotomous). To account for the proportion

of obese participants, adjustments for mid-life BMI were categorized as follows: BMI <25, 25-29.9 or ≥ 30 . Further analysis was performed to estimate the likelihood of being obese using logistic regression analyses by comparing tertile of childhood growth velocity as well as calculating risk estimates for growth velocity and CVD mortality, excluding obese adult participants, using Cox-regression analyses with adjustments for the same co-variables previously mentioned although this data is not presented. All analyses were performed using SPSS version 20.0 (IBM, New York).

RESULTS

Childhood growth

Table 1 summarizes the birth size and childhood growth of participants by sex and tertile of BMI-velocity from ages 8 to 13 years. Both boys and girls with the greatest BMI-velocity were heavier and taller during childhood compared to their slower growing counterparts.

At study recruitment

During a mean follow-up period of 25.9 years (SD 8.4), there were 202 CVD deaths with 137 from CHD among men and 90 CVD deaths with 44 from CHD among women (Table 2). At study entry, men with the highest childhood BMI-velocity were younger and also heavier at mid-life. Apart from cholesterol, these men also had more unfavorable levels of CVD risk factors including skinfold measures, systolic blood pressure and serum triglycerides and uric acid. Women with the greatest childhood BMI-velocity exhibited greater mid-life BMI, triglycerides and skinfold measures.

Childhood BMI-velocity and CVD mortality

Greater BMI-velocity from ages 8 to 13 was linked to CVD mortality with risk estimates for the highest vs. lowest tertile of BMI-velocity corresponding to HR (95% CI): 1.49 (1.03, 2.15) among men and 2.32 (1.32, 4.08) among women after full multivariate adjustment (Table 3, model 2). Although among men, the association between BMI-velocity and CVD mortality was attenuated after adjustment for mid-life BMI (P for trend 0.005 versus 0.062 after adjustment), the association with CHD mortality was more stable.

BMI z-score at a specific age and CVD mortality

When analyzing BMI at a specific age in childhood in relation to CVD mortality, there was a gradual rise in risk among both sexes with increasing age and BMI z-score though the relationship was only significant at age 13 among women (Table 4).

Sensitivity analyses

Participants in the highest tertile of childhood BMI-velocity were at greater risk of being obese in adulthood, odds ratio (95% CI), 2.88 (1.72, 4.82) among males and 1.87 (1.41, 2.48) among females. Due to the markedly higher percentage of obese adult individuals in the highest tertile of childhood BMI-velocity, we ran analyses excluding obese adults to determine whether they were driving the association with CVD mortality. We found the risk estimates for childhood BMI-velocity and CVD mortality to be unchanged (highest vs. lowest tertile) with corresponding HR (95% CI), 1.59 (1.06, 2.37) for men and 2.68 (1.47, 4.87) for women after full multivariate adjustment.

DISCUSSION

In the current analysis, we found that faster BMI-velocity from ages 8 to 13 years was strongly associated with greater risk of CVD death. After adjusting for mid-life BMI and traditional CVD risk factors, among men the rate of CVD death in the highest tertile of childhood BMI-velocity was 1.49 times higher (95% CI 1.03, 2.15) than that in the lowest tertile. Among women, the rate was more than double, HR 2.32 (1.32, 4.08), in the highest compared to the lowest tertile of childhood BMI-velocity.

The interesting aspect of this cohort is that the children were growing during a period prior to the obesity epidemic and yet, we still observe elevated risk in children of normal weight. Based on the International Obesity Task Force BMI-for-age cut-offs,¹⁶ less than 5% of the children would be classified as overweight at any age between 8 to 13 years. Furthermore, our sensitivity analyses show that the association between childhood BMI-velocity and CVD mortality persists not only among normal weight children but also among normal weight adults. When examining childhood BMI at a specific age, we also saw that the association with CVD mortality increased from 8 to 13 years and our estimates were similar to those previously reported in a cohort of Danish school children at the same age growing up from 1930-1976.⁷

Detailed childhood growth from ages 8 to 13 years in this cohort is a strength of this study which enabled us to determine the association of this growth period with CVD mortality. Furthermore, we were simultaneously able to consider the influence of mid-life CVD risk factors and anthropometry on this association with follow-up until death.

In our cohort, it appeared the differences in childhood BMI-velocity and CVD mortality risk differed by gender. Adjustment for mid-life BMI attenuated the risk estimates among men, but less so among women. Cardiometabolic risk factors were comparatively elevated among males with the greatest childhood BMI-velocity, although adjustments for select risk factors did not markedly affect the estimates we observed. Moreover, previous analysis from the larger Reykjavik Study has suggested alterations in coronary risk factors are not sufficient to explain the differences in CVD risk between men and women.¹⁴ It is possible that in our cohort, the tempo of prepubertal childhood BMI-velocity, which is inherently different between the sexes, may itself be associated with CVD risk and the potential mechanisms are further discussed.

Prior to entering puberty, there is a slow growth period which occurs between the ages of 9 to 12 years among boys.¹⁷ This stage is characterized by increases in weight but slower gains in height and is likely captured among the males we analyzed in this cohort. Limited research exists on pubertal timing among males and its effects on metabolic disorders, but accelerated prepubertal growth has been shown to be correlated with both timing of puberty and adult metabolic derangements.¹⁸ The faster childhood BMI-velocity among the males in our cohort, while not leading to obesity, likely reflects a greater proportion of fat gain which may hasten exposure to adverse metabolic effects. This is supported by a previous analysis in this cohort which linked faster gains in childhood BMI among males to adult hypertension, although similar to our findings with CVD mortality, adjustment for mid-life BMI attenuated the association.¹⁹ We observed only modest changes in CVD mortality risk after adjusting for systolic blood pressure; however, the mid-life measurements were taken at only one time point and thus it is difficult to precisely determine its contribution.

Compared to males, females enter puberty on average two years earlier and for most of the females in this cohort pubertal development had likely begun by 13 years of age. The greater risk estimates among girls compared to boys with the greatest childhood BMI-velocity may be a consequence of early development which can accelerate the timing of menarche and early menarche itself has been associated with CVD mortality.^{20,21}

It has previously been shown that genetics may play a greater role in explaining variation in body size among boys, while girls may be more prone to environmental influences.²² This may partially explain the gender differences in risk we observe, as the timing of the slow growth period may be genetically determined²³ while pubertal events such as age at menarche among girls can be modified by external exposures such as nutrition and other environmental influences. Thus, we speculate that the increased CVD mortality risk we observe may be a combination of faster BMI-velocity coupled with hormonal fluctuations associated with pubertal development. Large variations in childhood BMI have been reported with respect to pubertal onset such as timing of peak height velocity²⁴ suggesting that both height- and BMI-velocity may have distinct influences on later risk of disease and should be evaluated separately.

In a previous analysis from this cohort, uric acid levels were suggested to play a role in the association between faster childhood BMI gains and adult hypertension.¹⁹ Uric acid levels increase in both sexes at puberty^{25,26} and were elevated among the fastest growing males in our cohort. These levels can track from childhood into adult age, however, adjusting for serum uric acid in our analyses did not change the risk estimates in either sex (data not shown). Furthermore, the usefulness of uric acid in predicting CHD in the general population is still uncertain.²⁷

The present study has certain limitations that should be addressed. Data on gestational age was not available, however adjusting for maternal parity, a strong predictor of birth size, did not appear to affect our main findings. Information on growth prior to 8 years of age was not available for analysis. However, a systematic review of studies related to childhood BMI and later CHD risk reported that BMI in early childhood (2–6 years) showed a weak inverse association while BMI in later childhood (7 to <18 years) was positively related to later CHD risk.²⁸ Although the stronger associations with the later childhood years could be due to closer timing to the age of the outcome, this review suggests the latter childhood years may be a more applicable time period to investigate in terms of understanding adult disease risk. Furthermore, our source data included 4601 singletons born in Reykjavik between 1914 and 1935 only 1924 subjects were included in our analyses due to missing growth data. Adult characteristics of those with and without growth measures were similar, however, we acknowledge that a loss of more than 50% of subjects is a notable limitation to our study.

