Neurodevelopment and cardiovascular risk in 7-year old children born with marginally low birth weight

Josefine Starnberg
(Lindberg)
To my unborn child
# Table of Contents

**Abstract** ............................................................................................................................... iv  
Background ................................................................................................................................. iv  
Aim ........................................................................................................................................ iv  
Method ..................................................................................................................................... iv  
Results ....................................................................................................................................... iv  
Conclusions ................................................................................................................................ v  

**Original papers** ................................................................................................................... vi  

**Abbreviations** ....................................................................................................................... vii  

**Enkel sammanfattning på svenska** ...................................................................................... ix  
Bakgrund ................................................................................................................................ ix  
Syfte ....................................................................................................................................... ix  
Metod ....................................................................................................................................... x  
Studiedesign ............................................................................................................................... x  
Datainsamling ............................................................................................................................. x  
Resultat ...................................................................................................................................... x  
Slutsats ...................................................................................................................................... xi  

**Introduction** ............................................................................................................................ 1  
Low birth weight ......................................................................................................................... 1  
Short and long-term health in children born with low birth weight ........................................ 3  
  *Neonatal morbidity* ............................................................................................................... 3  
  *Growth* .................................................................................................................................. 4  
  *Nutrition* ............................................................................................................................... 5  
  *Health consequences beyond childhood* ........................................................................... 6  
Cardiovascular risk following low birth weight ........................................................................ 6  
  *The Barker hypothesis* ........................................................................................................ 6  
  *Accelerated postnatal growth hypothesis* .......................................................................... 7  
  *Metabolic syndrome* ............................................................................................................ 8  
  *Overweight and obesity* ......................................................................................................... 9  
  *Insulin resistance and glucose intolerance* ......................................................................... 10  
  *Blood lipids* .......................................................................................................................... 13  
  *Blood pressure* ..................................................................................................................... 15  
  *Marginally low birth weight and later cardiovascular risk* .................................................. 16  
Neurocognitive development following low birth weight ...................................................... 16  
  *Neurodevelopment* .............................................................................................................. 16  
  *Cognition in low birth weight children* .............................................................................. 18  
  *Early growth and later cognition* ......................................................................................... 19  
Iron – a possible mediator of long term programming ............................................................ 19  
  *Iron – an essential mineral* .................................................................................................. 19  
  *Iron homeostasis* ................................................................................................................ 20  
  *Iron and neurodevelopment* ............................................................................................... 21  
  *Iron and cardiovascular risk* ............................................................................................... 22  
  *Iron requirements in low birth weight children* ................................................................. 22
Conclusions and clinical implication .............................................. 68
  Cardiovascular risk ........................................................................................................ 68
  Early growth and nutrition ............................................................................................ 68
  Iron supplementation .................................................................................................... 69
  Neurocognitive results .................................................................................................. 70

Acknowledgements ......................................................................... 71

References .......................................................................................... 73
Abstract

Background
Being born preterm (<37+0 gestational weeks) or with low birth weight (LBW, <2500 g) has been associated with a number of adverse health outcomes later in life. Most studied are cardiovascular and neurodevelopmental consequences in those born preterm and with very LBW (<1500 g). However, a majority of LBW children are born with a birth weight between 2000 and 2500 g, herein referred to as marginally LBW. The long-term risk profile for this substantially large group of children, is not known.

Aim
The aim of this study was to explore cardiovascular risk and neurocognitive development in marginally LBW children born in Sweden.

Method
This was originally a randomized controlled double-blinded trial aiming to explore the effects of iron supplementation in 285 children born with marginally LBW. The children were randomized to receive 0 mg/kg/day (placebo), 1 mg/kg/day or 2 mg/kg/day of iron supplements between 6 weeks and 6 months of age. As part of this observational follow-up study, 95 matched control children born with normal birth weight (NBW, 2501-4500 g) were recruited former to the first follow-up at 3.5-years. This thesis presents data from a follow-up at 7 years, including anthropometric data, blood pressure (BP), body composition (from a dual-energy X-ray absorptiometry) and laboratory markers of cardiovascular risk such as fasting glucose, insulin and lipid profile. Also, the children were tested using the validated neurocognitive tests WISC-IV (Wechsler Intelligence Scale for Children), Beery VMI (Beery-Buktenica developmental test of visual-motor integration) and TEA-Ch (Test of Everyday Attention for Children).

Results
The marginally LBW children were thinner (15.1 vs 15.5 kg/m², p=0.046), shorter (122.4 vs 124.9 cm, p=0.001) and had a higher prevalence of underweight (10.7 % vs 2.9 %, p=0.050) compared to their NBW peers. In addition, the LBW children had a significantly larger prevalence of high fasting insulin levels (>90th percentile of the control group). The subgroup of children born small for gestational age (SGA) also had a higher mean fasting glucose level, compared to NBW controls. There were no differences in prevalence of overweight or having an adverse lipid profile between the groups. The marginally LBW children who
had received iron supplements, as part of the original intervention trial, had approximately 2 mmHg lower systolic BP, compared to the placebo group (p=0.026). The odds of having a high BP was lowered by 68 % (OR 0.32; CI 0.11-0.96) in the supplemented groups.

The marginally LBW children had 3.1 points lower verbal comprehension IQ (p=0.004), 3.5 points lower Beery VMI (p=0.028) and poorer selective attention compared to those born with NBW.

**Conclusions**

The marginally LBW children were thinner and shorter and they had an imbalanced glucose and insulin homeostasis, particularly those born SGA. Early iron supplements lowered systolic BP to a level similar to controls, suggesting a novel hypothesis regarding a long term protective effect against adverse programming. Finally, the children born with marginally LBW had poorer neurocognitive outcomes, prompting particular attention at school age.
Original papers

This thesis is based on the following papers, referred to in the text by their roman numerals (I-IV).


Paper I and III are reprinted with kind permission from the original publishers.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBW</td>
<td>Low birth weight</td>
</tr>
<tr>
<td>NBW</td>
<td>Normal birth weight</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>IQ</td>
<td>Intelligence quotient</td>
</tr>
<tr>
<td>Beery VMI</td>
<td>Beery-Buktenica developmental test of visual-motor integration</td>
</tr>
<tr>
<td>WISC</td>
<td>Wechsler Intelligence Scale for children</td>
</tr>
<tr>
<td>TEA-Ch</td>
<td>Test of Everyday Attention for Children</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>FMI</td>
<td>Fat mass index</td>
</tr>
<tr>
<td>FFMI</td>
<td>Fat-free mass index</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>IDL</td>
<td>Intermediate-density lipoprotein</td>
</tr>
<tr>
<td>ApoB</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>ApoA1</td>
<td>Apolipoprotein A1</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Glucose transporter type 4</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor-1</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>IGF binding protein 1</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>ID</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td>IDA</td>
<td>Iron deficiency anemia</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High-sensitive C-reactive protein</td>
</tr>
</tbody>
</table>
Enkel sammanfattning på svenska

**Bakgrund**

Barn födda med låg födelsevikt (<2500 g) löper en ökad risk för att drabbas av hjärtkärlsjukdomar senare i livet. Förklaringen bakom detta ligger i s.k. tidig metabol programmering. Man tror att fostret programmeras utifrån den omgivande miljön, något som senare kan bli ofördelaktigt om miljön efter födseln ser annorlunda ut. Till exempel; ett tillväxthämtat foster som utsätts för svält intrauterint anpassar sig och programmeras därefter. När barnet sedan föds och tillgången till näring inte längre är begränsad, finns en diskrepans mellan programmeringen och den faktiska miljön. Detta tror man leder till att vissa individer är mer benägna att drabbas av välfärdssjukdomar som övervikt och fetma, hypertoni, diabetes typ 2 och höga blodfetter, vilka alla ökar risken för att dö i hjärtkärlsjukdom.

De senaste åren har ytterligare studier föreslagit att det snarare är en accelererad tillväxt efter födseln som programmerar den ökade risken. Detta har öppnat upp för möjligheten att idag kunna styra riskprofiler genom att manipulera tidig tillväxt via nutrition. För att kunna ge barn födda med låg födelsevikt de bästa förutsättningarna för ett hälsosamt liv, behöver vi ta reda på mer om vilka barn som riskerar att drabbas och hur deras riskprofiler ser ut.

En annan risk med att födas med låg födelsevikt är att hjärnans utveckling och mognad inte är redo att möta extrauterina omgivningen. Man tror att i ett barn som föds för tidigt (<37 gestationsveckor) eller för litet prioriteras mognaden av de mest vitala organen, som lungor och cirkulationsorgan. Vilka som riskerar att drabbas av nedsatt kognitiv förmåga är ännu inte helt känt.

Hela 60 % av de barn som föds med låg födelsevikt är födda med bara en marginellt låg födelsevikt (2000–2500 g). Det saknas studier på hur det går för dessa barn senare i livet, både vad gäller risken för hjärtkärlsjukdomar och eventuella kognitiva svårigheter.

**Syfte**

Syftet med den här studien var att ta reda på om barn födda med marginellt låg födelsevikt har en ökad risk att drabbas av:

- Övervikt
- Högt blodsocker
- Insulinresistens
• Höga blodfetter  
• Högt blodtryck  
• Påverkad kognitiv förmåga  
• Lägre visuomotorisk förmåga  
• Sämre uppmärksamhetsförmåga

jämfört med barn som är födda med normal födelsevikt (2501–4500 g).

Dessutom undersökte vi huruvida tidigt järntillskott påverkade någon av ovanstående utfall.

Metod

Studiedesign
Den här studien var från början en interventionsstudie vars syfte var att undersöka resultaten av tidigt järntillskott till barn födda med marginellt låg födelsevikt. Barnen rekryterades i Umeå och i Stockholm när de var 6 veckor gamla, och randomiserades till att få placebo, 1 eller 2 mg järn/kg kroppsvikt/dag, upp till 6 månaders ålder.

Inför denna uppföljningsstudie upp till 7 års ålder rekryterades ytterligare 95 matchade kontrollbarn födda med normal födelsevikt.

Datainsamling
Vid 7 års ålder mättes barnens vikt, längd, midjeomfång samt blodtryck och puls. De lämnade blodprov som analyserades för bl.a. glukos, insulin, blodfetter och CRP. Barnen genomgick en DXA-undersökning (dual-energy X-ray absorptiometry) för att närmare kunna analysera kroppssammansättning. De fick också träffa en barnpsykolog som testade dem avseende på

• IQ: WISC IV (Wechsler Intelligence test for Children)  
• Visuomotorisk förmåga: Beery VMI (Beery-Buktenica developmental test of visual-motor integration)  
• Uppmärksamhetsförmåga: TEA-Ch (Test of Everyday Attention for Children)

Resultat
Delvis i motsats till hypotesen så var barnen födda med marginellt låg födelsevikt inte bara kortare (-2.5 cm, p=0.001), utan också smalare (-0.4 kg/m², p=0.046) och hade mindre fett (-0.3 kg/m², p=0.087) och fett-fri massa (-0.2 kg/m², p=0.033), jämfört med barnen födda med normal födelsevikt. De hade dessutom större risk för undervikt (10.7 vs 2.9 %, p=0.050).
Men de lågviktfödda barnen hade däremot i större utsträckning ett högt faste-
insulin (20.6 vs 8.6 %, p=0.038) och dessutom hade de barn som var födda för
små för sin gestationsålder (SGA, small for gestational age) ett högre faste-
blodsocker (4.7 vs 4.5 mmol/l, p=0.020), jämfört med kontrollbarnen.

Tidig viktuppgång korrelerade positivt med senare BMI (body mass index), faste-
blodsocker och ofördelaktiga blodfetter, vilket indikerar att det finns en möjlighet
att påverka senare riskfaktorer med tidiga nutritionsrekomendationer. Dessutom korrelerade amning negativt med blodsocker och ogynnsamma
blodfetter, vilket understryker den tidigare väl beskrivna skyddande effekten av
amning.

När vi undersökte effekterna av att ha fått järntillskott mellan 6 veckor och 6
månaders ålder, så fann vi ett 2.2 mmHg lägre systoliskt blodtryck hos de barn
som erhållit järn jämfört med de som erhållit placebo. Oddsen för att ha ett högt
blodtryck vid 7 års ålder minskade med 68 % hos järnsupplementgrupperna.

De psykologtester som genomfördes visade att de barn som var födda med
marginellt låg födelsevikt hade 3.1 poäng lägre verbalt IQ (p=0.004), 3.5 poäng
lägre visuomotorisk förmåga (p=0.028) samt sämre selektiv och total
uppmärksamhetsförmåga, jämfört med kontrollbarnen.

**Slutsats**

Sammanfattningsvis så löper marginellt lågviktfödda barn vid 7 års ålder snarare
en ökad risk för undervikt än för övervikt. Däremot verkar de ha en rubbad balans
för blodsockerkontroll, framför allt de barn som är födda små för tiden (SGA),
exempelvis p.g.a. tillväxthämnings. Detta kan vara tidiga tecken på en ökad risk
för hjärtkärlsjukdom även i denna för övrigt friska grupp barn. En risk som
möjlig kan manipuleras genom amning och genom att påverka tillväxten.

Järntillskott tidigt i livet verkar ha en skyddande effekt mot att senare drabbas av
högt blodtryck. Detta är ett tidigare okänt samband som möjliggör för ytterligare
strategier för att skydda lågviktfödda barn. Det indikerar också att järn har en
viktig roll i tidig programmering, vilket leder oss närmare att förstå
bakomliggande mekanismer.

Barn födda med marginellt låg födelsevikt har dessutom lägre verbalt IQ, sämre
visuomotorisk förmåga samt påverkad uppmärksamhetsförmåga, vilket kan
resultera i läs- och skrivsvårigheter och påverka skolresultat. Dessa barn bör
därför ges särskild uppmärksamhet i samband med skolstart.
**Introduction**

**Low birth weight**

The World Health Organization defines low birth weight (LBW) as birth weight less than 2500 g. Being born with a birth weight below this cut off is associated with a range of poor health outcomes, both early and later in life. Today, these children accounts for approximately 15 % of all births, ranging from 4 % in developed countries to 16 % in developing countries.\(^1\)\(^2\)

**Figure 1.** Definitions and overlap for being born preterm (<37 gestational weeks) or term (≥37 gestational weeks) vs being born small (<-2 SDS for birth weight) or appropriate (≥ -2 SDS for birth weight) for gestational age (SGA or AGA).