Adjustments for established CVD risk factors were included in our analyses; however, they were measured at only one time point. Moreover, although adjustment for adult BMI has been suggested to be misleading as it is in the causal pathway and has the potential for collider bias, it is necessary to consider as a mediator when analyzing adult mortality. Furthermore, a simulation study has shown that collider bias does not generally result in considerable bias to the effect estimates.²⁹ While correcting for these factors did not fully account for the association between childhood BMI-velocity and CVD death, they likely played an important role in the pathway to the outcomes we observed.

This study contributes to the ongoing research on obesity among youth and highlights the need to monitor accelerated growth even among normal weight children. Efforts to encourage positive

dietary and lifestyle choices should continue over the life course as there is some evidence that heavier children who return to a healthy adult weight may have a slightly better CVD risk profile compared to those who stay or become overweight or obese.³⁰

Conclusion

In our analyses, we have shown that the faster tempo of BMI-velocity from ages 8 to 13 years was associated with increased risk of CVD mortality even after adjustment for mid-life BMI and traditional CVD risk factors. Importantly, the association between faster BMI-velocity and CVD death remained even among individuals with normal child and adult body size. Our findings are, thus, also relevant among countries undergoing nutrition transitions which are often followed by increase in obesity where the long-term effects of early accelerated growth need to be more thoroughly investigated.

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Conflict of Interest: None declared.

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Table 1. Anthropometrics at birth and during childhood, mean and SD, split by tertile of BMI-velocity (Δ kg/m² per year) between ages 8 to 13 years.

Anthropometrics	Boys (N=979)				Girls (N=945)			
	Tertile 1	Tertile 2	Tertile 3	P for trend	Tertile 1	Tertile 2	Tertile 3	P for trend
<i>At birth</i>								
Birth weight (kg)	3.81 (0.58)	3.77 (0.57)	3.84 (0.55)	0.534	3.64 (0.67)	3.68 (0.54)	3.72 (0.55)	0.044
<i>During childhood</i>								
Weight age 8 (kg)	25.3 (3.1)	25.6 (2.8)	26.9 (3.5)	<0.0001	24.8 (3.4)	25.4 (3.0)	27.0 (3.5)	<0.0001
Weight age 13 (kg)	38.4 (4.6)	41.9 (4.9)	49.1 (8.0)	<0.0001	39.7 (5.6)	44.6 (4.9)	52.1 (6.6)	<0.0001
Height age 8 (cm)	126.9 (5.2)	127.9 (5.0)	129.3 (5.4)	<0.0001	125.7 (5.1)	127.2 (4.8)	128.4 (4.7)	<0.0001
Height age 13 (cm)	151.6 (6.6)	153.9 (7.1)	157.6 (7.7)	<0.0001	152.6 (6.8)	155.6 (6.2)	157.6 (5.6)	<0.0001
BMI age 8 (kg/m ²)	15.7 (1.2)	15.6 (1.0)	16.1 (1.3)	<0.0001	15.7 (1.4)	15.7 (1.2)	16.3 (1.5)	<0.0001
BMI age 13 (kg/m ²)	16.7 (1.2)	17.6 (1.0)	19.7 (2.0)	<0.0001	17.0 (1.5)	18.4 (1.3)	20.9 (2.1)	<0.0001
BMI-velocity 8 to 13 years (Δ kg/m ² per y)	0.20 (0.10)	0.40 (0.05)	0.73 (0.23)	<0.0001	0.27 (0.12)	0.54 (0.07)	0.93 (0.26)	<0.0001

Table 2. Mid-life anthropometrics and cardiometabolic factors, mean and SD or %, split by tertile of BMI-velocity between ages 8 to 13 years.

	Boys (N=979)				Girls (N=945)			
Adult CVD risk factors	Tertile 1	Tertile 2	Tertile 3	<i>P</i> for trend [†]	Tertile 1	Tertile 2	Tertile 3	<i>P</i> for trend [†]
Age (years)	50.4 (5.6)	50.2 (5.3)	49.1 (5.3)	0.002	51.8 (5.9)	51.1 (5.9)	51.2 (6.2)	0.2
BMI (kg/m ²)	25.1 (3.4)	26.0 (3.3)	27.5 (3.9)	<0.001	24.2 (3.6)	24.7 (3.6)	26.5 (4.5)	<0.001
Obese (%)	7.6	8.0	22.4	<0.001	6.4	7.6	18.4	<0.001
Subscapular skinfold (mm)	16.0 (7.3)	17.0 (7.3)	18.9 (8.0)	<0.001	17.9 (8.5)	19.3 (9.8)	21.2 (10.9)	<0.001
Triceps skinfold (mm)	10.6 (7.1)	11.8 (7.2)	13.4 (8.2)	<0.001	21.1 (9.3)	21.0 (9.6)	22.7 (10.0)	0.04
Systolic BP (mm Hg)	135 (18)	140 (21)	142 (21)	<0.001	128.0 (17.2)	130 (19)	128 (18)	0.9
Diastolic BP (mm Hg)	87 (10)	89 (11)	92 (12)	<0.001	82 (9)	82 (10)	82 (10)	0.9
Total cholesterol (mmol/L)	6.3 (1.0)	6.4 (1.1)	6.3 (1.0)	0.9	6.2 (1.1)	6.2 (1.1)	6.3 (1.2)	0.2
Triglycerides (mmol/L)	1.4 (0.9)	1.4 (0.7)	1.6 (1.1)	0.002	1.0 (0.5)	1.1 (0.5)	1.2 (0.5)	<0.001
Fasting glucose (mmol/L)	4.6 (0.6)	4.6 (0.7)	4.7 (1.0)	0.04	4.3 (0.6)	4.4 (0.9)	4.4 (0.9)	0.6
Uric acid (μmol/L)	328 (63)	335 (65)	344 (68)	0.002	258 (59)	261 (55)	263 (66)	0.4
Family history								
Hypertension (%)	19.3	24.8	25.8	0.1	26.7	36.2	31.4	0.04
Diabetes, type 2 (%)	7.6	10.7	10.4	0.3	8.3	8.9	12.4	0.2
Myocardial infarction (%)	18.0	19.9	19.6	0.8	25.1	28.6	26.3	0.6
Stroke (%)	10.7	13.2	11.3	0.6	16.5	15.6	13.7	0.6

[†]*P*-values are derived from t-tests for continuous variables and chi-square tests for categorical variables.

Table 3. Association between BMI-velocity from 8 to 13 years and fatal CVD or CHD events in unadjusted and adjusted models.

All Fatal CVD Events					Fatal CHD Events			
Tertiles (T) of BMI-velocity ¹	T1 (n=327)	T2 (n=326)	T3 (n=326)	P for trend*	T1 (n=315)	T2 (n=315)	T3 (n=315)	P for trend*
Boys	Hazard ratios (95% Confidence intervals)				Hazard ratios(95% Confidence intervals)			
Mean age at event, years (cases)	70.7 (n=53)	72.3 (n=67)	68.2 (n=82)		69.0 (n=33)	70.6 (n=44)	66.3 (n=60)	
Unadjusted	1.0	1.38 (0.96, 1.98)	1.68 (1.19, 2.37)	0.013	1.0	1.44 (0.92, 2.26)	1.97 (1.29, 3.02)	0.006
Adjusted model 1 [†]	1.0	1.31 (0.91, 1.89)	1.70 (1.19, 2.43)	0.005	1.0	1.38 (0.88, 2.18)	1.93 (1.25, 3.00)	0.005
Adjusted model 2 [‡]	1.0	1.28 (0.89, 1.85)	1.49 (1.03, 2.15)	0.062	1.0	1.35 (0.86, 2.14)	1.72 (1.10, 2.70)	0.037
Girls								
Mean age at event, years (cases)	76.5 (n=21)	75.6 (n=31)	72.8 (n=38)		76.2 (n=11)	75.4 (n=14)	69.4 (n=19)	
Unadjusted	1.0	1.48 (0.85, 2.57)	2.07 (1.22, 3.53)	0.013	1.0	1.30 (0.59, 2.86)	2.03 (0.96, 4.26)	0.038
Adjusted model 1 [†]	1.0	1.51 (0.85, 2.67)	2.38 (1.36, 4.16)	0.005	1.0	1.32 (0.58, 2.99)	2.26 (1.03, 4.96)	0.017
Adjusted model 2 [‡]	1.0	1.43 (0.80, 2.54)	2.32 (1.32, 4.08)	0.007	1.0	1.29 (0.57, 2.95)	2.12 (0.96, 4.72)	0.030

¹BMI-velocity 8 to 13 ($\Delta\text{kg/m}^2$ per year), mean (SD): Boys, T1: 0.20 (0.10), T2 0.40 (0.05), T3 0.73 (0.23); Girls: T1: 0.27 (0.12), T2: 0.54 (0.07), T3: 0.93 (0.26)

*P for trend estimated by entering the BMI-velocity from 8 to 13 years as a continuous variable in the regression model.

[†]Adjusted for birth year, maternal parity, birth weight, BMI at age 8, adult age at examination, current and previous smoking, total cholesterol, systolic blood pressure and familial hypertension

[‡]Adjusted for co-variates in model 1 with further adjustment for mid-life BMI at recruitment

Table 4. Adjusted hazard ratios for CVD death per 1-unit increase in BMI z-score at ages 8, 10, and 13 years.

	Boys			Girls		
	<i>Model 1*</i>	<i>Model 2**</i>	<i>Model 3[†]</i>	<i>Model 1*</i>	<i>Model 2**</i>	<i>Model 3[†]</i>
Age 8	1.11 (0.96, 1.27)	1.12 (0.98, 1.28)	1.00 (0.86, 1.16)	0.95 (0.76, 1.19)	0.95 (0.76, 1.20)	0.92 (0.72, 1.18)
Age 10	1.15 (0.89, 1.49)	1.09 (0.84, 1.42)	1.02 (0.78, 1.34)	1.46 (0.97, 2.21)	1.37 (0.90, 2.09)	1.35 (0.88, 2.07)
Age 13	1.40 (1.13, 1.73)	1.33 (1.07, 1.64)	1.19 (0.96, 1.49)	1.63 (1.20, 2.20)	1.56 (1.15, 2.12)	1.55 (1.13, 2.13)

*Model 1: Adjusted for birth year, maternal parity, birth weight, BMI at age 8 (except at age 8), participant age at recruitment, current and previous smoking

**Model 2: All variables in model, with further adjustments for familial hypertension, total cholesterol and systolic blood pressure

[†]Model 3: All variables in model 2 with further adjustment for mid-life BMI at recruitment

PAPER III

ORIGINAL ARTICLE

Early peak height velocity and cardiovascular disease mortality among Icelandic women

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Introduction. Early pubertal onset among girls has been associated with cardiovascular disease (CVD) risk factors. We examined whether timing of peak height velocity (PHV), an early marker of maturity, was associated with CVD mortality.

Materials and methods. We analysed 973 Icelandic women, born 1921–1935, with annual childhood growth measures from ages 8–13 years, recruited into the longitudinal Reykjavík study 1968–1991. CVD deaths from recruitment to December 2009 were recorded.

Results. Eighty-six women died from CVD, 42 deaths from coronary heart disease (CHD). Compared to girls with PHV after age 12, girls with PHV < 11 years and between 11 and 12 years had greater risk of CVD mortality, hazard ratio 1.87 (95% confidence interval 1.07–3.26, $P = 0.028$) and 2.56 (1.52–4.31, $P < 0.001$), respectively. Comparable associations were observed with CHD cases 2.27 (1.17–4.44, $P = 0.016$) as well as non-CHD CVD cases 2.21 (1.17–4.19, $P = 0.015$) when comparing girls with PHV after versus prior to age 12. Timing of PHV was not associated with traditional CVD risk factors in mid-life including body mass index and adverse lipid profiles or with all-cause mortality.

Discussion. Earlier timing of PHV in girls may increase the lifetime risk of CVD mortality and may be an important determinant for later cardiovascular health.

Key words: Cardiovascular diseases, cohort studies, epidemiology, growth, mortality, risk factors

Introduction

Cardiovascular disease (CVD) continues to be the leading cause of death worldwide. Women tend to have different CVD risk profiles compared to men (1,2), and there is a growing need to provide risk assessment to identify factors that specifically impact women.

Key messages

- In this Icelandic cohort of women, reaching peak height velocity prior to age 12 was associated with increased risk of cardiovascular disease mortality.
- The association between peak height velocity and cardiovascular disease mortality was independent of mid-life adult body mass index and traditional risk factors.
- Timing of peak height velocity, a pubertal event that occurs prior to age at menarche among girls, may be an important determinant for later cardiovascular health.

Early menarche has been associated with risk factors for CVD such as high blood pressure and elevated plasma lipids at adult age (3,4). Recent reports also suggest an inverse relationship with menarcheal age and adult CVD mortality (5,6). However, previous studies investigating puberty in girls and later disease risk have largely relied on retrospective self-reported age at menarche (3–8). Although the use of recalled menarcheal age has been somewhat validated, the reliability of such retrospective methods vary greatly depending on characteristics of the population including age at recall and education (9).

Peak height velocity (PHV) is a pubertal event achieved prior to menarche (10,11) and has been used as a marker for early development (12). To our knowledge, the timing of PHV and its association with CVD mortality has not been previously investigated. While childhood body mass index (BMI) has been linked to coronary heart disease (CHD) (13), the influence of pre-pubertal height acceleration itself on later disease risk has been less well examined. Furthermore, few studies have been able to take into account the simultaneous influence of mid-life BMI and conventional CVD risk factors.

As early onset of puberty among girls is an ongoing concern due to its associations with adverse health outcomes (14), it is of interest to determine whether timing of height acceleration among girls can provide insight into cardiometabolic risk markers and CVD mortality in adulthood. To achieve this aim, we utilized childhood growth measures gathered from school health records to examine the association between age at PHV among girls and mortality from CVD, including CHD, in adulthood.

Materials and methods

The study population

The longitudinal Reykjavik Study was initiated by the Icelandic Heart Association in 1967 to assess and manage cardiovascular diseases in Iceland (15). In this analysis, we studied a sample of women who were born in Reykjavik and attended the two main schools in Reykjavik where childhood growth data were also collected. The source data for this paper consist of 4601 subjects born between 1914 and 1935 and who resided in Reykjavik when recruited into the study from 1967 to 1991.