As illustrated in figure 1, newborn infants are divided into partly overlapping subgroups, either according to range of birth weight or, most commonly by gestational age at birth.

**Subgroups of birth weights:**

- Extremely LBW: <1000 g
- Very LBW: <1500 g
- Moderately LBW: 1500-2500 g
- Marginally LBW: 2000-2500 g (the focus of this project)
- Normal birth weight (NBW): 2501-4500 g
- High birth weight: >4500 g

Subgroups of gestational ages:

- Preterm: <37 gestational weeks, which can be further divided into:
  o Late preterm: 34+0 to 36+6 gestational weeks
  o Moderate preterm: 32+0 to 33+6 gestational weeks
  o Very preterm: 28+0 to 31+6 gestational weeks
  o Extremely preterm: <28 gestational weeks
- Term: 37+0 to 41+6 gestational weeks, which can be further divided into:
  o Early term: 37+0 to 38+6 gestational weeks
  o Late term: 41+0 to 41+6 gestational weeks
- Post term: >42 gestational weeks

Depending on intrauterine growth, children can be grouped according to being born small or appropriate for gestational age (SGA and AGA), mostly defined as having a birth weight of ≤-2 standard deviations (SD) or above, respectively. Moreover, children born SGA can be further divided into intrauterine growth restricted (IUGR) children, or being constitutionally born SGA.3-4 IUGR is more common and is a pathological condition caused by maternal, fetal or placental deficiencies during pregnancy.

Consequently, LBW children is a heterogeneous group, including a wide range of gestational ages and birth weights, which further on leads to a range of different long-term challenges. The last decades, research have mostly been focusing on extremely preterm birth or children born with very LBW. However, a vast majority (more than 60 %) of all LBW children born in Sweden today are born with a birth weight between 2000 and 2500 g (figure 2). We have defined this group as marginally LBW children.2 The long-term consequences these children encounter is not entirely known.

**Summary:** LBW is a birth weight <2500 g, normally due to preterm birth or being born SGA, the latter often caused by IUGR. The majority of LBW children are born only with marginally LBW (2000-2500 g).
Figure 2. Distribution of birth weights in Sweden.

Short and long-term health in children born with low birth weight

Following the increased survival rates of preterm and LBW children, research regarding short and long-term health outcomes has earned increased interest during the last decades.

Neonatal morbidity

Children born with LBW and/or born preterm are at increased risk of multiple adverse perinatal outcomes, owing to immature organs and systems that are not able to meet the demands of extraterine life. Development and maturation of organs and tissues are continuous during early life and are dependent on several factors such as gestational age at birth and maternal nutrition, health status, lifestyle and medications. These early life events are not only important for the immediate health consequences, but may also have long-term impact since studies have shown that postnatal morbidity is associated with increased risk of later childhood morbidity and even mortality.

Some of the most common early complications following LBW are:

- **Hypothermia**: a condition more common in LBW and premature children due to immature thermo-regulatory systems, such as the hypothalamic function. In addition, these children have less subcutaneous fat and brown adipose tissue, which is an important site of heat production in newborns.
• **Respiratory conditions**: respiratory distress syndrome is caused by deficient surfactant, which is needed for pulmonary compliance and to prevent alveolar atelectasis. Another condition is transient tachypnea of the newborn, caused by failure to clear the alveolar space from fluid. Both conditions are more common in those born preterm and even in infants born early term.⁵

• **Hypoglycemia**: infants born preterm or with LBW have lower amounts of hepatic glycogen stores and more difficulties in lowering the insulin levels postnatally due to immature regulatory systems, which leads to problems in maintaining a vital glucose level. Also, these children more often have feeding problems, another risk factor for hypoglycemia.⁵

• **Jaundice**: postnatally, there is a rapid red blood cell (RBC) turnover, caused by a shorter life span in fetal RBCs and a relative polycythemia in neonates. The breakdown of RBCs and hemoglobin results in the degradation product bilirubin, which needs to be conjugated by hepatic enzymes and excreted in the bile. In newborns, and especially in children born preterm, the hepatic enzymes and excretion system is immature and cannot meet the demands, leading to hyperbilirubinemia and jaundice, which in turn can be neurotoxic and lead to brain damage.⁹

**Growth**

Growth of infants and children are normally divided into different phases including the intrauterine, infancy, childhood and adolescent growth phase. These phases will have distinct characteristics for growth pattern and regulation depending on gestational age, birth weight and potential growth restriction.¹⁰

Children born preterm usually experience an initial growth restriction, suggested to be caused by the immature organs and an inadequate nutritional intake.⁴, ¹¹ After this initial growth faltering, healthy preterm born children, and especially those born after 32 gestational weeks, usually fall back to the reference growth curve, following that of term born babies.¹² For children born SGA, 80% will experience a relative catch-up growth within the first 6 months of life.⁴ However, infants born SGA still have an increased risk of remaining growth restricted later in life, both in height and in weight.¹³ In addition, the children born SGA who are also born preterm will have even more difficulties in catching up and will more likely remain short throughout adulthood.⁴, ¹³, ¹⁴

Of particular interest in the matter of early growth research, is to determine how these growth traits can be affected by environmental factors, particularly
nutrition. The goal is to find the ideal growth standards for each individual, to be able to give the newborn child the right preconditions to reach his or her optimal potential without increasing the risk for adverse events. This balance is a current challenge to pediatric research.

**Nutrition**

Breastmilk includes all the nutritional requirements for a healthy term born infant and has also been associated with other beneficial outcomes such as improved neurodevelopment and a decreased risk of infectious diseases.\(^{15-17}\) Likewise for LBW children, breastmilk is recommended due to the established short and long-term effects. However, depending on gestational age and birth weight, these children may need additional supplementation. For instance, LBW children have low iron stores and require iron supplementation since the breastmilk cannot meet those demands.\(^{18, 19}\) For very LBW children it is also recommended to add vitamin D, phosphorous and calcium. The smallest and most premature babies often require feeding support, for example being fed by gastric tubes or parentally.\(^{20}\)

Breastfed and formula fed infants have different growth pattern. Breastfed infants are leaner and shorter than formula fed children at 12 months of age, a pattern probably derived through lower protein and total energy intake in breastfed infants than in those fed formula.\(^{15}\) It has been suggested that the differences in growth pattern, contributes to a protective effect against later development of obesity in breastfed infants compared to those fed formula.\(^{21}\)

Apart from the effect on later overweight, early nutrition also affects the brain development. According to a meta-analysis, breastfeeding has positive effects on later cognitive outcomes, detectable from 6 months of age and persistent until adulthood.\(^{16}\) The correlation is dose-dependent, with higher cognitive scores in children breastfed for longer duration. The magnitude of the difference in cognitive performance were even larger for children born with LBW, suggesting that LBW children benefit even more from breastfeeding. The mechanisms behind these neurocognitive effects are unknown. However, one possible mechanism could be the fat content in the diet. Human breastmilk provides about 50% of the energy as lipids, which enable growth and fat-soluble vitamin uptake.\(^{22}\) The most important lipids are omega-3 and 6 fatty acids, both balanced in the breastmilk according to the infant’s needs. The high level of n-3 has for instance been suggested to explain the better cognitive functions observed in breastfed infants, since it accumulates in the membrane of neurons during infancy.\(^{23}\)
**Health consequences beyond childhood**

The last decades, long-term consequences of being born preterm or with LBW have been studied. The most studied outcomes are the risk of cardiovascular disease (CVD) and neurodevelopmental consequences. Being born with LBW has been associated with increased risk of overweight, and especially larger proportions of abdominal fat mass, high BP, glucose intolerance and insulin resistance, conditions that increase the risk of CVD.\(^{24-26}\) Today, cardiovascular events are one of the most common causes of death world-wide, and there is an increasing need for early prevention. In addition, LBW has been associated with behavioral problems, such as attention difficulties, executive functions and cognitive problems.\(^{27-29}\) This could not only affect school achievement, but also mental health status, socioeconomic status as well as affect social skills.

**Summary:** Children born with LBW or preterm are at increased risk of postnatal morbidity. Apart from the immediate consequences, there are evidence suggesting that also long-term health could be compromised and that they for instance present with an increased risk of CVD and neurocognitive deficiencies.

---

**Cardiovascular risk following low birth weight**

**The Barker hypothesis**

In 1977, Forsdahl found an association between the regional rate of death caused by arteriosclerotic diseases and rates of infant mortality 40-70 years earlier.\(^{30}\) Following this, David Barker and colleagues observed an inverse correlation between birth weight and systolic BP in 10-year-old children and in 36 year old adults, independent of current body weight.\(^{31}\) In another study they found epidemiological evidence suggesting that fetal and postnatal health is associated with later cardiovascular risk.\(^{32}\) These results gave rise to the Barker hypothesis, which is based on the idea that events or insults during critical periods in early life can effect long-term metabolism through *early programming*. Later in life, this programming could affect the risk of developing non-communicable diseases, such as stroke and ischemic heart disease or other CVDs (figure 3). The programming events are believed to take place during critical windows when the cells and organs exhibit plasticity and adapt their phenotypic features according to environmental requirements, i.e. according to nutritional intake.

For instance, the adaption of a child exposed to IUGR due to nutritional deprivation, programs the fetus to manage scarce conditions. After being born
SGA, the infant will most likely experience a greater catch-up growth postnatally, compared to an infant born AGA. When these children are exposed to an abundance of nutrition postnatally, it will lead to an accelerated growth. Thus, there is a discrepancy in the intrauterine environment and programming and the extraterine conditions, making these children prone to adverse developmental programming.25

The Barker hypothesis laid ground for a large field of research that has been given lots of focus during the last decades. However, there are still unanswered questions. This is mainly due to difficulties in establishing causal relationships, since the risk of CVD is multifactorial. Also, due to obvious ethical dilemmas, the possibilities to perform randomized controlled trials (RCTs) are limited. In addition, there seem to be different risk panoramas depending on gestational age, degree of IUGR, birth weight, etc., with different response to growth in and extrauterine, creating a complex picture to grasp.

---

**Summary:** LBW children are at increased risk of developing non-communicable diseases such as CVD. The mechanisms are based on the theory that early plasticity of organs and tissues, adapt to scarce intrauterine conditions. Postnatally, this programming might be a mismatch to extrauterine conditions, leading to an increased risk of adverse metabolic programming.

---

**Accelerated postnatal growth hypothesis**

It has been suggested that not only the intrauterine conditions, but also the early growth trajectories correlates to long-term health within several aspects, including the risk of overweight/obesity, cardiovascular risk, neurodevelopment and short stature.4, 33 These observations suggest an opportunity to affect the health outcomes with nutritional interventions. Of particular interest are the clinical studies that have revealed that preterm and LBW infants with greater early postnatal weight gain seem to be at increased risk for later adverse cardiovascular outcomes, such as insulin imbalance and endothelial dysfunction.34, 35

This theory, *the accelerated postnatal growth hypothesis* or *the growth acceleration hypothesis*,36 suggests that the correlation to small size at birth might just be a proxy for early postnatal weight gain, which would be the main component generating the association to later cardiovascular risk. The founders of the theory propose that the critical windows suggested during intrauterine life,
is rather a continuum of a sensitive period all through early organ and tissue maturation (figure 3).\textsuperscript{36}

Accelerated growth or excessive catch-up growth in infancy is thus associated with an increased cardiovascular risk. On the other hand, insufficient catch-up is associated with other adverse outcomes, such as impaired neurocognition.\textsuperscript{37} Different subgroups of children born with LBW present different risk panoramas and there is still a gap of knowledge for caretakers to be able to tend to these children in an optimal way.

**Figure 3.** A schematic picture of how early fetal programming and postnatal accelerated growth can affect later risk of CVD.

**Summary:** Different pre- and postnatal growth pattern affect later metabolic outcomes. *Accelerated postnatal growth* in children born SGA or preterm is believed to increase the risk of CVD, however, growth faltering is associated with impaired neurocognition. Thus, the optimal postnatal growth within different subgroups of LBW is yet not entirely known.

**Metabolic syndrome**

Metabolic syndrome is a condition of overweight (particularly central adiposity), hypertension, dysglycemia (resulting in glucose intolerance and insulin resistance) and dyslipidemia (high levels of cholesterol, triglycerides and low-density lipoprotein [LDL], low levels of high-density lipoprotein [HDL]). The
name metabolic syndrome describes the trend of these risk factors to be clustered rather than occurring alone. The condition is strongly associated to an increased risk of type 2 diabetes mellitus (T2DM) and CVD.\textsuperscript{38}

The exact mechanisms behind the clustering and the risk of developing CVD, is not entirely clear. There is an abundance of suggested mechanisms behind the pathological process in the metabolic syndrome, including structural changes in organs, changes in cellular transporter expression and epigenetic mechanisms.\textsuperscript{39} The tendency to cluster is seen both in children, adolescents and adults, and it has been suggested that the syndrome is underdiagnosed in children.\textsuperscript{40} There is still no accepted method or criteria for diagnosing children with metabolic syndrome, limiting the possibilities for the condition to be confirmed.

**Summary:** Overweight, hypertension, dysglycemia and dyslipidemia have a tendency to cluster and form a condition called metabolic syndrome, which increases the risk of CVD. The clustering is seen in children as well as in adults and mechanisms behind is partly unknown.

**Overweight and obesity**

Overweight and obesity in childhood is an increasing problem worldwide. Today it is estimated that approximately 24 \% of children in developed countries are overweight or obese, whereas the corresponding prevalence in developing countries is 13 \%.\textsuperscript{41} In 2010, overweight or obesity alone was estimated to cause over 3 million deaths worldwide, numbers that increased when combined with other risk factor causing ischemic heart disease.\textsuperscript{42}

Overweight and obesity tend to track from childhood until adulthood, which is of big concern since prolonged overweight generates an even larger risk to develop CVD.\textsuperscript{43, 44} Treatment of overweight and obesity in children has shown to be challenging. To be successful, the intervention must permeate the environments that the child is exposed to, ranging from the school to the household and last for 6-12 months.\textsuperscript{45, 46} Hence, the need for early prevention in children at risk is crucial.

**Low birth weight and overweight**

Several studies have shown that LBW, and especially when followed by accelerated postnatal growth, associates to an increased risk of overweight and obesity in childhood and adulthood.\textsuperscript{47} Also, those born with LBW have an increased proportion of abdominal fat tissue, which is more associated to
cardiovascular risk than peripheral fat is. However, the results vary with different study designs and settings. For instance, the authors of a meta-analysis recently questioned the causality and found that LBW was associated with lower or no risk of obesity and that the opposite association was only seen in studies with smaller sample size and lower quality.

Several mechanisms have been suggested in the studies that found an increased risk. For instance, an abnormal regulation of leptin, which is a hormone that sends signals to the brain regarding the current energy status of the body. It is mainly regulated and secreted from adipose tissue. Eating is followed by increased levels, which have direct effect on centers in the brain further signaling satiety and promoting energy expenditure. Early in life, factors such as IUGR and breastfeeding, have shown to affect the leptin regulatory effect, which could lead to changes in regulating body weight later in life.

**Summary:** The high prevalence of overweight among children is an increasing global health problem. The need for early prevention is crucial. Being born with LBW could be a risk factor for overweight and obesity, possibly modifiable by early growth patterns – but previous data are diverging, probably due to different settings.

**Insulin resistance and glucose intolerance**

Insulin resistance and glucose intolerance is closely associated with overweight and obesity, both conditions increasing the risk of developing T2DM and CVD. This association is also seen in children, where greater obesity increases the risk of glucose intolerance. Figure 4 represents the normal process taking place when the glucose level rises in the blood stream. When plasma glucose increases, the beta-cells in the pancreas release insulin.

**Figure 4.** The normal process of glucose and insulin homeostasis.
Insulin, which is an anabolic hormone, reach the intercellular space through transendothelial transport. The main site of action is liver, muscle and adipose tissue, where it facilitates glucose uptake, promote glycogen storage as well as synthesis of triglycerides and proteins. In addition, insulin has a hemodynamic role, possibly derived by nitric oxide (NO), which cause vasodilation.

Insulin resistance is believed to be one of the key mechanisms to develop arteriosclerosis, which is the underlying cause to events such as ischemic heart disease and stroke. A range of different risk factors and conditions, such as overweight, lifestyle factors, diet, physical activity, genetic variance and more, play a part in developing insulin resistance. For instance, with obesity comes an abundance of lipid storage and large adipocytes, especially in the visceral and central fat depots. The enlarged size of the adipocytes causes impaired cellular uptake, resulting in deficient insulin action. Lack of intracellular insulin further on results in lipolysis and release of free fatty acids (FFA) and at the same time, cause a dysfunction in glucose uptake, which in turn leads to hyperglycemia. Hyperglycemia has been suggested to cause toxicity derived by reactive oxygen species (ROS), which is proinflammatory, further adding to the cellular and vascular damage. In addition, inflammation and excess of FFA starts the process of atherosclerosis, which inhibit transendothelial uptake of insulin, resulting in impaired hemodynamic regulation (figure 5).

![Figure 5](https://example.com/figure5.png)

**Figure 5.** The development of insulin resistance in adipocytes following obesity.

*Low birth weight and later glucose and insulin imbalance*

There is an abundance of studies that have confirmed the association between poor fetal growth and later impaired insulin sensitivity. Evidence point towards IUGR being the most pronounced risk factor. Although, this does not
seem to be the case in all subgroups and populations. In a study of adults born with very LBW, no difference in glucose homeostasis between children born SGA and AGA was observed. However, the authors did confirm a higher insulin secretion, possibly driven by poorer insulin sensitivity, in the overall LBW group compared to controls. Due to the lack of difference between the LBW children born SGA and AGA, the authors suggested that the abnormal insulin sensitivity could be derived by postnatal factors, such as stress or growth, which supports the theory of early accelerated growth. Also supportive of this theory is a RCT in which preterm infants were assigned standard term formula, enriched preterm formula or breastmilk. The enriched diet resulted in larger weight gain and at the follow-up at 13-16 years of age, fasting 32-33 split proinsulin levels (a form of proinsulin that could indicate greater insulin resistance) was higher in those who received nutrient-enriched diets.

Several mechanisms initiating the observed glucose and insulin imbalance following LBW have been suggested. In a review from 2012, Martin-Gronert and Ozanne discussed the main mechanistic components so far revealed. One of them is a reduced number of GLUT4 (glucose transporter type 4), which have been observed in muscle and adipose tissue following IUGR in both animal and human studies (figure 6). The reduction of glucose transporters results in an impaired glucose uptake, which might cause elevated levels of plasma glucose and hyperglycemia. In addition, a reduced number of beta-cells in the pancreas have been observed after IUGR, which might lead to insulin imbalance and also result in hyperglycemia. Another factor that could play a part is insulin-like growth factor 1 (IGF-1), a protein that mediates the effect of growth hormone. Lower levels of IGF-1 have been observed in children born SGA as well as in adults with CVD.

**Summary:** LBW is associated with greater risk of developing insulin resistance and glucose intolerance, which is one of the key mechanisms in developing CVD.
\textbf{Blood lipids}

Elevated levels of triglycerides and LDL (or Apolipoprotein B, [ApoB]) and lowered levels of HDL (or Apolipoprotein A1, [ApoA1]) are three markers included in the “lipid triad”, a concept often used when discussing metabolic risk. These are all related to atherosclerosis and CVD, a risk that increases with prolonged exposure.

The atherogenic lipoproteins (LDL, very low-density lipoprotein [VLDL], intermediate-density lipoprotein [IDL]) are more prone to penetrate the vessel wall, where they bind to proteins in the extracellular matrix and triggers macrophages and inflammation. This process is the pathophysiological process that causes endothelial damage and atherosclerosis. Furthermore, HDL is an anti-atherogenic lipoprotein that can bind to the vessel wall and remove fat molecules from cells, thereby protecting them from excessive storage. On every atherogenic lipoprotein, there is a protein called ApoB, while every HDL particle contains ApoA1. Therefore, measuring these proteins correlates well to the lipid profile.

Adipocytes in overweight and obese individuals become enlarged and saturated with lipids, which leads to impaired insulin sensitivity and release of FFA in the blood (figure 5 and 7). The elevated levels of FFA further on result in an increased flux of FFA to hepatocytes in the liver, as well as increased storage in both the hepatocytes as well as in myocytes in muscle tissue. The FFAs in the liver are esterified to VLDL and IDL and released into the blood stream. The raised fat storage in the hepatocytes and myocytes damage the insulin sensitivity, further progressing the insulin resistance.
Figure 7. Process of lipolysis in adipocytes without and with insulin resistance, the latter leading to endothelial damage.

Low birth weight and later lipid profile
Though less studied compared to insulin resistance, children and adults born with LBW have been suggested to have an adverse lipid profile, compared to those born with NBW, a correlation accentuated with increasing age. However, the evidence is scarce, especially from prospective studies, and systematic reviews have not yet established a consensus.

Children born with LBW have an increased risk of central adiposity and overweight. According to the theory of metabolic programming and the accelerated growth hypothesis, the intrauterine or early postnatal environment affect adipogenesis, although the exact mechanisms are unknown. The adipogenesis, both with regard to function and number of cells, is sensitive to insulin levels, IGF-1 and other hormones. Altered levels of these factors could therefore lead to abnormal development of adipocytes during early life.

Summary: An adverse lipid profile refers to elevated levels of LDL, cholesterol and triglycerides and lowered levels of HDL, resulting in a high ApoB/ApoA1 ratio. This condition contributes to the development of insulin resistance. Whether the risk of dyslipidemia is elevated in LBW children is still unknown.
**Blood pressure**

The exact underlying mechanisms to why high BP evolves as a part of metabolic syndrome, is not exactly known. However, high BP is estimated to be the number one risk factor for global disease burden, and caused almost 10 million deaths in 2010, a number that is increasing.42

One underlying contributing factor might be the endothelial dysfunction that follows the process of insulin resistance and atherosclerosis, including the impaired vasodilation signaling (figure 6 and 7). In addition, reports from animal and human studies have suggested that there is an increased activity of the peripheral sympathetic nervous system in obese subjects. This has been suggested to be derived by enhanced signaling of norepinephrine as well as an increased sensitivity of adrenergic receptors, in response to high-caloric intake, hyperinsulinemia, hyperglycemia, elevated levels of FFA and high leptin levels, resulting in vasoconstriction.64 Visceral adiposity leads to physical compression and stress to the kidney cells, resulting in impaired function and eventually, glomerulosclerosis, permanent renal damage and consequently hypertension.

**Low birth weight and later blood pressure**

A lot of studies have confirmed a negative correlation between birth weight or gestational age and later BP, an association that have been suggested to enhance over time.26, 31, 61 For instance, one cross-sectional national study from the US showed an inverse association between birth weight and BP in children and adolescents (6-15 years of age). The authors found that for girls, every 1000 g decrease in birth weight increased the systolic BP with 1.4 mmHg.65 Another study found that birth weight Z-scores correlated negatively with systolic BP at 5 years of age, with the highest BP observed in SGA born children with fastest postnatal weight gain.66

In a RCT of enriched formula vs standard formula or breastmilk to preterm born infants, those fed with nutrient-enriched diets had the greatest weight gain in infancy. At the follow-up at 13-16 years of age, those fed breastmilk (the group that also had lower daily weight gain) had lower diastolic (62 vs 65 mmHg) and mean arterial pressure (82 vs 86 mmHg), compared to those fed enriched preterm formula.67 This supports the *early accelerated growth hypothesis* – that accelerated weight gain early in life might be harmful in the aspect of cardiovascular risk.

A number of different mechanisms have been suggested.68 Some of them are an increased response to vascular constrictors (angiotensin II and phenylephrine), impaired response to vascular dilators (NO), arterial stiffness due to impaired elastin synthesis within the vessel walls or a decrease in number of nephrons in the kidney. Another possible underlying mechanism could be the impaired
placental function in IUGR fetuses, failing to inactivate glucocorticoids which would result in increased levels of glucocorticoids to the fetus. This would program the hypothalamic-pituitary-adrenal axis and results in increased levels of cortisol in adulthood.\textsuperscript{68}

\begin{center}
\textbf{Summary:} High BP is a major risk contribution to global burden of disease. There are evidence suggesting that LBW increases the risk of high BP, but the exact mechanisms and risk panorama are still unclear.
\end{center}

\textit{Marginally low birth weight and later cardiovascular risk}

Most studies of early programming focus on very LBW or extremely LBW, even though the majority of all LBW children are born only with marginally LBW.\textsuperscript{2} In Sweden, this accounts for over 2200 children every year. What these children potentially are at risk of is still unclear. Some studies have shown an inverse relationship between birth weight and cardiovascular risk, suggesting that also marginally LBW would be a risk factor, although lower. However, the pathogenesis behind CVD is multifactorial, and for health care providers to be able to assess the risk of each individual, we need to know more about this common subgroup.

\textit{Neurocognitive development following low birth weight}

\textit{Neurodevelopment}

Knowledge regarding normal or healthy brain development is essential to understand the consequences of early insults to the central nervous system (such as stress, malnutrition or illness). The development of the brain begins early in embryogenesis, emerging from progenitor cells called neuroepithelial cells, located in the outermost tissue layer already at 16 days of gestation. From here, neurons and glial cells are formed and migrate to their predefined locations, guided by neurotrophic factors. The neurons reach their proliferation peak as well as their final location at the 16\textsuperscript{th} week of gestation.\textsuperscript{69} By then, the neurons branch and extend dendrites and axons, reaching other locations to receive and conduct signals between regions of the brain. To enable signalling, synapses are shaped at the dendrites and axonal trees. Before birth, a programmed cell death takes place, eliminating approximately 50 \% of all the neurons for morphological rearrangements to take place.\textsuperscript{70} After birth, the brain continues to develop while functional abilities advance. Myelination of the neurons takes place from approximately the 24\textsuperscript{th} gestational week and continues during childhood. This enables the neurons to function properly.\textsuperscript{69}
At birth, important subcortical regions in the brain as well as larger cortical structures, are definable in imaging studies. However, large part of the cortex is still immature. An increase of the activity level (glucose uptake) in different regions occur at different time points and is believed to mark functional development and maturation. The subcortical regions peak at the time of birth, such as the thalamus and brainstem. Basal ganglia, parietal, temporal and occipital cortex matures at around 3 months of age, regions that for instance control sensory and motor functions. Frontal cortex peaks at around 6 months, a region controlling more complex functions such as memory and cognition. Regions with the most complex functions matures during longer periods of time, for instance the synaptogenesis in the visual cortex compared to the prefrontal cortex, of which the latter takes longer time to mature completely.\textsuperscript{71, 72}

\textbf{Figure 8.} The cortical regions of the brain and what it controls. During development, the most complex cortical regions takes longer time to mature, making them vulnerable for a longer period of time.

The brain continues to mature during childhood and adolescence. From age 7-15, a second synaptic elimination and rearrangement takes place, resulting in loss of 40\% of synaptic density, with the same regional order as the maturation at birth, leaving the complex cortical regions until last (figure 8).\textsuperscript{70} The cortex of the brain is the outermost layer containing neuronal bodies, called the grey matter, which is folded in gyri and sulci to fit as much area possible. Beneath the grey matter is the white matter, in which the axonal tracts are located. Like other structures, cortical regions mature at different pace, and some studies have shown a continuous structural change up until 60 years of age, for instance in the frontal region.\textsuperscript{72} Hence, the development and maturation of different regions and functions in the human brain is a dynamic and ongoing process, reaching beyond childhood.
**Summary:** The development of the human brain starts days after conception and continues throughout adulthood. The cortex, containing complex cognitive functions, develops and matures last. Insults during different periods of development would result in different consequences.