Annual recording of height and weight measures began in two main schools in Reykjavik in 1929 for children 8 years of age and older (birth year ≥ 1921). There were 781 subjects born prior to 1921, and 1699 subjects lacked school health records due to migration as not all subjects attended the main schools, leaving us with 2120 participants available for analysis (16). Girls enter puberty, on average, 2 years earlier than boys (17). In this cohort, childhood growth measures were recorded annually from ages 8 to 13 years. For this reason, the 1085 men in the cohort were excluded from the analysis because pubertal timing would not be identifiable based on the growth data we had available. The remaining sample size of 1035 women was further narrowed as PHV could not be accurately identified from growth measures ($n = 62$). Thus, the final sample size was 973 women.

Comparisons between the women in the source cohort who entered the study versus those who did not ($n = 1253$) revealed no indication that adult anthropometrics or markers for CVD differed markedly—adult height (165.2 versus 164.7 cm), total cholesterol (6.3 versus 6.3 mmol/L), serum triglycerides (1.1 versus 1.1 mmol/L), fasting glucose (4.4 versus 4.4 mmol/L), systolic blood pressure (129 versus 128 mmHg), and diastolic blood pressure (82 versus 81 mmHg). Informed consent was obtained from all participants, and the study received approval from the Icelandic National Bioethics Committee and the Data Protection Commission.

Collection of birth and growth measures

Birth weight was recorded to the nearest ± 50 g, and birth length from crown to heel (in cm) was obtained from midwives' birth records stored in the National Archives of Iceland. Childhood growth measures from ages 8 to 13 were recorded in school health records at regular yearly intervals beginning in 1929 at two main schools in Reykjavik. At each yearly exam, the child's height and weight were recorded along with the year and month of measurement. On average, there were 5.5 height measures available per subject from ages 8 to 13 years, with 92% of the cohort having 5 or more measurements.

Timing of peak height velocity

From the growth measures which were collected yearly from ages 8 to 13 in two main Reykjavik schools, height velocity was defined as difference in height between two consecutive annual measurements divided by the time between the two measurements

($\Delta\text{cm}/\Delta\text{time}$). The changes in height were visually assessed for each participant by two independent reviewers. The maximum height acceleration after 8 years of age was identified as PHV (12) if it was followed by a clear increase in height velocity from the previous year (if after age 8) followed by at least a 1 cm/y decrease the next year with a consistent decrease in height velocity at the subsequent years. If the greatest height acceleration occurred at the measurement taken between 12 and 13 years or there was no marked increase in height velocity, the women were categorized as having late PHV ($n = 476$). PHV prior to age 12 was identifiable in 497 women (51%), of which one-half ($n = 248$) had clearly reached PHV prior to 11 years of age (early) and the second half ($n = 249$) between ages 11 and 12 (middle), leading to the categories used in our analyses (see Supplementary Figure 1 available online at <http://informahealthcare.com/doi/abs/10.3109/07853890.2013.852347>).

Anthropometrics and blood measures at adult age

Methods on data collection from the Reykjavik Study have been previously described in detail (15). In brief, participants were randomly selected through the National Register and invited by letter or telephone to the Heart Preventive Clinic in Reykjavik to complete a medical examination.

The women in this cohort were recruited between 1968 and 1991, and the mean age of study participants at examination was 51 years (± 6 , range 36–65 years). Each participant's height was recorded to the nearest 0.5 cm and weight to the nearest 100 g, without shoes and in light undergarments. Subjects were instructed to consume no food or drink after 10 pm the night prior to the exam. Fasting blood samples and blood pressure after a 5-minute rest in a sitting position were collected by trained nurses. Skinfold measures were collected at two areas, triceps and subscapular, with calibrated callipers to the nearest 1.0 mm (18). Information on medical history, family history, use of medication, and smoking habits was assessed by a standardized health questionnaire completed by the participant and verified by study personnel.

CVD and CHD mortality

The date and cause of all deaths in the cohort were obtained through linkages with the Icelandic Statistical Bureau. Death from any cause and fatal cardiovascular deaths, including CHD and non-CHD, that occurred from the time of recruitment starting from 1968 in this cohort to 31 December 2009 were considered. Death certificates were reviewed and coded by an official government pathologist (19). Death from CVD or CHD was ascertained if death certificates included the following International Classification of Diseases: code 420 (1967–1970, 7th revision), codes 410–413 (1971–1980, 8th revision), codes 410–414 (1981–2009, 9th revision), and codes I20–I25 (2010, 10th revision).

Statistical analysis

The median, mean \pm SD, or proportion was used to describe participant characteristics. The associations between variables were evaluated using *t* tests and chi-square for trend statistics. Cox regression analyses were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) for the association between age at PHV and CVD, CHD and non-CHD CVD mortality, as well as all-cause mortality. The underlying time-scale was time from study recruitment to fatal CVD event or until 31 December 2009, whichever came first. PHV was categorized as early (prior to age 11), middle (between ages 11 and 12), and late (after age 12). PHV after 12 years of age was used as the reference category.

Due to the low number of cases, analyses for CHD and non-CHD CVD mortality were performed with two categories

comparing girls with PHV prior to versus after age 12. Age-adjusted analyses were performed along with further variable adjustments for birth year (dichotomous 3-year intervals from 1921 to 1935), maternal parity, previous and current smoking (dichotomous), age at clinical examination, total cholesterol, systolic blood pressure, and familial hypertension, and additionally for birth weight, BMI at age 8, and mid-life adult BMI. We used SPSS version 20.0 (IBM Corp., NY, USA) for all statistical analyses. The significance level was $P < 0.05$, two-sided.

Results

The median height gain of girls achieving PHV in the early (< 11 years) and middle (between 11 and 12 years) groups was 7.9 and 8.2 cm/y, respectively (Table I). Among the girls in the late PHV group, there was a trend toward increasing height velocity, median 7.2 cm/y between the ages of 12 and 13 years, but the velocity was lower in comparison to girls with earlier PHV as expected. This also indicates a combination of girls who had reached PHV between the ages of 12 and 13 along with those who had not.

Characteristics of the participants in mid-life adulthood are presented in Table II by PHV category. Differences in age, age at mortality from CVD, and anthropometric measures in adulthood were negligible between the PHV groups, although the mean BMI and proportion of obese women were higher in the middle PHV category (11 to 12 years). At mid-life, though the trends were insignificant, women in the middle PHV category had higher uric acid concentrations, while those in the earliest PHV category had lower diastolic blood pressure and a greater percentage used pain medication.

At the end of 2009, there were 348 total deaths in the cohort. Eighty-six women died from CVD, of which 42 were from CHD. After full multivariate adjustment (Table III), compared to girls with late PHV, girls with early and middle PHV were at greater risk of overall CVD mortality with corresponding HRs of 1.87 (1.07–3.26, $P = 0.028$) and 2.56 (1.52–4.31, $P < 0.001$). There were comparable associations with CHD mortality when evaluating girls with PHV after versus prior to 12 years of age, HR 2.27 (1.17–4.44, $P = 0.016$) as well as for non-CHD CVD mortality 2.21 (1.17–4.19, $P = 0.015$). These associations remained stable after adjustment for both childhood BMI at age 8 and mid-life adult BMI. Timing of PHV was not associated with all-cause mortality.

Discussion

In this current analysis of 973 Icelandic women, we found that earlier PHV, i.e. prior to 12 years of age, was associated with higher rate of death from CVD but not all-cause mortality. Comparable associations were observed with CHD and non-CHD CVD mortality. Variations in our findings by cause of death suggest distinct processes are involved with respect to the influence of childhood height acceleration. Furthermore, this association did not appear to be fully mediated through childhood BMI or well-established risk factors for CVD in mid-life such as total cholesterol, systolic blood pressure, or BMI.