**Cognition in low birth weight children**

LBW and preterm birth, especially the smallest and the most preterm born babies, are at risk of cognitive deficits later in life. Apart from direct injuries to the central nervous system, such as intraventricular hemorrhage or periventricular leukomalacia, these children are also at increased risk of more diffuse impairments. The risk of injuries and adverse neurocognitive deficits increase with decreasing gestational age and birth weight.

Different subgroups of preterms and LBW children are faced with somewhat different challenges. In a meta-analysis of adolescents born with low or NBW, the authors found approximately 5 points lower IQ in those born with LBW, without further exploring the different LBW subgroups. Furthermore, poorer cognitive abilities within several aspects, such as IQ, visual-motor integration, attention control, executive functions and behavior, resulting in disadvantage in academic achievement, have been observed in studies of extremely preterm children (born before gestational week 28) and children born with very LBW (<1500 g at birth). Likewise, one prospective cohort study that compared children born only moderately preterm (mean birth weight of 2239 g) to those born at term found that total IQ, performance IQ, visuospatial reasoning, attention control and inhibition were significantly poorer in those born moderately preterm, also after adjusting for parental education. In addition, according to a review of Baron et al., those born late preterm are at risk for long-term neurological consequences. These results are in consistency with findings from other reviews of children born moderate-late preterm. Consequently, being born with LBW associates to an increased risk of neurocognitive deficits, although the possible impact of being born only with marginally LBW is not yet known.

**Summary:** Preterm and LBW children are at increased risk of poorer intelligence, attention and behavioral problems as well as compromised school achievement. However, studies mostly focus on those born very preterm or those with very LBW, and does not always consider the influence of socioeconomic factors. The possible risk of poorer neurocognitive outcomes in marginally LBW children is not entirely known.
Early growth and later cognition

Although there are somewhat inconsistent results in the literature, early growth faltering is a risk factor for poorer cognitive development, while breastfeeding is a protective factor. Some clinical studies have recently shown that enhanced nutritional intake in children born very preterm or with very LBW resulted in improved brain maturation including both gray matter areas as well as connectivity in white matter structures at term equivalent age. However, when studying the later neurocognitive outcomes, results vary. A recent review from Ong et al concluded that in most observational studies there was a positive correlation between postnatal growth and later neurocognitive results in preterm infants. However, these results were not reproducible in clinical trials. Another review found the same results when studying children born AGA. A review of postnatal growth on later neurocognitive outcomes in children born SGA at term found no effect on neurocognition in RCTs promoting faster weight gain. However, similar to other reviews, an association indicating positive effects of early postnatal growth on neurodevelopment was repeatedly shown in observational studies reviewed.

On the other hand, a large observational study looking at early growth in term born children found that growth faltering from birth to 9 months of age was associated with poorer cognitive results later in life, with the most sensitive period being the first 8 weeks.

To summarize, early growth is important for neurodevelopment in LBW and preterm children, especially in those born SGA. However, the exact impact and effects later in life of early accelerated growth is not entirely clear. Growth faltering might lead to adverse neurocognitive consequences. This emphasize the need of further knowledge to be able to promote the ideal postnatal growth for each individual to reach their optimal cognitive ability.

Summary: Early growth faltering have negative effects on neurodevelopment, although the effect of accelerated growth is not known. To be able to promote the most optimal balance for each individual child, more knowledge is needed.

Iron – a possible mediator of long term programming

Iron – an essential mineral

Iron is essential for health, growth and development. The mineral is crucial for oxygen transport in the blood, for DNA synthesis and for electron transport. Approximately 66 % of all the iron is found in hemoglobin, the oxygen carrying protein in RBCs.
Iron deficiency (ID) is the most common micronutrient deficiency, affecting more than 2 billion people worldwide. Symptoms of ID and ID anemia (IDA) are non-specific and rarely correlates to the degree of deficiency. Clinically, patients may present with fatigue, breathlessness, palpitations, dizziness, headache and restless legs. Biochemical markers are often used to evaluate the iron level. However, there are no well validated ways to assess iron status in children, and especially not in infants due to their rapid growth and cell turnover. ID progresses in three stages; iron storage depletion, ID and IDA. In the second state, organs and tissues might already be suffering consequences by the low iron availability, which is why there is a need for diagnostic tools to identify the earlier stages.

ID commonly occurs in pregnant women and preschool children living in low and middle income countries. In developed countries, one of the largest risk groups for ID are infants born with LBW. Therefore, iron is of particular interest when exploring possible nutritional factors in infancy that may affect long-term health in LBW children.

**Summary:** Iron is a crucial mineral for development and health. ID is the most common micronutrient deficiency. Those born with LBW are a risk group for developing ID, which could affect their long-term health.

**Iron homeostasis**

Iron is available for uptake in the gastrointestinal tract as heme-bound iron (meat) and non-heme iron (vegetables and plants), with bioavailability of both being low, 12-25 % and <5 % respectively. The regulation of iron uptake is not entirely known, but the regulatory system is believed to be mature after approximately 6 months of age.

After the enterocytes in the gastrointestinal tract have absorbed iron molecules, further transport to the blood stream is mainly regulated by the peptide hepcidin, which is produced by the liver as iron levels are sufficient. Hepcidin inhibits the transport protein ferroportin, placed on the membrane of enterocytes and other iron storage cells, resulting in inhibition of iron flux to the blood stream. If hepcidin is not present to inhibit ferroportin, iron is transported to the blood stream. Here, iron is bound to transferrin, a transport protein with a high affinity to the transferrin receptor located on the surface of developing RBCs. The uptake and utilization of iron in these precursor cells are regulated by erythropoietin (EPO), a hormone produced by the kidney in response to increased oxygen demands. Each RBC contains approximately 300 million hemoglobin molecules, build up by four heme fragments each, all with an iron atom enabling the molecule to carry oxygen (figure 9).
Summary: Iron regulation matures at approximately 6 months of age. The regulation is only partly known and controlled by multiple enzymes and systems in the body.

Iron and neurodevelopment
Iron is essential for brain development. Several previous studies have reported that ID in infancy is associated with impairments to the central nervous system, resulting in poorer cognitive function and school achievement, attention deficits, behavioral problems and lower muscular strength and mass, although support from clinical trials are limited. Iron is foremost prioritized to RBCs, at expenses of other tissues, for instance the brain. Human studies have shown a significant decrease of iron levels in the brain after IUGR, a condition leading to a reduction of total iron stores. Other neurophysiologic human studies have found slower neurotransmission after ID in infancy. The degree of negative effects of ID depends on the timing and the severity of the condition during brain development. A critical window of vulnerability of different regions in the brain has been suggested. The more severe ID and the longer duration, the lower the chances are to recover iron stores in the brain and restore function.
Suggested origins to these impairments mostly come from animal studies and include altered neurotransmission, changed dendritic structure and impaired myelinisation. These changes have not been completely reversed despite treating the deficiency with iron supplements, indicating that ID early in life could lead to irreversible impairments.

**Summary:** Iron is essential for brain development. ID in infancy could lead to irreversible impairments such as altered motor function, behavioral problems and poorer cognitive abilities.

**Iron and cardiovascular risk**

Even though the main reason for avoiding ID is related to neurocognitive impairment, there are several previous reports regarding iron intake or iron status and the correlation to cardiovascular risk. A cross-sectional international study showed an inverse relationship between non-heme iron intake and systolic BP in adults, even after adjusting for confounders, indicating a protective effect of iron intake. In addition, intravenous iron supplementation to patients with coexisting chronic heart failure and ID have shown to improve quality of life parameters, exercise capacity and reduce hospitalization. Also, animal studies of dams to iron restricted rats have shown that the offspring have an increased systolic BP, persisting into adulthood, suggesting that iron might have a part in the early programming of later cardiovascular risk.

As summarized by Lapice in a systematic review, both ID and iron overload have been associated with increased risk of CVD, and the authors emphasize the risk of confounding in the complex multi-factorial risk panorama of cardiovascular health. Hence, as of today, the role of iron and its impact on cardiovascular risk is not entirely known, and more studies, especially clinical trials, are needed.

**Summary:** The role of iron in the pathogenesis of CVD is not yet known, although there are several previous studies that suggest a correlation.

**Iron requirements in low birth weight children**

Iron requirements in a newborn is approximately 10 times higher per kg body weight compared to that of an adult, mainly due to the rapid growth. Iron stores in NBW children (75 mg/kg) with addition of iron yielded from the hemoglobin
turnover the first 6 weeks, is sufficient for the first 6 months of life. The current recommendations state that iron-rich complementary food should be given from at least 6 months of age, for instance iron-fortified formula.\textsuperscript{19} Other protective interventions recommended to reduce the risk of ID is late umbilical cord clamping (>3 minutes), administer iron supplement from 4 months of age in high-risk individuals and not supply more than 500 ml/day of cow’s milk before the age of 12 months.

However, iron stores in LBW children are not sufficient to last for 6 months, resulting in increased risk of ID. Therefore, 1-3 mg/kg/day of iron supplements from birth to 6 months of age are recommended for LBW children.\textsuperscript{19} Still, since iron regulation systems are not matured until approximately 6 months of age, there is a risk of iron overload when supplementing.\textsuperscript{19} There are few previous studies exploring this risk in LBW infants.

\underline{Summary:} Children born with LBW have lower iron stores than children born with NBW, and they benefit from supplementation with positive long-term effects on behavior and possibly on cognition. Other possible long-term effects of iron intake in infancy are less studied.
Objectives

The objectives of the present thesis were to answer the following research questions:

- How does being born with LBW affect later cardiovascular risk? (paper I and II)
- How does being born with LBW affect later neurocognitive function? (paper IV)
- Are the risk profiles different for children being born preterm vs being born SGA? (paper I, II, IV and kappa)
- Are early growth and nutrition predictors of later cardiovascular risk and neurocognitive performance? (paper I and kappa)
- How does early iron supplementation affect later cardiovascular risk? (paper III and kappa)
Materials and Methods

Study design
This study was originally a double-blinded RCT of early iron supplementation administered to 285 children born with marginally LBW. For a prospective observational follow-up approach, an addition of 95 matched control children born with NBW were recruited (figure 10). In this thesis, consequences of being born with marginally LBW were studied using the observational study design (paper I, II and IV). However, when studying possible effects of iron supplementation (paper III), the original interventional design was used.

![Figure 10. A schematic picture of the study design.](image)

Study population

The marginally low birth weight children
The marginally LBW children were recruited before 6 weeks of age, at Karolinska University Hospital and Umeå University Hospital, two tertiary hospitals in Sweden. Inclusion criteria were a birth weight between 2000 and 2500 g, no diagnosed congenital disease, not having been treated with blood transfusion or supplemented with iron. The infants were identified using medical birth records and the parents to the subjects fulfilling the inclusion criteria were invited to participate in the study. The parents who accepted gave written consent.

Any congenital diseases that might influence the results diagnosed before the 7-year follow-up led to exclusion in all analyses (n=4 in paper I, n=3 in paper II-IV). Also, when analyzing results of the randomized intervention trial (paper III), children with anemia (Hb <90 g/l) at inclusion (n=16) or those diagnosed with hemolytic disease (n=2) during the neonatal period, were excluded from the trial.
The control group
As part of the observational follow-up study design, 95 control children were recruited before the 3.5-year visit. To do this, every third born LBW child was chosen as an index case. For each index case, a list was made of the 10 children born closest in time at the same study center, with the same sex and fulfilling the inclusion criteria of: birth weight between 2501 and 4500 g; born at term (gestational week 37-42) and not having been admitted to the neonatal ward. The parents to the first child on each list, born closest in time to the index case, was contacted and invited to join the study. If they declined, the parents to the next child on the list were contacted, until each index case had a corresponding control child.

Intervention
Before 6 weeks of age, the marginally LBW infants were stratified according to sex and study center and by computerized randomization they were allocated to 3 different groups receiving 0 (placebo), 1 or 2 mg iron/kg/day. Parents and all staff involved in the data collection were blinded regarding group allocation. The iron supplement (ferrous succinate drops of Ferromyn S; Astra Zeneca) was given between 6 weeks and 6 months of age. Dose adjustments were done according to weight at 12 and 19 weeks of age. According to intention-to-treat principle, the children diagnosed with anemia at 12 weeks of age and prescribed non-blinded iron supplements (n=9) were still included in the analyses. These cases were excluded in per-protocol analyses, together with those with low compliance (n=43).

All parents received identical bottles and were instructed to administer the dose between meals using syringes. The total daily dose was divided and given twice daily. The placebo drops were of similar taste and color, prepared by Apoteket Production and Laboratories. The parents were asked to fill in daily checklists, and those parents giving <70 % of the total dose were considered low compliance.

To identify possible side effects, parents were asked to register symptoms such as hard stools, respiratory symptoms and fever, as part of the daily checklists.

Data collection
At inclusion, information regarding the parents age and birth country as well as perinatal data such as iron supplements during pregnancy, smoking habits, obstetrical complications, birth weight and length, gestational age and Apgar score were collected. Using a Swedish gestational age-corrected growth standard, weight for age standard deviation score (SDS) was calculated. Those with SDS for birth weight less than -2 SDS were defined as SGA, all others cases as AGA.
**Early growth**
The children were followed up at 6, 12 and 19 weeks and at 6 and 12 months of age. The weight, length and head circumference was assessed at each visit and the change in SDS (ΔSDS) for weight and length was calculated, from last visit and from birth.

**Anthropometrics at follow-up**
At 3.5 and 7 years of age, the weight, height and waist circumference of the children was measured. BMI was calculated according to the International Obesity Task Force (IOTF) as body weight/height² (kg/m²). Obesity or overweight (paper I) was defined using the age- and sex specific reference by Cole et al (BMI >25kg/m²). In addition, underweight was defined using a Swedish reference (< -2 SD). Normal weight was defined as not being overweight or underweight.

BP measurement and pulse was assessed using an electronic device. Three measurements, one minute apart, were performed and the mean systolic and diastolic BP as well as mean pulse was calculated. To identify cases with BP within the hypertensive range (paper III), a Swedish reference was used.

![Figure 11. Body mass index, fat mass index and fat-free mass index.](image)

**Body composition**
At age 7, the body composition of the children was assessed using a dual-energy X-ray absorptiometry (paper I). From each examination, fat-free mass was calculated as bone mineral content + lean mass. Fat-free mass index (FFMI) and fat mass index (FMI) were further calculated as fat-free mass/height² (kg/m²) and fat mass/height² (kg/m²) respectively (figure 11). Truncal adipose tissue (%) was calculated as truncal fat mass (g)/total truncal mass (g).

**Laboratory markers of cardiovascular risk**
At the 3.5 and 7 year visits, the children came after overnight fasting and was offered a local anesthesia patch containing 25 mg Prilocaine and 25 mg Lidocaine.
Blood glucose was analyzed on the spot, while blood lipids (triglycerides, cholesterol, LDL, HDL, ApoB and ApoA1) was analyzed at the two hospital laboratories (paper II). ApoB/ApoA1 ratio was used as a marker of lipid status. A second serum tube was centrifuged and stored in a freezer for later analyses. This serum was used for analyses of insulin and high-sensitive C-reactive protein (hs-CRP). Hs-CRP is an inflammatory marker elevated in children with higher BMI and central adiposity.

HOMA-IR was calculated using the formula \((\text{insulin [IU/ml]} \times \text{glucose [mmol/L]})/22.5\). This is a surrogate equation often used in studies to evaluate peripheral insulin sensitivity due to its close correlation to golden standard methods.

**Questionnaires**

At age 7, the parents to the child were asked to fill in the Nordic Health Questionnaire. The parents were also asked to fill in CBCL (Child behavioral checklist), SDQ (strength and difficulties questionnaire) and 5-15. These questionnaires have been described and the results have been presented previously.

**Neurocognitive assessment**

At the 7-year visit, a pediatric psychologist tested the children with regard to three validated cognitive tests (paper IV). The total test time was approximately 30 minutes. The psychologists were blinded throughout the study. The tests used were as follows.

**Wechsler Intelligence test for Children IV**

WISC IV is a test with four domains for different intellectual abilities in children ages 6-16 year.

- **Verbal comprehension**: The first domain tested verbal apprehension, including subtests for vocabulary, similarities and comprehension.
- **Perceptual reasoning**: The second tested perceptual reasoning with tasks concerning block designs, picture concepts and matrix reasoning, mainly testing the non-verbal apprehension, logic thinking and visual-spatial perception.
- **Working memory**: The third domain tested working memory, including subtests for digit spans with number repetition and coding.
- **Processing speed**: The fourth domain tested processing speed, containing subtests for letter-number sequencing and symbol search.

Summarizing these four domains, a final full scale index was calculated by the psychologist. The result from each subtest yielded a raw point, which was then converted to a gender- and age specific scale point. Total scale points for each subtest resulted in a domain scale point, yielding an IQ score for each domain. Based on a reference population, the IQ scoring has a mean (SD) of 100 (15). IQ <85 was considered suboptimal in dichotomized variables.

**Beery Visual-Motor Integration test**

Beery VMI is a test for visual perception and motor integration. The child’s task was to copy 24 figures by hand, thereby assessing the ability to coordinate visual perception and hand-finger movements. The result was registered as raw points, which were then converted into age specific standard scores, with a mean (SD) of 100 (15).

**Test of Everyday Attention in Children**

TEA-Ch is a test for attention ability, designed to minimize demand on verbal comprehension, memory, reasoning and other abilities accounting for intelligence rather than attention. The test is build-up of 9 sections, of which 2 sections were used in this study; *Sky search* testing selective attention and *Score!* testing sustained attention. In the first section, the child’s task was to identify targets among distractors on a sheet, getting points for the sum of targets found, time per identified target as well as overall attention while performing the task. In the second part, the child was asked to listen to and count tunes. The results were registered as raw points and converted to age- and sex specific scale scores, with a mean (SD) of 10 (3). The mean score for each section was calculated and presented as selective and sustained attention respectively.

**Statistical analyses**

SPSS 22.0 and 23.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. For group comparisons of continuous variables, independent t-test or analyses of variance (ANOVA) was used when comparing two and more groups respectively. For confounder or covariate adjusted comparisons, analyses of covariance (ANCOVA) was used. For categorical comparisons, Chi-Square test was used, Fisher’s exact when appropriate (n <5 in any of the groups compared).

Multivariate linear regression model was used to explore possible associations between early predictors and later outcomes, such as early postnatal growth and diet on later BMI. These analyses were adjusted to preselected covariates.
Power analyses

From the initial 285 included LBW children we expected remaining minimum of 60 children in each intervention group at 7 years of age, giving 180 LBW children in total. For the 95 control children recruited former to the 3.5-year visit, we expected a drop out of 25% until 7 years of age, giving a remaining sample size of 70 control children at 7 years. Using such sample size, the 7 year follow-up study was powered (assuming a power of 80% and a significance level of 5%) to detect a group difference of 0.42 SD between LBW children and controls. This corresponded to a difference of 1.4 kg in weight and 6 points in cognitive scores, levels that we considered being of clinical significance.
Results

Study subjects at follow-up

Figure 12 presents the study flow chart. Three children were excluded in all analyses due to congenital diseases (DiGeorge syndrome, Becker’s muscular dystrophy and Williams syndrome). In total, 219 children were included in the follow-up study at 7 years. However, 17 only participated by sending in questionnaires (13 marginally LBW children and 4 control children) and did not attend to the visit at the study center.

For a more detailed description of the RCT follow-up, please see paper III. As presented elsewhere, there were no significant difference in reported side effects or dropout rate between the iron intervention groups.\textsuperscript{18}

![Flow chart of included subjects at the 7-year follow-up in the observational study and the randomized intervention trial respectively.](image)

**Drop-out analyses**

Those 63 marginally LBW children who dropped out before the 7-year control, had significantly higher mean Apgar score (9.7 vs 9.4, \(p=0.003\)) than the remaining study subjects. For other perinatal characteristics, as well as postnatal morbidity, there were no difference between the drop outs and the included children. With regard to parental characteristics, there was a higher prevalence of mothers from a non-Scandinavian country (30 % of the drop outs vs 17 % of the included, \(p=0.026\)). Also, there was a higher prevalence of drop outs in
Stockholm compared to Umeå (24 vs 9 %, p=0.024). For the control children, there were no difference in any of the background or baseline factors between the drop outs and the included children.

When comparing the cognitive test results from the 3.5-year follow-up, there were no difference in verbal, performance or total IQ between those marginally LBW children remaining in the study and those who dropped out (data available for 19 of the LBW children leaving the study). Neither did we observe differences in cognitive tests in the drop outs from the control group (data available for 13 of the control children dropping out).

**Summary:** A total of 76 % of the LBW children and 78 % of the controls remained to the follow-up at 7 years. There was a selective drop out of LBW children with mothers born in a non-Scandinavian country.

**Description of the cohort**

**Background data**
The baseline and background characteristics of the 219 marginally LBW children and 74 controls included in the follow-up study in any way are presented in table 1. The marginally LBW children were by definition smaller in size at birth, than the control group. The mothers in the marginally LBW group born in non-Scandinavian countries came from Asia (17), Europe (13), North America (5), South America (4) and Africa (3).
Table 1. Baseline and parental data of the included marginally LBW children compared to the controls.

<table>
<thead>
<tr>
<th>Perinatal characteristics</th>
<th>Marginally LBW</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (girl)</td>
<td>110 (50 %)</td>
<td>39 (53 %)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2290 (150)</td>
<td>3550 (430)**</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>45.3 (1.4)</td>
<td>50.6 (1.9)**</td>
</tr>
<tr>
<td>Head circumference at birth (cm)</td>
<td>32.2 (1.2)</td>
<td>35.1 (1.5)**</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>36.5 (1.9)</td>
<td>40.0 (1.2)**</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>125 (57 %)</td>
<td>52 (71 %)*</td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>66 (30 %)</td>
<td>1 (1 %)**</td>
</tr>
<tr>
<td>Parity</td>
<td>1.6 (0.8)</td>
<td>1.6 (0.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parental characteristics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother's age at delivery (years)</td>
<td>32.3 (4.8)</td>
<td>32.4 (4.4)</td>
</tr>
<tr>
<td>Mother born outside Scandinavia</td>
<td>38 (17 %)</td>
<td>5 (7 %)*</td>
</tr>
<tr>
<td>Mother's BMI (kg/m²)</td>
<td>23.9 (4.8)</td>
<td>23.7 (3.8)</td>
</tr>
<tr>
<td>Father's BMI (kg/m²)</td>
<td>25.4 (3.1)</td>
<td>25.4 (3.2)</td>
</tr>
<tr>
<td>Mother with university education</td>
<td>128 (62 %)</td>
<td>42 (58 %)</td>
</tr>
<tr>
<td>Father with university education</td>
<td>118 (61 %)</td>
<td>39 (57 %)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or numbers (%). **p <0.001; *p <0.05 using independent t-test for continuous variables and Chi-Square test for categorical. Fisher’s exact when n<5 in any cell.

Subgroups

Figure 13 demonstrate the partly overlapping subgroups of analyzed marginally LBW children born preterm or term and SGA or AGA respectively. A majority of children born AGA were born preterm (87 %), while most of the SGA born children were born term (82 %).

Figure 13. Distribution of children born preterm or term as well as small or appropriate for gestational age (SGA and AGA respectively). Reprinted from Staffan Berglund’s doctoral thesis “Effects of iron supplementation on iron status, health and neurological development in marginally low birth weight infants” (2012).
Early growth

Figure 14. Weight- and length growth during the first year of life. Data is presented as SD score (SDS). *p<0.05 for change in weight (∆SDS between the visits) between those born SGA and AGA. ‡p<0.05 for change in length (∆SDS between the visits) between those born SGA and AGA.

The early growth patterns in the marginally LBW cohort have been explored in detail in manuscripts that are not included in the present thesis.18, 109 Between birth and 6 weeks of age, there was a significant difference in growth rate between children born SGA and AGA respectively, both with regard to weight (+0.12 for AGA, +1.1 for SGA, p<0.001) and length (+0.90 for AGA, +1.2 for SGA, p=0.004) (figure 14). This indicates that the most pronounced growth difference between the subgroups was present the first 6 weeks after birth, with those born SGA presenting the most accelerated growth. The children born SGA did not completely catch-up to those born AGA, however, after 6 weeks of age the growth curves took more similar shapes.

The difference between the groups remained and at 3.5 years of age, the SGA born children were at higher risk of being underweight and short.109
**Postnatal morbidity**

Among the marginally LBW children, 49% were admitted to a neonatal clinic (table 2), while the controls by definition were not. There was an overall high prevalence of breastfeeding among the LBW infants with 91% being breastfed at 6 weeks of age (55% exclusively) and 67% at 6 months of age (data not presented in the table). There were no data available concerning breastfeeding among the controls.

**Table 2.** Early morbidity and breastfeeding in marginally LBW subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Apgar score</th>
<th>Neonatal ward</th>
<th>Respiratory distress</th>
<th>Hypoglycemia</th>
<th>Icterus</th>
<th>Breastfed at 6 wks</th>
<th>Breastfed at 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGA</strong></td>
<td>9.3 (1.2)</td>
<td>79 (66)</td>
<td>25 (21)</td>
<td>34 (29)</td>
<td>112 (94)</td>
<td>81 (68)</td>
<td></td>
</tr>
<tr>
<td><strong>SGA</strong></td>
<td>9.6 (0.8)</td>
<td>27 (27)**</td>
<td>2 (2)**</td>
<td>4 (4)**</td>
<td>85 (88)</td>
<td>62 (65)</td>
<td></td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td>9.4 (0.8)</td>
<td>26 (27)</td>
<td>1 (1)</td>
<td>19 (19)</td>
<td>3 (3)</td>
<td>83 (87)</td>
<td>61 (65)</td>
</tr>
<tr>
<td><strong>Preterm</strong></td>
<td>9.4 (1.1)</td>
<td>80 (67)**</td>
<td>26 (22)**</td>
<td>25 (21)</td>
<td>35 (29)**</td>
<td>114 (94)</td>
<td>82 (68)</td>
</tr>
</tbody>
</table>

Data are mean (SD) for Apgar score, number of children (%) for others. **p<0.001 using independent t-test and Chi-Square test respectively.

**Household and morbidity at age 7**

Table 3 presents the results regarding family situation at age 7 in both study groups, with results mainly from the Nordic Health Questionnaire. There were no differences in any of the analyzed data regarding household or morbidity. In addition, 9 of the parents in the marginally LBW group (mothers and fathers) were unemployed and 5 were on long-term sick leave. The corresponding numbers among the controls were 2 and 1 respectively. All other parents were either employees, entrepreneurs, on parental leave or studying (data not shown).

**Summary:** The marginally LBW children had higher prevalence of non-Scandinavian born mothers. A majority (87%) of marginally LBW children born AGA were also born preterm, and most (82%) of those born SGA were born term, hence overlapping subgroups. Rapid postnatal growth was observed between birth and 6 weeks of age, particularly in those born SGA.
**Table 3.** Data regarding household and morbidity reported from questionnaires at age 7.