There have been no previous studies evaluating PHV among girls and later risk of disease, so we compare our findings to reports on early age at menarche. Large-scale epidemiological studies support our main finding that early maturity and CVD mortality are linked independent of adult body size (5,6). The European EPIC-Norfolk study of Caucasian women found that menarche before age 12 was associated with increased risk of CVD mortality, HR 1.28 (1.02–1.24), which was only partly mediated by increased adult adiposity (5). In a population of Singaporean Chinese women, early menarche was associated with CVD and CHD mortality with corresponding HRs of 1.17 (1.07–1.27) and 1.23 (1.06–1.43) after adjustment for adult BMI (6). Both studies used retrospective recall of menarche and the age category prior to 12 years to indicate early maturation. It is likely that these early-maturing girls were experiencing PHV prior to age 12, which would be similar to the girls in the early PHV group in our cohort. The greater risk estimates in our cohort may imply that PHV is more strongly related to CVD mortality compared with age at menarche. However, given our modest number of cases and the broad confidence intervals observed in our analyses, our findings may be compatible with a smaller hazard ratio.

Associations between age at menarche and cardiometabolic risk factors have been more ambiguous. Findings from the Fels Longitudinal Study and the Bogalusa Heart Study showed that women with early menarche exhibited higher blood pressure (20) and a greater prevalence of syndrome X (21) in early adulthood. In a Finnish cohort, using standardized height growth as a proxy for early puberty, subjects with early pubertal timing had increased blood pressure, higher BMI, and shorter stature in adulthood compared to individuals who experienced puberty later (7). Other cohorts have found no connection between early menarche and adult blood pressure or diabetes (22,23). In our

Table I. Median values for height and height velocities from ages 8 to 13 by timing of peak height velocity.

Age (y)	Timing of peak height velocity					
	Early < 11 years ($n = 248$)		Middle 11–12 years ($n = 249$)		Late > 12 years ($n = 476$)	
	Height (cm)	Height velocity (cm/y)	Height (cm)	Height velocity (cm/y)	Height (cm)	Height velocity (cm/y)
8 ^a	129.0		127.1		126.5	
9	134.7	5.5	132.2	5.1	131.0	5.0
10	140.0	6.3	138.0	5.2	135.6	5.0
11	148.0	7.9	143.5	5.5	141.0	5.3
12	154.0	5.5	152.0	8.2	147.0	5.5
13	158.0	4.2	157.0	5.1	154.0	7.2 ^b

^aBMI at age 8, mean (SD): PHV < 11 years 16.1 (1.4), 11–12 years 16.0 (1.3), > 12 years 15.7 (1.4).

^bLate PHV category (> 12 years) reflects the median height velocity of girls with PHV identified between ages 12 and 13 and those who had not reached PHV.

Table II. Anthropometrics and cardiometabolic risk factors at mid-life adulthood, mean \pm SD or per cent, by timing of peak height velocity.

	Timing of peak height velocity			P for trend
	Early (< 11 years) n = 248	Middle (11–12 years) n = 249	Late (> 12 years) n = 476	
Age	50.6 \pm 6.1	51.6 \pm (5.7)	51.6 \pm 6.0	0.06
Age at death (any cause)	72.3 \pm 8.9	71.6 \pm 9.9	71.0 \pm 9.7	0.27
Age at CVD death	72.0 (10.0)	75.0 (9.2)	76.3 (8.5)	0.09
Height (cm)	165.7 \pm 5.6	165.2 \pm 5.7	165.1 \pm 5.2	0.2
BMI (kg/m ²)	25.3 \pm 4.2	25.6 \pm 4.5	24.8 \pm 3.9	0.1
Triceps skinfold (mm)	22.4 \pm 9.6	22.0 \pm 10.6	21.1 \pm 10.0	0.09
Subscapular skinfold (mm)	19.9 \pm 10.0	19.9 \pm 10.5	18.9 \pm 9.5	0.1
Previous smoker (%)	16.5	16.9	17.9	0.9
Current smoker (%)	45.2	38.2	41.2	0.3
Blood and medical parameters				
Total cholesterol (mmol/L)	6.3 \pm 1.2	6.3 \pm 1.1	6.3 \pm 1.1	0.7
Triglycerides (mmol/L)	1.1 \pm 0.5	1.1 \pm 0.5	1.1 \pm 0.5	0.4
Fasting glucose (mmol/L)	4.3 \pm 0.7	4.4 \pm 0.9	4.4 \pm 0.9	0.5
Uric acid (μ mol/L)	255 \pm 59	271 \pm 67	256 \pm 57	0.7
Systolic BP (mmHg)	127 \pm 18	131 \pm 20	129 \pm 18	0.2
Diastolic BP (mmHg)	81 \pm 10	82 \pm 10	82 \pm 10	0.06
Diabetes, type 2 (%)	2.4	3.6	2.3	0.6
Obese (BMI \geq 30) (%)	10.5	15.3	9.5	0.06
Medication				
Anti-hypertensives (%)	10.9	12.4	12.0	0.9
Hormone replacement (%)	7.7	8.4	6.7	0.7
Pain (%)	9.3	4.8	4.8	0.04
Thyroid (%)	2.0	3.2	3.2	0.6
Family history				
Maternal parity	3.1 \pm 2.3	3.0 \pm 1.9	3.2 \pm 2.2	0.4
Stroke (%)	15.7	17.3	13.7	0.4
Myocardial infarction (%)	30.6	27.7	24.4	0.2
Hypertension (%)	29.4	35.7	29.2	0.2
Diabetes (%)	9.7	13.7	8.2	0.07

P values are derived from *t* tests for continuous variables and chi-square tests for categorical variables.

cohort we did not find marked differences by timing of PHV for select CVD risk factors, and adjustments for these variables strengthened the associations observed. We also found no differences between timing of PHV and adult height which is in line with existing reports on pubertal growth (17,24). Adverse CVD risk factors did not appear to influence the association between childhood height velocity and CVD death, at least at mid-life. However, we note that these factors were measured at only one time point and likely contributed to the outcomes we observe.

While PHV and onset of menarche are both downstream events of initial pubertal onset, in populations where childhood growth measures are available, identification of PHV may be a useful objective marker for CVD risk similar to age at menarche (4). It is probable that earlier PHV leads to earlier onset of menarche, and the timing of PHV may be the growth phase during which mechanisms are triggered that accelerate both height gain and simultaneously contribute to adult metabolic disorders (7). Whether this occurs due to fluctuations in sex hormones

Table III. Hazard ratios for CVD, non-CHD CVD, and CHD mortality by category of peak height velocity.

PHV category	Cases/n	Age-adjusted, HR (95% CI)	Adjusted model 1 ^a , HR (95% CI)	Adjusted model 2 ^b , HR (95% CI)	Adjusted model 3 ^c , HR (95% CI)	P value for model 3
All fatal CVD events						
Late (> 12 y)	32/476	Referent				
Middle (11–12 y)	31/249	1.89 (1.15–3.09)	2.58 (1.54–4.30)	2.58 (1.53–4.33)	2.56 (1.52–4.31)	< 0.001
Early (< 11 y)	23/248	1.64 (0.96–2.81)	1.85 (1.07–3.22)	1.87 (1.07–3.26)	1.87 (1.07–3.26)	0.028
Fatal non-CHD CVD events						
> 12 years	17/461	Referent				
< 12 years	27/470	1.74 (0.95–3.20)	2.30 (1.22–4.34)	2.23 (1.18–4.21)	2.21 (1.17–4.19)	0.015
Fatal CHD events						
> 12 years	15/459	Referent				
< 12 years	27/470	1.85 (0.98–3.48)	2.14 (1.11–4.13)	2.28 (1.17–4.45)	2.27 (1.17–4.44)	0.016
All-cause mortality						
Late (> 12 y)	176/476	Referent				
Middle (11–12 y)	91/249	1.01 (0.78–1.30)	1.10 (0.85–1.43)	1.09 (0.84–1.41)	1.08 (0.83–1.40)	0.570
Early (< 11 y)	81/248	0.99 (0.76–1.28)	1.00 (0.76–1.31)	0.98 (0.75–1.29)	0.98 (0.75–1.29)	0.882

BMI = body mass index; CHD = coronary heart disease; CVD = cardiovascular disease; PHV = peak height velocity.