<table>
<thead>
<tr>
<th></th>
<th>Marginally LBW</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly income of the household(Kr)</td>
<td>48 000 (38-55 000)</td>
<td>49 000 (40-56 000)</td>
</tr>
<tr>
<td>Economic difficulties last 12 mo</td>
<td>13 (6.2 %)</td>
<td>2 (2.7 %)</td>
</tr>
<tr>
<td>Living area (m²)</td>
<td>138 (59)</td>
<td>135 (43)</td>
</tr>
<tr>
<td>Study child with own room</td>
<td>135 (64 %)</td>
<td>52 (70 %)</td>
</tr>
<tr>
<td>Number of children in household</td>
<td>2.2 (0.8)</td>
<td>2.2 (0.6)</td>
</tr>
<tr>
<td>Diagnosed with atopic†† diseases</td>
<td>43 (20 %)</td>
<td>19 (26 %)</td>
</tr>
<tr>
<td>Diagnosed with ADHD or other</td>
<td>9 (4.3 %)</td>
<td>2 (2.7 %)</td>
</tr>
<tr>
<td>psychological diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing impairment, visual or speech defects</td>
<td>28 (13 %)</td>
<td>8 (11 %)</td>
</tr>
<tr>
<td>Diagnosed with gastrointestinal</td>
<td>20 (9.5 %)</td>
<td>6 (8.2 %)</td>
</tr>
<tr>
<td>disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of drugs on prescription†</td>
<td>38 (18 %)</td>
<td>14 (19 %)</td>
</tr>
<tr>
<td>Use of over-the-counter drugs†</td>
<td>84 (40 %)</td>
<td>30 (41 %)</td>
</tr>
</tbody>
</table>

Data are mean (SD), median (IQR) for income, or numbers (%) of children. No significant differences were observed using independent t-test or Chi-Square test (Fisher’s exact when appropriate). †For the last 3 months. ††Atopic diseases were defined as eczema, allergies or asthma.

**Anthropometric results**

Early iron supplements did not affect later anthropometric outcomes (table 8), which is why the observational study design approach was used.

**Table 4.** Anthropometric characteristics and body composition at 7 years of age in marginally LBW children compared to controls.

<table>
<thead>
<tr>
<th></th>
<th>Marginally LBW</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>22.7 (3.6)**</td>
<td>24.3 (3.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>122.4 (5.4)**</td>
<td>124.9 (5.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.1 (1.6)*</td>
<td>15.5 (1.5)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>54.3 (4.9)</td>
<td>55.2 (4.7)</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>2.5 (1.3)</td>
<td>2.8 (1.3)</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>12.4 (0.8)*</td>
<td>12.6 (0.8)</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>16.1 (6.4)</td>
<td>17.6 (5.9)</td>
</tr>
<tr>
<td>Truncal adipose tissue (%)</td>
<td>14.5 (7.1)</td>
<td>15.7 (6.6)</td>
</tr>
</tbody>
</table>

Data are mean (SD). **p<0.001 and *p<0.05 using independent t-test.
As presented in table 4 and paper I, the marginally LBW children were 1.6 kg lighter and 2.5 cm shorter than their NBW peers at 7 years of age and had lower BMI, FMI and FFMI (although not significantly lower for FMI). There were no differences between the groups when analyzing total body fat percentage or truncal adipose tissue.

Furthermore, as presented in figure 15, the marginally LBW group had significantly higher prevalence of being underweight (10.7 vs 2.9 %) similar to the results from the 3.5-year follow-up published elsewhere. Adjusting for whether the mother was born in Scandinavia, in a logistic regression model for predictors of being underweight, rather accentuated the significance of group allocation. In addition, we observed significantly lower prevalence in the marginally LBW group with a BMI within the reference range (-2SD – 25kg/m²) compared to controls (82.5 vs 92.9 %). Although not significant, there was also a slightly higher prevalence of LBW children at 7 years of age who were overweight or obese (6.8 vs 4.3 %).

![Figure 15. Prevalence of children who were underweight, overweight/obese or had a normal BMI at 7 years of age. P-value using Chi-Square test, Fisher’s exact when appropriate.](image)

**Summary:** The marginally LBW children were shorter, lighter and thinner than the controls. They also had a higher prevalence of underweight, and a lower prevalence of having a BMI within the normal range.
Laboratory markers of cardiovascular risk

Since the early iron intervention did not affect any of the laboratory cardiovascular risk markers (table 8), the intervention groups were studied as one combined cohort using the observational design.

As presented in table 5 and paper II, there were no differences between marginally LBW children and controls in the mean/median values of any of the analyzed cardiovascular risk markers, including glucose, insulin, HOMA-IR, lipids (cholesterol, triglycerides, ApoB, ApoA1, LDL and HDL) or hs-CRP (paper II). This did not change when adjusting the model for sex or BMI.

Table 5. Laboratory markers of cardiovascular risk in marginally LBW children and NBW controls.

<table>
<thead>
<tr>
<th></th>
<th>Marginally LBW</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.6 (0.5)</td>
<td>4.5 (0.5)</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>2.7 (2.3-3.8)</td>
<td>2.8 (LD-3.5)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.56 (0.4-0.8)</td>
<td>0.60 (LD-0.7)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.4 (0.7)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.60 (0.2)</td>
<td>0.57 (0.2)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.7 (0.6)</td>
<td>2.8 (0.7)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 (0.4)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.82 (0.2)</td>
<td>0.81 (0.2)</td>
</tr>
<tr>
<td>Apo A1 (g/L)</td>
<td>1.4 (0.3)</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td>ApoB/ApoA1</td>
<td>0.61 (0.3)</td>
<td>0.57 (0.1)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.23 (0.1-0.7)</td>
<td>0.19 (0.1-0.5)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median (IQR). No significant differences were observed using independent t-test, Mann-Whitney U (insulin, HOMA-IR and hs-CRP) or analysis of covariance (ANCOVA) adjusted for sex and BMI (glucose). LD is below lowest detection limit (LD for insulin 0.2 µU/mL and 0.078 mg/L for hs-CRP).

In addition, we compared the prevalence of having laboratory markers above an alert threshold, (defined as the 90th percentile of the control group). As presented in figure 16, the prevalence of LBW children with high fasting insulin was 20.6% compared to 8.6% for the NBW control group (p=0.038). In a logistic regression model, adjusted for BMI and sex, the odds ratio (confidence interval) (OR [CI]) for having an insulin level above the 90th percentile was 3.2 (1.2-8.9) for LBW children compared to those with NBW. To further investigate possible confounding from background differences, we added whether mother was born in Scandinavia to the model. This attenuated the significance of group allocation to an OR (CI) of 2.6 (0.9-7.2). No other significant differences in prevalence of adverse laboratory levels were observed between the groups.
Summary: There was a significantly higher prevalence of children with high fasting insulin in the marginally LBW group, indicating early signs of insulin resistance. No other cardiovascular risk marker differed between the groups.

SGA vs. AGA – effects on anthropometry and cardiovascular risk

All main outcomes were also assessed separately for marginally LBW children born SGA and AGA respectively (table 6, paper I and paper II). Height, weight, BMI, FMI, FFMI and total body fat percentage was significantly lower in children born SGA compared to controls, as opposed to those born AGA who were more similar to controls. For truncal adipose tissue, no significant difference was observed.

For cardiovascular laboratory risk markers, the children born SGA had significantly higher mean fasting glucose level compared to the controls (p=0.020) as well as compared to the AGA subgroup (p=0.018, data not shown in the table). This difference did not change when adjusting the analyses for sex and BMI (p=0.010) or when adjusting for whether the mother was born in Scandinavia (p=0.011). Neither did removing the 5 children with a glucose level above 5.6 mmol/L (data not shown).
Table 6. Anthropometrics, body composition and laboratory cardiovascular risk markers in marginally LBW children born SGA and AGA compared to controls.

<table>
<thead>
<tr>
<th>Marginally LBW subgroups</th>
<th>SGA</th>
<th>AGA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>22.1 (3.6)**</td>
<td>23.2 (3.5)</td>
<td>24.3 (3.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>121.2 (5.1)**</td>
<td>123.3 (5.5)*</td>
<td>124.9 (5.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.0 (1.5)*</td>
<td>15.2 (1.5)</td>
<td>15.5 (1.5)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>53.7 (5.4)</td>
<td>54.7 (4.5)</td>
<td>55.2 (4.7)</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>2.4 (1.3)*</td>
<td>2.6 (1.3)</td>
<td>2.8 (1.3)</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>12.3 (0.8)*</td>
<td>12.5 (0.8)</td>
<td>12.6 (0.8)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>3.6 (2.2)*</td>
<td>4.0 (2.1)</td>
<td>4.5 (2.3)</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>15.5 (6.6)*</td>
<td>16.5 (6.3)</td>
<td>17.6 (5.9)</td>
</tr>
<tr>
<td>Truncal adipose tissue (%)</td>
<td>14.2 (7.1)</td>
<td>14.9 (7.1)</td>
<td>15.7 (6.6)</td>
</tr>
<tr>
<td><strong>Laboratory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.7 (0.5)**†</td>
<td>4.5 (0.5)</td>
<td>4.5 (0.5)</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>2.9 (2.2-4.1)</td>
<td>2.6 (2.1-3.7)</td>
<td>2.8 (LD-3.5)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.62 (0.4-0.8)</td>
<td>0.55 (0.4-0.8)</td>
<td>0.60 (LD-0.7)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.4 (0.7)</td>
<td>4.4 (0.8)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.58 (0.2)</td>
<td>0.61 (0.3)</td>
<td>0.57 (0.2)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.6 (0.7)</td>
<td>2.7 (0.6)</td>
<td>2.8 (0.7)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 (0.4)</td>
<td>1.4 (0.3)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>ApoB (mg/L)</td>
<td>0.80 (0.2)</td>
<td>0.84 (0.2)</td>
<td>0.81 (0.2)</td>
</tr>
<tr>
<td>ApoA1 (mg/L)</td>
<td>1.5 (0.3)</td>
<td>1.4 (0.2)</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.22 (0.1-0.7)</td>
<td>0.24 (0.1-0.8)</td>
<td>0.19 (0.1-0.5)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median (IQR). **p<0.001 and *p<0.05 using independent t-test or Mann-Whitney U (insulin, HOMA-IR and hs-CRP). †p<0.05 adjusted for sex and BMI (glucose) using analysis of covariance (ANCOVA). WC is waist circumference. Lowest detection (LD) limit for insulin was 0.2 µU/mL, 0.078 mg/L for hs-CRP.

When exploring the prevalence of underweight, weight within normal range and overweight/obesity among the stratified groups, the marginally LBW children born SGA had the highest prevalence of both underweight and overweight, and the lowest prevalence of weight within the normal range (figure 17). This did not change when adding whether the mother was born in Scandinavia in the model, to adjust for possible confounding.
Summary: The children born SGA were lightest, shortest and thinnest. Also, they had a more adverse profile concerning glucose and insulin, indicating an increased risk of glucose intolerance and insulin resistance among these children, compared to controls and to marginally LBW children born AGA.

Early growth and nutrition – effects on cardiovascular risk

As presented in table 7 and paper I, early growth (analyzed as ∆SDS) in weight was a positive predictor to later BMI. Significantly predicting periods of growth were found both at 6-12 weeks and at 19 weeks-12 months of age. Furthermore, postnatal weight gain also predicted later FMI, whereas early growth in length predicted FFMI.

With regard to laboratory markers of cardiovascular risk, it was observed that postnatal growth between 0-6 weeks was a positive predictor of fasting glucose at 7 years of life. To further explore this association, a logistic regression model of predicting a glucose level above the 90th percentile was performed. In this model, growth in weight between 0-6 weeks (ΔSDS) of one SD increased the risk of
having a high glucose at 7 years of age with an OR (95% CI) of 2.1 (1.3-3.6). Furthermore, breastfeeding until 6 months of age was a negative predictor for later mean fasting glucose, indicating a protective effect. There were no significant association between breastfeeding and having a high glucose in a logistic regression model. For HOMA-IR and insulin, no significant predictors were detected (not presented in table 7).

For ApoB/ApoA1, a marker of lipid profile, early growth in weight (6-12 weeks) and length (19 weeks-6 months) was a weak positive predictor. Also for lipid profile, breastfeeding was a negative predictor.

**Table 7.** Linear regression model assessing the predictive value of postnatal growth and early nutrition on later cardiovascular risk. A similar model for predictors of HOMA-IR and fasting insulin did not identify any significant predictors.

<table>
<thead>
<tr>
<th></th>
<th>BMI (kg/m²)</th>
<th>Fasting glucose (mmol/l)</th>
<th>ApoB/ApoA1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>r²</td>
<td>B (95% CI)</td>
</tr>
<tr>
<td><strong>Early growth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (ΔSDS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 6 wks</td>
<td>NS</td>
<td>0.09 (0.01;0.16)</td>
<td>0.185</td>
</tr>
<tr>
<td>6 - 12 wks</td>
<td>0.70 (0.30;1.10)</td>
<td>0.061</td>
<td>NS</td>
</tr>
<tr>
<td>12 - 19 wks</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>19 wks - 6 mos</td>
<td>0.71 (0.16;1.27)</td>
<td>0.034</td>
<td>NS</td>
</tr>
<tr>
<td>6 - 12 mos</td>
<td>0.70 (0.34;1.05)</td>
<td>0.074</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Length (ΔSDS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 6 wks</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6 - 12 wks</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>12 - 19 wks</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>19 wks - 6 mos</td>
<td>NS</td>
<td>NS</td>
<td>0.08 (0.002;0.16)</td>
</tr>
<tr>
<td>6 - 12 mos</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Early nutrition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively breastfed at 6 wks</td>
<td>NS</td>
<td>-0.14 (-0.28;-0.01)</td>
<td>0.173</td>
</tr>
<tr>
<td>Breastfed at 6 mos</td>
<td>NS</td>
<td>-0.22 (-0.36;-0.08)</td>
<td>0.204</td>
</tr>
<tr>
<td>Iron supplementation</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Analyses for BMI and ApoB/ApoA1 were adjusted for age and sex. Analyses for HOMA-IR, fasting insulin and glucose was adjusted for age, sex and BMI. B is unstandardized regression coefficient with 95% confidence interval (CI) using regression model. r is the standardized regression coefficient for full model including covariates. NS is not significant.
Summary: Postnatal weight growth positively predicted later BMI, fasting glucose and ApoB/ApoA1 ratio, while breastfeeding correlated negatively to both later fasting glucose and ApoB/ApoA1 ratio.

Effects of early iron supplementation on cardiovascular risk – follow-up of the RCT

The short-term analyses of the randomized iron intervention have been published elsewhere and summarized in the doctoral thesis *Effects of iron supplementation on iron status, health and neurological development in marginally low birth weight infants* by SK Berglund.

Herein, we assessed the effect of iron, as one of many perinatal factors, on the cardiovascular risk profile, including anthropometrics, BP and laboratory cardiovascular risk markers.

There were no significant differences between the iron supplementation groups (0, 1 or 2 mg/kg/day) regarding anthropometric data, body composition, or laboratory markers of cardiovascular risk. However, a non-significant trend in higher BP in the placebo group was observed (paper III). To further analyze the results, the iron supplementation groups were combined to one intervention group and was compared to placebo (table 8).

As further illustrated in figure 18, there was a significantly lower systolic BP in the group receiving iron supplements compared to placebo in adjusted analyses (101 vs 103 mmHg, p=0.026). The adjusted mean difference was 2.2 mmHg. A confounder adjusted logistic regression model revealed that the OR (CI) for having a high systolic BP at 7 years of age was 0.32 (0.11-0.96) in children who had received early iron supplementation (detailed description in paper III). This indicates that the odds of having a high BP was lowered by 68 %.

Figure 18.
Boxplot of the systolic BP in those marginally LBW children who received placebo or iron supplements. *P*-value is adjusted for sex, age, height and heart rate.
Table 8. Anthropometrics, body composition, BP and laboratory markers of cardiovascular risk in marginally LBW children receiving placebo or iron between 6 weeks and 6 months of age.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Iron supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>22.0 (3.2)</td>
<td>23.0 (3.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>121.5 (5.4)</td>
<td>122.5 (5.4)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>14.8 (1.6)</td>
<td>15.2 (1.6)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>53.5 (4.9)</td>
<td>54.6 (4.9)</td>
</tr>
<tr>
<td>FMI (kg/m$^2$)</td>
<td>2.4 (1.3)</td>
<td>2.5 (1.3)</td>
</tr>
<tr>
<td>FFMI (kg/m$^2$)</td>
<td>12.3 (0.8)</td>
<td>12.5 (0.8)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>82 (12)</td>
<td>83 (10)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>103.0 (8)</td>
<td>101.2 (8)$^\dagger$</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>63.0 (5)</td>
<td>62.9 (5)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.6 (0.5)</td>
<td>4.6 (0.5)</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>2.9 (2.3-3.5)</td>
<td>2.6 (2.0-3.8)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.59 (0.4-0.7)</td>
<td>0.54 (0.4-0.8)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.5 (0.7)</td>
<td>4.3 (0.8)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.58 (0.2)</td>
<td>0.59 (0.3)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.8 (0.6)</td>
<td>2.7 (0.6)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.4)</td>
</tr>
<tr>
<td>ApoB/ApoA1</td>
<td>0.63 (0.4)</td>
<td>0.60 (0.3)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.20 (0.1-0.6)</td>
<td>0.24 (0.1-0.8)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median (IQR). $^\ast p<0.001$ and $^\ast p<0.05$ using independent t-test or Mann-Whitney U (insulin, HOMA-IR and hs-CRP). $^\dagger p<0.05$ adjusted for sex and BMI (glucose) and sex, age, height and heart rate (BP) using analysis of covariance (ANCOVA). Lowest detection (LD) limit for insulin was 0.2 µU/ml, 0.078 mg/L for hs-CRP.

**Summary:** Marginally LBW children given iron supplementation in infancy had 2.2 mmHg lower systolic BP at 7 years of age compared to placebo. Iron supplementation between 6 weeks and 6 months of age reduced the risk of having a high systolic BP by 68%, suggesting that iron supplements might have a protective effect against adverse metabolic programming.
Neurocognitive results
The early iron intervention did not affect any of the herein studied neurocognitive outcomes, although as presented elsewhere, those who received iron had less externalizing behavioral problems at age 7. When compared in an observational design between marginally LBW children and controls, all neurocognitive outcomes were adjusted for preselected possible confounders (sex of the child, parents’ educational level, maternal age at child birth and whether or not the mother was born in Scandinavia).

**Figure 19.** WISC IV and Beery VMI in marginally LBW children compared to NBW controls at 7 years of age. *p < 0.05 using independent t-test. ‡p < 0.05 using analyses of covariance (ANCOVA) adjusted for sex, mother and father with university education, maternal age at child birth and if the mother was born in Scandinavia.

**Figure 20.** TEA-Ch in marginally LBW children compared to NBW controls at 7 years of age. *p < 0.05 using independent t-test. ‡p < 0.05 using analyses of covariance (ANCOVA) adjusted for sex, mother and father with university education, maternal age at child birth and if the mother was born in Scandinavia.
The marginally LBW children had lower scores in all IQ domains, reaching significance for verbal comprehension IQ (crude mean difference: -3.1, p=0.004, adjusted mean difference: -3.9, p=0.004) (figure 19 and paper IV). A similar difference was observed for Beery VMI (crude and adjusted mean difference respectively: -3.5, p=0.031 and -3.7, p=0.030) and for the domains of TEA-Ch testing selective attention (crude and adjusted mean difference: -1.36, p=0.001 and -1.26, p=0.004) as well as overall mean TEA-Ch scores (crude and adjusted mean difference: -1.24, p=0.001 and -1.1, p=0.006) (figure 20 and paper IV).

**Summary:** The marginally LBW children had lower verbal comprehension IQ and visual-motor integration scores as well as poorer selective attention compared to NBW controls.

**Preterm vs. term – effects on neurocognition**

When comparing preterm and term born marginally LBW children, those born at term had the lowest scores of verbal comprehension IQ (adjusted mean difference of -3.6, p=0.019) as well as of visual-motor integration (adjusted mean difference -6.0, p=0.002) (figure 21 and paper IV). However, the preterm born children had significantly poorer results at the attention tests (TEA-Ch) (figure 22 and paper IV).

**Summary:** Being born term (whereof 84 % were born SGA) contributed more significantly to the lower neurocognitive outcomes of marginally LBW children compared to those born preterm (whereof 85 % were born AGA).
Figure 21. Cognitive test results (WISC for IQ and Beery VMI for visual-motor integration) in marginally LBW children stratified for being born preterm or term, vs controls born with NBW. Data are mean. *p<0.05 using independent t-test, ‡p<0.05 using analyses of covariance (ANCOVA) adjusted for sex, mother and father with university education, maternal age at child birth and whether the mother was born in Scandinavia.

Figure 22. Test of everyday attention (TEA-Ch) in marginally LBW children stratified for being born preterm or term, vs controls born with NBW. Data are mean. *p<0.05 using independent t-test, ‡p<0.05 using analyses of covariance (ANCOVA) adjusted for sex, mother and father with university education, maternal age at child birth and whether the mother was born in Scandinavia.
Early growth and nutrition – effects on neurocognition

When exploring the effect of early postnatal growth and nutrition on later neurocognitive outcomes (table 9), early growth in length (0-6 weeks) was a positive predictor to verbal comprehension IQ. To further explore this, a logistic regression model for having a verbal comprehension IQ below 1 SD (<85) was performed, adjusted for preselected variables. Here, there were no significant impact of early growth in length on later suboptimal verbal comprehension IQ (OR [95 % CI]: 0.43 [0.12-1.51]). For full scale IQ, no effect of early growth or nutrition was detected (not presented in table 9).

Table 9. Linear regression model of postnatal growth and nutrition and later neurocognitive outcomes. Those outcomes with non-significant predictors are not presented in the model (full scale IQ, selective and sustained attention).

<table>
<thead>
<tr>
<th>Early growth</th>
<th>Verbal comprehension IQ</th>
<th>Beery VMI (standardized score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95 % CI)</td>
<td>r²</td>
</tr>
<tr>
<td><strong>Weight (ΔSDS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 6 wks</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6 - 12 wks</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>12 - 19 wks</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>19 wks - 6 mos</td>
<td>NS</td>
<td>-6.1 (-10.5; -1.8)</td>
</tr>
<tr>
<td>6 - 12 mos</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

| **Length (ΔSDS)** | | | | |
| 0 - 6 wks | 1.70 (0.19; 2.3) | 0.129 | NS                  |
| 6 - 12 wks | NS                  | NS         | NS                  |
| 12 - 19 wks | NS                  | NS         | NS                  |
| 19 wks - 6 mos | NS               | NS         | NS                  |
| 6 - 12 mos | NS                  | NS         | NS                  |

| **Early nutrition** | | | | |
| Exclusively breastfed at 6wks | NS                  | NS         | NS                  |
| Breastfed at 6mos          | NS                  | NS         | NS                  |
| Iron supplementation       | NS                  | NS         | NS                  |

Analyses adjusted for age, sex, mothers age at child birth, mother and fathers having a university education and whether the mother was born in Scandinavia. B is unstandardized regression coefficient with 95 % confidence interval (CI) using regression model. r is the standardized regression coefficient for full model including covariates. NS is not significant.
For Beery VMI, early growth in weight was a negative predictor, suggesting that accelerated early weight gain had a negative impact on later visual-motor function. Moreover, the findings were further explored in a logistic regression model for having a Beery VMI score below 1 SD (<85). In these analyses, early growth in weight did not affect having a suboptimal visual-motor integration (OR [95% CI]: 1.6 [0.5-6.0]).

For selective and sustained attention, no effect of early growth pattern was observed (not presented in table 9). There were no significant correlations between the neurocognitive outcomes and breastfeeding or iron supplementation between any of the studied neurocognitive outcomes.

**Summary:** The correlation between postnatal growth and early nutrition to later neurocognitive outcomes was weak, although early growth in length seems to be a positive predictor to later verbal comprehension and weight gain associated unexpectedly to a lower VMI score.
Discussion

Summary of main results
In the present study we found that (table 10):

- Marginally LBW children were shorter, thinner and more underweight compared to NBW controls. In addition, those born SGA showed signs of higher fasting glucose, suggesting that this subgroup have early signs of imbalanced glucose homeostasis.
- Weight gain in infancy correlated positively to BMI, fasting glucose and ApoB/ApoA1, whereas breastfeeding correlated negatively to later glucose and ApoB/ApoA1 levels.
- The marginally LBW children who received iron supplements had a lower systolic BP, indicating that early iron supplements could protect LBW children against the risk of increased BP.
- The marginally LBW had poorer neurocognitive scores for verbal comprehension IQ, visual-motor integration and selective attention.

Table 10. Main findings from paper I, II and IV.

<table>
<thead>
<tr>
<th>Outcomes at age 7</th>
<th>Marginally LBW</th>
<th>Marginally LBW subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Underweight</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Thin</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Shorter</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>High fasting glucose</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Insulin imbalance</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Dyslipidemia</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Poor verbal comprehension</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Poor visual-motor integration</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Poor attention</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Preterm</th>
<th>Term</th>
<th>SGA</th>
<th>AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Thin</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Shorter</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>High fasting glucose</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin imbalance</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor verbal comprehension</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor visual-motor integration</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor attention</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

X is when there is a significant risk or difference compared to controls born with NBW.
The cohort
This study was conducted in two university cities that most likely have a better socioeconomic status than the average in Sweden. This might influence the results since socioeconomic status matters to both intelligence and to cardiovascular risk. To evaluate this, background data as well as household data from the 7-year control were explored.

According to statistics from the Swedish medical birth register, the mean maternal age at child birth in Sweden is 30.3 years, which is approximately 2 years younger than mean maternal age in our study. Older mothers usually have a higher grade of education and income, which could contribute to cognitive and behavioural advantages in the current cohort.

The same source concluded that approximately 24% of children born in Sweden have a mother who is not a native Swede. This proportion is higher than in our study cohort, especially compared to our control group (from 20% at study start to 17% at 7 years of age in the LBW group, and 7% in the control group both at inclusion and at 7 years). Moreover, there was a higher proportion of children with mothers from a non-Scandinavian birth country among the marginally LBW children dropping out before 7 years of age (30%), suggesting a selective drop-out that might bias the results to our marginally LBW children’s advantage. The lower proportion in the control group is most likely also due to selection bias, despite the thoroughly matching that was performed before recruitment.

According to Statistics Sweden (www.statistikdatabasen.scb.se) median disposable income for a household with children and two adults was 45 000 Swedish kronor per month for ages 30-39 (year 2011). In this cohort, median disposable income in participating households at the 7-year follow-up, single households excluded, was also 45 000 kr. This suggests that the current cohort does not represent a high-income group, which strengthens the external validity of the results and suggests that they, from that point of view, are applicable to other regions than Swedish university cities.

Cardiovascular risk

Overweight
In this cohort of Swedish children, the overall prevalence of overweight and obesity was generally low (6.9% in the marginally LBW group, 4.3% in the control group), whereas the prevalence of underweight was high (11% in the marginally LBW group, 3% in the control group). In comparison, another study found that the prevalence of overweight and obesity among 7-year old children in
Sweden was 14 %, while the prevalence of underweight was just above 2.5 %, similar to our control group. In addition, Sjöberg et al found that the prevalence of overweight was 16 %, while the prevalence of thinness was 7.5 %. They also concluded that the risk of both overweight and underweight in children are greater for those living in rural areas compared to urban areas, probably reflected by socioeconomic status. Hence, the differences between the cohorts may partly be explained by geographic and/or socioeconomic factors. Another possible explanation is that the prevalence of childhood overweight in Sweden is actually decreasing, which has been observed in other studies.

Our results suggest, in contrast to the theory, that the LBW children at 7 years of age had an increased risk of underweight rather than overweight. Compared to the prevalence of underweight in the present cohort at 3.5 years of age (9.5 %), a minor but yet increasing trend among the marginally LBW children can be observed. The same trend was seen for the prevalence of overweight, from 4.7 % at 3.5 years of age to 6.9 % at age 7. Together, these observations indicate that the increased risk of having an abnormal body size may become more prominent later in life.

When it comes to body composition, our study found that marginally LBW children had lower FMI, FFMI and total proportion of fat, which is in contrast with the theory of adverse metabolic programming. The subgroup of children born SGA had the lowest mean values, despite the rapid growth the first year of life (figure 14), in contrary with the accelerated growth hypothesis. In addition, there were no difference between truncal adipose tissue between the groups, which also support that marginally LBW children in Sweden do not experience an increased risk of overweight and central adiposity.