^aAdjusted for birth year, maternal parity, smoking (current and previous), age at clinical examination, total cholesterol, systolic blood pressure, and familial hypertension.

^bAdjusted for the same covariates as in model 1 and additionally for birth weight and BMI at age 8.

^cAdjusted for the same covariates as in model 2, and additionally for adult BMI.

such as estrogen or changes in adiposity and/or adverse blood lipid profiles needs to be elucidated and is an area where further follow-up is required.

Other environmental exposures, e.g. organic pollutants, have also been implicated in progressing onset of puberty (25). All participants in this current analysis were born in Reykjavik and went to school and were living there as adults when recruited into the study. Although not a completely isolated environment, the women would have been exposed to similar external environments. Immigration into the country was also very minimal during this period, and different racial backgrounds would not be a source of bias. Early maturation among current youth has also been associated with unhealthy behaviours such as smoking which continue into adulthood (26). Associations remained significant after adjustments for former and current smoking, suggesting that it was not a major confounder in our analyses.

Additional factors not controlled for might be involved in the outcomes we observed. For example, age at menopause is also associated with risk of CVD (27). In women of Northern European descent, the average age at menopause is between 50 and 51 years (28). When we truncated our analyses to women ≤ 50 years of age as an estimate of women who had likely not entered menopause, the risk estimates for the association between PHV (late versus early) and total CVD mortality after multivariate adjustment went from 1.87 (1.07–3.26) to 2.11 (0.81–5.52). The central risk estimate does not change dramatically, and the wider confidence intervals can be attributed to the lower numbers of cases (30 versus 86 CVD deaths in the total cohort). Although menopause may have had some effect on later CVD death, we still find an increased risk of CVD mortality among women who likely had not entered menopause.

In recent years, the role of the adipocyte-derived hormone leptin on pubertal timing has been of growing interest. Animal studies have shown that inadequate energy intake in pubertal rats halts the development of reproductive organs (29), and in leptin-deficient mice introduction of the hormone induces puberty (30). As leptin is known to have atherogenic effects (31), earlier exposure to higher leptin levels could be an unexplored mechanism for the increased CVD mortality seen among the women who had earlier PHV.

There are some limitations in the present study. Growth measures after 13 years of age were not available for analyses, and thus we were unable to determine the timing of PHV if it occurred after this age. Measurement error is also a possibility; however, the mean childhood height measures in our cohort were found to be a fair representation of school children in Iceland during this period when compared to reference data for all public schools in Reykjavik (32). Furthermore, each child's height was clinically measured in regular yearly intervals by a trained health professional and did not rely on self-reported information, making it possible to compare with relative accuracy girls with early PHV to those with PHV after age 12. Longitudinal growth studies have shown that the PHV in average-growing girls is 8.1 cm/y (33). A more recent study comparing average PHV among American girls reported the average to be 8.3 cm/y and not different from British girls (17). These numbers are consistent with the median height velocity in our cohort in the early and middle PHV categories. The original design for the Reykjavik Study (1968–1991) did not include data on age at menarche. To truly determine if PHV is independently associated with CVD mortality it is essential to compare it alongside menarche data which is an important area for future studies.

We also acknowledge that the number of CHD and non-CHD CVD cases is modest, yet we still observe similar risk estimates

in these categories despite the limited sample size. The lack of a dose-response when evaluating all CVD deaths could be attributed to both a lower number of cases and to the fact that almost 50% of our cohort were in the PHV category > 12 years of age. The change in risk likely could not be captured with the growth data we had available and may be observed if growth was collected beyond 13 years of age.

Conclusions

Our findings suggest earlier PHV can increase the lifetime risk of CVD mortality independent of BMI at age 8, mid-life adult BMI, and CVD risk factors. In general, there appears to be no beneficial effect of early maturity on later health. Early height acceleration, even among normal-weight children, is an easily tracked measurement, and by closely monitoring pre-pubertal and adolescent growth we may be able to recognize growth patterns associated with later disease and identify appropriate timing of clinical interventions.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online

Supplementary Figure 1.

PAPER IV

Title: Introduction of solid foods prior to 6 months of age is associated with faster growth during infancy and increased body mass index in childhood

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Abstract:

Background and aim: Rapid growth during infancy is associated with increased risk of overweight and obesity and differences in weight gain are at least partly explained by means of infant feeding. The aim of this study was to assess growth rate in infancy and up to 6 years of age according to infant feeding practice at 5 months of age.

Methods and Materials: Subjects were 154 children born 2005 who were prospectively followed from birth to 12 months and again at 6 years of age. Information on infant feeding practices was collected at 0-5 months and dietary records were collected at 5 months of age via a 24-hour food record. Infant feeding practice at 5 months of age was categorized as exclusively breastfed, only formula fed, or started on solid foods. Changes in infant weight gain were calculated at various intervals between birth to 18 months. Linear regression analyses were performed to examine associations between infant feeding practice at 5 months and body mass index (BMI) at 6 years of age adjusted for sex, birth weight, and maternal education.

Results: Infants who were formula fed or had been provided solid foods grew faster, particularly between 2 to 6 months of age with mean difference and 95% confidence interval (CI) of 512g (147, 877) and 336g (101, 571), respectively, compared to exclusively breastfed infants. The addition of solid foods at 5 months predicted greater BMI at 6 years, with BMI being on average 0.7 kg/m² (95% CI 0.0, 1.3) higher among infants provided solid foods compared to those exclusively breastfed at 5 months of age.

Conclusion: Infants who were formula fed and who were provided solid foods at 5 months grew faster during early infancy than children who were exclusively breastfed. Further studies are needed to differentiate whether rapid infant growth is the cause or effect of early solid food introduction.

MeSH terms: growth, infant, breastfeeding, weaning, overweight, child

Introduction

Childhood obesity is an ongoing public health concern (1). Infant growth patterns are becoming better understood and the timing and tempo of growth rate appears to be important variables in predicting childhood obesity (2, 3). Rapid growth during the first year of life has been associated with increased risk of overweight and obesity in children (4, 5) and differences in weight gain during early infancy are at least partly explained by means of infant feeding (6-8).

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life and its benefits are well accepted worldwide (6, 9). However, there is evidence that most mothers in European countries begin to introduce complementary foods before 6 months of age (10). Studies have shown that bottle fed children grow more rapidly than children who are exclusively breastfed (11, 12) and are at greater risk of childhood overweight or obesity (6) but less is known about the long-term effects of introduction of solid food in early infancy. In a recent randomized controlled trial, introduction of complementary feeding at 4 months of age did not increase weight gain in infancy and did not appear to affect the risk of overweight or obesity at 18 months or 29-38 months of age compared to infants exclusively breastfed for 6 months (13-15). The aim in this current analysis was to assess growth rate in infancy and up to 6 years of age by early infant feeding practices with focus on the age of 5 months.

Materials and Methods

The study population, recruitment and data collection have previously been described in detail (16). In brief, a random sample of 250 Icelandic infants born in 2005 was collected by

Statistics Iceland. The inclusion criteria were Icelandic parents, singleton birth, gestational length of 37-41 weeks, birth weight within the 10th and 90th percentiles, no birth defects or congenital long-term diseases, and the mother had early and regular antenatal care. In this current analysis, eligible subjects were those with complete infant data and dietary record at 5 months, and weight and height measurements at 6 years of age. Informed written consent was obtained from all parents. The study was approved by the Icelandic Data Protection Authority (S5099/2011), Local Ethical Committee at Landspítali- University Hospital (1104Ref.16 2011) and the Bioethics committee (VSNb2011010008/037).

Infant and childhood growth data collection

Birth information on weight and length was gathered from the maternity wards. Infant anthropometric measurements were gathered from healthcare centers monthly from 1-6 months, then singularly at 9, 12, and 18 months. As close to the child's sixth birthday as possible (mean 73.4±3.2 months) weight (Marel M series 1100, Iceland; ± 0.1 kg) and height (Ulmer stadiometer, Prof. Heinze, Busse design Ulm; ± 0.5 cm) were measured in a clinical examination at the Landspítali-University Hospital. BMI was calculated as weight (kg)/height (m²). When the infant was 12 months of age, the parent or caregiver was asked to complete a questionnaire regarding information on age, education, and physical characteristics including self-reported height and weight of both parents.

Dietary assessment

Information on breastfeeding was gathered monthly during the first 12 months. The parents or caregivers completed a 24-h food record monthly from 5-8 months and 10-11 months using common household measures such as cups and spoons. At 9 and 12 months of age,

weighed food records were kept for three consecutive days on accurate scales (PHILIPS HR 2385, Austria; PHILIPS HR 2385, Hungary; ± 1 g accuracy).

Average daily consumption of energy and the contribution of energy providing nutrients at the age of 9 and 12 months were estimated using ICEFOOD, a software program used by the Icelandic Nutrition Council. Special infant products, such as cereals and purées were added to the database and nutrient losses due to food preparation were taken into account in the calculations.

Statistical analysis

Mean and standard deviation (SD) or proportion was used to describe infant and maternal characteristics. Our analysis are mainly based on the feeding practice at the age of 5 months where we compare the growth in infancy between those children who were exclusively breastfed at this time point to those who were either exclusively bottle fed or those who had been introduced to solid food at the age of 5 months. The reason why we chose to use the 5 month registration for our primary analysis is that this was the earliest detailed food registration available in the present study and it gives information on variations in duration of exclusive breastfeeding. Linear regression analyses were used to determine associations between infant feeding practice at 5 months of age and BMI at 6 years. All regression analyses were adjusted for sex, birth weight, and maternal education level categorized as completion of elementary school, high school or vocational school, or university. Changes in growth were calculated from crude differences in measurements at the two different time points. All statistical analyses were carried out using SPSS version 20.0 (IBM Corp., NY, USA). The level of significance was set at $P < 0.05$.

Results

Table 1 shows the participants' characteristics comparing infants at 5 months of age who were exclusively breastfed (n=62), exclusively formula fed (n=12), or started on solids (n=80).

Table 1. Participant characteristics and dietary variables by infant feeding practice at 5 months of age.

	Infant feeding practice at 5 months		
	Exclusively breastfed (n=62)	Exclusively formula fed (n=12)	Solid foods (n=80)
	Mean±SD	Mean±SD	Mean±SD
<i>Maternal characteristics</i>			
Age (years)	31.5±4.5	30.4±4.0	30.7±4.8
BMI (kg/m ²)	23.7±3.2	29.2±9.1	25.3±4.8*
University education [n (%)]	31 (50)	8 (67)	34 (43)
<i>Infant characteristics</i>			
Girls [n (%)]	34 (55)	24 (55)	38 (48)
Exclusive breastfeeding (mon.)	5.0±0.9	1.7±1.7*	2.6±1.7*
Breastfeeding duration (mon.)	9.5±1.9	3.9±2.7*	6.9±3.2*
<i>Food groups introduced at 5 months</i>			
Porridge [n (%)]	-	-	72 (42.1)
Legumes [n (%)]	-	-	4 (8)
Dairy products [n (%)]	-	-	2 (1.2)
Meat, organs [n (%)]	-	-	0
Eggs [n (%)]	-	-	0
Fruits and vegetables [n (%)]	-	-	35 (21)
<i>Dietary composition at 9 months</i>			
Energy (kcal)	733±176	755±114	753±190
Protein (E%)	12±3	14±4*	13±4*
<i>Dietary composition at 12 months</i>			
Energy (kcal)	839±203	818±165	833±171
Protein (E%)	14±3	15±3	16±4*

*Significantly different from exclusively breastfed infants

Maternal characteristics were not markedly different although mothers who exclusively breastfed their child at 5 months had lower BMI. The total duration of breastfeeding was shortest among infants who were formula fed at the age of 5 months but had not been introduced to solid foods at this time point. Among infants who were provided solid foods at

5 months, the food items with the highest frequency of consumption were porridge (42%) and fruits and vegetables (21%). No children were eating meat, fish, poultry, liver, or eggs at 5 months. Although no difference was observed in total energy intake at 9 or 12 months based on infant feeding practice at the age of 5 months, the contribution of protein to total energy intake was higher, as expected, in infants who had been introduced solids foods at both 9 and 12 months of age compared with the group being exclusively breastfed at the age of 5 months.

There were no differences in birth weight between the different infants groups (Table 2). However, infants who had been provided solid foods at 5 months grew faster after birth, particularly between 2 and 6 months of age and these infants were heavier at both 6 and 12 months of age compared to exclusively breastfed infants (Table 3). In a secondary analysis, the group that had been introduced to solid foods at the age of 5 months were split into two groups based on whether the infants were still partially breastfed or not (n=57 versus 23). The analysis revealed faster growth between 2 and 6 months in the group not partially breastfed at the age of 5 months, mean difference 787g (95% CI 472, 1102) compared with exclusively breastfed infants, while the difference was smaller and non-significant for the group on solid foods and partially breastfed (Table 3). The addition of solid foods at 5 months predicted greater BMI at 6 years, with BMI being on average 0.7 kg/m² (95% CI 0.0, 1.3) higher among infants provided solid foods compared to those exclusively breastfed at 5 months of age (Table 4).

Table 2. Anthropometrics from birth by infant feeding practice at 5 months of age.

	Exclusively breastfed (n=62)	Exclusively formula fed (n=12)	Solid foods (n=80)
	Mean±SD	Mean±SD	Mean±SD
Weight (kg)			
At birth	3.8±0.4	3.8±0.4	3.7±0.3
2 months	5.6±0.6	5.5±0.7	5.7±0.5
6 months	7.8±0.8	8.2±1.0	8.3±1.0*
12 months	9.6±0.9	10.3±1.4	10.2±1.8*
18 months	11.3±1.0	11.5±1.2	11.7±1.3
Length (cm)			
At birth	51.9±1.5	52.0±1.7	51.7±1.7
2 months	59.7±1.5	59.4±1.8	59.6±1.9
6 months	68.3±2.0	69.4±2.7	68.9±2.1
12 months	76.2±2.5	77.4±2.9	77.2±2.6*
18 months	83.0±2.5	83.8±3.2	83.7±2.7

*Significantly different from exclusively breastfed infants

Table 3. Changes in weight from birth to 18 months by infant feeding practice at 5 months of age comparing infants on formula or solid foods, Δ (95% CI), to exclusively breastfed infants.

	Infant feeding practice at 5 months				
	Exclusively breastfed (referent) (n=62)	Exclusively formula fed (n=12)	Solid foods (n=80)	<i>Solid foods with partial breastfeeding</i> (n=57)	<i>Solid foods with no breastfeeding</i> (n=23)
Changes in weight (g)	Mean \pm SD	Δ (95% CI) ^a	Δ (95% CI) ^a	Δ (95% CI) ^a	Δ (95% CI) ^a
Birth to 2 months	1859 \pm 500	-188 (-531, 155)	79 (-91, 250)	156 (-31, 343)	-119 (-369, 132)
2 to 6 months	2208 \pm 480	512 (147, 877)*	336 (101, 571)*	161 (-63, 385)	787 (472, 1102)*
6 to 12 months	1775 \pm 550	172 (-193, 538)	170 (-30, 368)	122 (-85, 328)	283 (-8, 574)
12 to 18 months	1649 \pm 563	-56 (-450, 338)	-152 (-352, 47)	-90 (-297, 116)	-314 (-634, 6)
Birth to 6 months	4081 \pm 745	308 (-168, 785)	473 (187, 759)*	360 (58, 661)*	755 (367, 1143)*
Birth to 12 months	5876 \pm 884	510 (-108, 1129)	626 (280, 972)*	462 (92, 831)*	1019 (566, 1471)*

^aMean difference in weight compared to exclusively breastfed infants (referent).

*Significantly different from exclusively breastfed infants.

Table 4. Effects of infant feeding practice at 5 months on BMI at 6 years of age.

	Δ (95% CI) ^a	<i>p</i> [*]
Exclusively breastfed	Referent	-
Formula fed	0.3 (-0.8, 1.3)	0.630
Solid foods	0.7 (0.0, 1.3)	0.034

^aAdjusted difference in BMI at 6 years with respect to exclusively breastfed infants.

^{*}Analyses adjusted for sex, birth weight, and maternal education

Discussion

In this current analysis, we found that infants who had started solids at 5 months of age had faster growth prior to the introduction of solid foods and up to 12 months of age. Differences in weight were most pronounced between the ages of 2 to 6 months. The addition of solid foods prior to the age of 6 months predicted greater BMI at 6 years.

The strength in this cohort lies in the longitudinal nature and the detailed information on infant feeding practices. In this way we are able to describe dietary intake in infancy in greater detail than possible in many other studies in relation to BMI at 6 years of age. It is difficult to separate the effects of exclusive breastfeeding (and total duration of breastfeeding) from the introduction of solid foods at the age of 5 months. However, our findings are in line with existing evidence that show exclusively breastfed infants grow slower compared to infants who have started complementary feeding (2, 6) and that longer duration of breastfeeding may protect against later obesity potentially due to slower weight gain in infancy (6).

There are several factors that contribute to variations in infant feeding practices. Predictors of early introduction of solids include young maternal age, low maternal education and short

(<4 weeks) duration of breastfeeding (17). Interestingly, the shortest duration of both exclusive and total duration of breastfeeding was seen in the group exclusively formula fed at the age of 5 months in the present study, indicating that very short duration of breastfeeding does not seem to predict early introduction of solid food in the population studied. There were no significant differences with maternal age, and maternal education also did not appear to predict introduction of solid foods at 5 months of age in the present study.

We note that the mothers who started their infants on formula feeding had a higher mean BMI compared to mothers of exclusively breastfed infants, although the numbers are too few to detect a significant difference. There is evidence from epidemiological studies that women who are overweight or obese are less likely to breastfeed compared to normal weight women (18, 19).

A probable explanation for the observed difference in growth rate between breastfed and formula fed infants is the relatively higher protein content of infant formula compared to breast milk. High protein intake may have a stimulating effect on insulin-like growth factor 1 which can accelerate growth (20). Furthermore, higher protein intake from complementary feeding is associated with faster weight gain and higher adiposity in infancy (21) and there is evidence that this is associated with greater BMI in childhood (4, 7, 22).

Results from a randomized controlled trial performed in Iceland, showed no significant differences in energy intake (15), growth (14), or risk of being overweight (13) between those exclusively breastfed for 4 versus 6 months. The reason may be a low amount of energy from complementary foods (14, 15) mainly consisting of infant cereals (67%) and median protein intake of only 0.9 g/day (14). In other studies, exposing infants to solid foods prior to the age of 4 months was associated with being overweight or obese in early childhood (23, 24), but it was suggested that the risk associated with the timing of introduction of solid food might be

greater among children who were no longer breastfed (23). In the present study, partially breastfed infants who had been introduced to solid food at the age of 5 months did not grow as fast as the infants who were no longer breastfed at 5 months. Together these findings suggest that in addition to the timing of complementary foods, the type of food, and the protein content of the infant formula introduced (25) may influence infant growth. However, limited data still exists on the effects of specific food groups introduced during the complementary feeding period in related to infant growth and childhood BMI.

In this cohort, 70% of infants in the solid food group were exclusively breastfed at 2 months of age. Mothers may introduce solid foods earlier if they find their infant appears hungry or worry that breast milk is inadequate for their infant's needs (26). Infants who had already started on solid foods at 5 months of age had a small but non-significant tendency towards faster growth from birth to 2 months, especially those who were still partially breastfed at the age of 5 months, and growth differences became significant between 2 to 6 months compared to exclusively breastfed infants. Our findings might indicate a separate possible explanation, that mothers began introducing food earlier to the children as a result of rapid growth as opposed to the growth being a secondary effect of the early solid food introduction. Further studies are needed in this area to determine whether rapid infant growth leads to children demanding more feedings or whether the introduction of solid foods is the main contributor.

The search for simple indicators of a healthy infant diet is ongoing. In 2008, the WHO published indicators of infant and young child complementary feeding practices (27). The dietary diversity indicator was used in the present study to get an overview of the difference solid food groups introduced at the age of 5 months. Although these indicators were based largely on studies in developing countries, they may serve as a useful guideline for future infant studies making comparisons with other countries possible. Knowing when and which food groups are introduced into complementary feeding is important for several reasons. For

example, the late introduction of eggs and nuts is associated with greater risk of food sensitivities (28). Delaying solid food introduction until 17 weeks of age may protect against food allergies (29) while the early introduction of vegetables during infancy is a good indicator of later frequent consumption (30). The type of food item consumed earlier in infancy may play a larger role in influencing later infant growth. Better mapping of the timing of introduction of different food groups during the complementary feeding period will aid to further develop guidelines on infant nutrition.

Some limitations exist in this current analysis. The sample size is a possible limitation however, the thorough data on dietary intake and growth variables provides valuable information and the number is sufficient to analyze differences in infant growth. Information on reasons affecting the duration of breastfeeding would have been useful, particularly among the infants who had been provided solid foods at 5 months to better understand the observed association with BMI at 6 years. Furthermore, the complementary feeding period continues until 2 years of age and it would have been valuable to have dietary records beyond 12 months of age. It is necessary to evaluate the composition of food later in infancy. However, the aim of this present analyses was to determine the growth differences based on infant feeding practices when solid foods were introduced, although even more detailed data related to diet and nutrient composition before the age of 5 months would have been beneficial. It appears that both formula and solid food introduction effect infant growth compared to exclusively breastfed infants.

Conclusion

In this current analysis, we found that infants who started complementary feeding prior to 6 months of age had faster growth during infancy and greater BMI at 6 years of age compared to exclusively breastfed infants. The introduction of solids predicted greater BMI at 6 years

of age. In a developed country where mothers are introducing solid foods prior to 6 months of age, better breastfeeding promotion strategies may help reduce the incidence of childhood overweight. Further studies with more detailed information on infants feeding practices and statistical power are needed to determine the appropriate composition of infant formula and solid foods introduced especially if mothers are for some reasons unable to breastfeed exclusively for 6 months.

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