To summarize, from what we can interpret so far, our data suggests that marginally LBW children in Sweden today are at risk for developing an aberrant body size, foremost due to an increased risk of underweight. This risk is most pronounced the subgroup of children born SGA.

**Early growth and later overweight**

In this study, we found positive associations between early growth and later BMI, FMI and FFMI. Also, as presented in paper I, size at birth was a positive predictor to FFMI. This is in agreement with several previous studies reporting a strong positive correlation between size at birth and early growth, and later body composition.

In a review, Kelishadi et al. stated that in LBW children, early growth is a stronger predictor than size at birth to later adverse metabolic programming, indicating a possibility to modify the risk by early nutritional recommendations.
“Dutch famine” and the “siege of Leningrad” are two famous real life applications supporting the hypothesis of accelerated growth. From these periods of regional starvation, those who were exposed to intrauterine nutritional restriction followed by catch-up growth (as in the Dutch example), had a greater risk of developing overweight and obesity, as oppose to those with a continuous scarce nutritional supply postnatally (as in Leningrad). In our study, early weight was a positive predictor to FMI, although those with the fastest postnatal growth (those born SGA) were still thinnest at 7 years of age, suggesting a failed attempt to catch-up growth.4

One Danish study of term born infants found that change in weight for age Z-scores was positively associated with BMI, FMI and FFMI at 3 years of age. However, the association between early growth and FMI was attenuated by breastfeeding, suggesting that breastfeeding was an effect mediator. In our cohort, those marginally LBW children who were fed breastmilk had slower weight and length gain the first year of life analyzed using linear regression model (data not shown). However, when adding breastfeeding at 6 weeks or 6 months to the regression model of early weight gain and later BMI or FMI, the significance of early weight gain did not change. This indicate that in our cohort, the association between early weight gain and later BMI and FMI, was not confounded by breastfeeding the first 6 months of life, strengthening the theory of programming by postnatal accelerated growth.

In consistency with the Danish study, several other studies have suggested an association between intra- and extrauterine growth, and later lean mass rather than fat mass. For instance, a longitudinal study of 9 year old boys born in a city in Brazil in 1993 showed that early weight gain (0-6 months) was positively associated with later height and lean mass, but not with fat mass. In their cohort, weight gain during childhood (1-4 years) was positively associated with obesity in the children. The authors suggested that the early weight gain results in an early programming of lean mass and physique, that influence the correlation to later BMI, rather than being influenced by programming of fat mass. To test this suggestion in our marginally LBW children, we added FFMI to the regression model of early predictors to later BMI and FMI, which did not change the significance of early weight gain, indicating that there was a FFMI-independent correlation between early weight gain and later fat mass. Nevertheless, we cannot exclude possible impact of other non-identified mediators or confounders.

Similar to our results, the association between early growth and later fat mass have been observed in several studies of children born with LBW, preterm and/or SGA. For instance, in a study of children born SGA and AGA, those born SGA had greater accumulation of total and abdominal fat mass between 2 and 4 year of
age. Both total and abdominal fat mass were associated with weight gain the first 2 years of life. According to a recent systematic review of children born SGA at term, early weight gain was associated with increase in fat mass in the majority of observational studies as well as in 3 RCTs. The authors emphasized the lack of early postnatal growth data, obstructing the possibilities to identify actual early critical windows.

In a study of preterm infants (mean gestational age of 32 weeks), positive associations with growth in weight between birth and term age, as well as between term age and 3 months with fat mass percentage and waist circumference in young adulthood was observed. Those with weight gain of the highest quartile had the largest fat mass percentage. This has been confirmed in other studies of preterms as well.

The mechanisms behind programming of different tissues are unknown. It has been proposed that the development of fat depositions, which takes place during the third trimester, gets interrupted by growth restriction or preterm birth. The adipose tissue development might then be more prone to program according to events after birth than in children born at term with NBW who have had a normal intrauterine adipose tissue development and programming.

Early nutrition and later overweight
Several previous studies have found an association between lower risk of overweight and less abdominal fat mass in breastfed children compared to those fed formula. In this cohort, no association between breastfeeding and later BMI, FMI or FFMI was seen (paper I). There were no associations between overweight at 7 years and breastfeeding during the first 6 months of life. Perhaps this was due to the overall low risk of overweight and a lack of power to detect any correlations.

Insulin
We did not detect a difference in median fasting insulin level between controls and the overall marginally LBW group, or the stratified SGA or AGA subgroups. This is in contrast with other studies that have shown a higher fasting insulin in children born SGA or with LBW. In addition, clinical trials have shown that after oral or intravenous glucose tolerance test, the insulin levels are higher in adults born with very LBW, indicating a partly compensated insulin resistance that results in increased insulin levels when strained. Since we did not perform glucose tolerance tests, we cannot exclude that an insulin resistance would become more prominent when strained in our marginally LBW children as well. Our results of fasting levels could indicate a type II error, or that differences will become more prominent and detectable later in life.
We did however detect a higher prevalence of suboptimal fasting insulin levels among the marginally LBW children compared to the controls. The level of significance was attenuated when adjusting for whether the mother was born in Scandinavia, even though a non-significant trend remained. This could indicate that we lack power to show a significant difference when considering ethnical background differences between the groups, or that maternal birth country is a confounder explaining our finding. According to previous studies, the risk of T2DM is higher among immigrants from Middle East compared to native Swedes, which could explain the confounding effect in our logistic regression model of high insulin levels. Consequently, from our results we can only conclude that the hereditability of insulin sensitivity from the mother is at least as important as being born a little bit too small when assessing the insulin level at 7 years of age.

**HOMA-IR**

We did not detect any differences in HOMA-IR in crude analyses. Since insulin had a lowest detection limit of 0.2 µIE/ml, we could not assume normal distribution or use the natural logarithm to create a normal distribution. This also yielded in some samples of HOMA-IR not being detectable. Due to the non-parametric assumption, the results could not be further evaluated with adjusted analyses.

**Early growth and nutrition and later insulin**

In our analyses, no associations between early growth or breastfeeding and later insulin or HOMA-IR levels were observed. This could be due to a lack of power for detecting differences this early in life. It could also suggest that early weight gain has a stronger effect on glucose homeostasis rather than on insulin secretion. If this is the case, an initial glucose resistance due to reduced insulin sensitivity could be the mechanism (as shown in figure 25). A previously suggested pathway is by reduced numbers of GLUT4 (glucose transporter type 4) and other insulin signaling proteins in both muscle and in adipose tissue after growth restriction, previously shown in animal and human studies. As shown in the present study, LBW children had lower FFMI than their NBW peers, probably representing a reduced muscle mass relative to body size, which could influence the results.

**Glucose**

Our laboratory results points towards an imbalanced glucose and insulin homeostasis in the marginally LBW children, with the highest risk among those born SGA. This suggests that the children born SGA are more prone to abnormal metabolic events, a conclusion in concordance to several previous studies. Interestingly, this subgroup of children was rather underweight than overweight, despite the rapid weight and length gain the first year of life (figure 14). The rapid
weight gain and later glucose imbalance gives support to the *early accelerated growth hypothesis*, suggesting that early growth is a contributor to the later risk, an effect that is not driven by increased risk of overweight.

**Mechanisms**

In this present study, due to its observational approach, we can only speculate regarding possible mechanisms behind these events. The elevated mean glucose level in the children born SGA could demonstrate the onset of glucose intolerance caused by impaired insulin secretion from the beta-cells in pancreas or by impaired insulin sensitivity resulting in reduced glucose uptake, the latter often associated with overweight (figure 5). We did not detect a difference in HOMA-IR between the groups, rather suggesting that the high glucose levels could be caused by a deficient insulin secretion. In fact, insulin secretion has been shown to be more closely correlated to size at birth than insulin sensitivity has. The compromised insulin secretion, would further on lead to hyperglycemia, a condition that can cause a vascular proinflammatory state, triggering endothelial dysfunction and promoting arteriosclerosis and insulin resistance. This pathway could explain the increased fasting glucose levels in SGA born children, possibly demonstrating the onset of hyperglycemia, although it would not explain the observed risk of having a high insulin level in the overall marginally LBW group.

To further support this theory, animal studies have been conducted to find mechanistic pathways. These studies often induce IUGR by nutritional restrictions, arterial ligations or by giving glucocorticoids during pregnancy. From these, altered microvascular structure as well as a reduced beta-cell mass have been observed in the pancreas, causing a deficient insulin secretion after being exposed to IUGR (figure 23).

![Figure 23](image)

**Figure 23.** A theoretical model of how IUGR leads to a decreased beta-cell mass in the pancreas, which results in less secretion of insulin and insufficient glucose uptake.

In contrast to the impaired insulin secretion observed in animal studies, studies in humans have rather suggested an increased insulin resistance in children born
SGA. For instance, in a Swedish study, SGA born children had higher fasting glucose, insulin and HOMA-IR (adjusted for current body size) at 8-10 years of age compared to those born AGA at term.\textsuperscript{55} This suggests an increased insulin resistance in those born SGA. In addition, the authors found evidence that those born preterm (<30 weeks of gestation) had signs of hepatic insulin resistance (figure 24), which would lead to compromised glucose uptake. Thus, they suggested different mechanistic pathways behind the glucose and insulin imbalance observed in different subgroups of LBW children.

**Figure 24.** A model of how preterm birth cause hepatic insulin resistance, altering the glucose and insulin homeostasis which causes an insufficient glucose uptake.

A study by Ong et al further support the theory of different mechanisms behind the increased risk of glucose and insulin imbalance observed in different subgroups of LBW children.\textsuperscript{126} In their study, 8-year-old children who grew the most during their first 3 years of life had poorer insulin sensitivity and higher insulin secretion after oral glucose intake (figure 25). This suggests that rapid growth is followed by an increased risk of insulin resistance, with a compensatory increase of insulin secretion, a mechanism proposed in studies of LBW children as well.\textsuperscript{25, 56} They also found a correlation between smaller size at birth and later insulin secretion, which had a stronger correlation than insulin sensitivity did, strengthening the theory of beta-cell mass reduction. The lowest insulin secretion was observed in the children with catch-down growth and who were shortest, which also partly could be explained by decrease in beta-cell mass.
Figure 25. Postnatal growth could alter the effect of insulin in muscle and adipose tissue, leading to insufficient glucose uptake.

Another rather newly discovered mediator that has been suggested to be part of the pathway to develop glucose intolerance, is the lower IGF-1 levels that have been observed in SGA born children as well as in adults with CVD (figure 26).\textsuperscript{4, 127} Levels of IGF-1 also correlates positively with height gain in childhood.\textsuperscript{126} IGF-1 mediates the effect of GH and regulates glucose homeostasis and insulin sensitivity, but the exact mechanisms are so far unknown. IGF-1 is a key factor for intrauterine growth, and children born with LBW, both due to preterm birth or SGA, have lower levels in cord blood sample, compared to infants with NBW.\textsuperscript{127} In a review, Jensen et al concluded that IGF-1 levels increases in children born SGA with rapid postnatal growth, but still remain at lower levels in those who are still underweight or short (< -2 SD). In our study, those born SGA were shorter, lighter and thinner, which may indicate that they had lower IGF-1 levels than the children born AGA, despite the rapid growth.\textsuperscript{127} Hence, lower levels of IGF-1 might be involved in the mechanisms of increased cardiovascular risk in those born SGA. Adding analyses of IGF-1 to the present study would have contributed to further mechanistic clues and should be considered in future similar studies.
Figure 26. The consequences of elevated or decreased IGF-1 levels.

**Early growth and later glucose**
In our study, we found a positive association between early postnatal weight gain and later glucose levels. This correlation did not seem to be present exclusively in those born growth restricted and had a greater catch-up growth postnatally.

Several previous studies have found associations between early postnatal weight gain and later glucose or insulin. For instance, one RCT of nutrient-enriched formula vs standard formula administered to preterms found positive associations between growth in weight the first two weeks of life and 32-33 split pro-insulin levels 13-16 years later, indicating signs of insulin resistance. Even though they did not detect an association with glucose levels, the results still confirm an imbalance caused by early growth.

A clear majority of observational studies report associations between postnatal growth and later insulin resistance, with different results regarding glucose levels. However, as in the studies of early weight gain and later body composition, there is a lack of studies investigating the effect of the early postnatal growth, to find critical windows. Also, not all studies adjust for current body size, which makes the results difficult to interpret.
Early nutrition and later glucose
We found that breastfeeding was associated with lower glucose levels at 7 years of age. This is in agreement with several other studies. A systematic review by Horta and colleagues found that breastfeeding lowered the risk of developing T2DM with an OR (95% CI) of 0.65 (0.49-0.86).128 Some of the suggested mechanisms are an improved maturation of the immune system by components of the breastmilk or by mediating a lowered risk of overweight and obesity, the latter not observed in the current cohort.

Lipid status and hs-CRP
According to our results, marginally LBW children does not have an increased risk of an adverse lipid status or higher hs-CRP, as a marker of low-grade inflammation, compared to peers born with NBW. This is in accordance to several previous studies of LBW children, for instance the report by Kistner.25, 55

Other studies have shown that lipid profile correlate to body fat, and the low risk of overweight in the present cohort could most likely explain the lack of an adverse lipid profile among the herein studied LBW children.129

Early growth and later lipid status
We found positive associations between early postnatal weight and length gain and later ApoB/ApoA1 ratio, indicating that early accelerated growth could lead to a more adverse lipid profile.

In a prospective cohort study of very preterm and very LBW children, lipid status and carotid intima-media thickness at 19 years of age were not correlated to early postnatal growth (0-3 months or 3 months-1 year).130 Instead, there were positive correlations between current BMI and lipid profile. The authors argue that the lipid profile was determined by the current body size, rather than being programmed early on. In our current cohort, the correlation to early growth remained after adding current BMI or FMI to the model, suggesting that the association was not mediated by current body size.

In a review by Ong et al, few studies investigated the effect of early postnatal growth in preterms and later lipid profile and only 1 of 3 three studies exploring cholesterol levels found a positive association.82 Almost no study adjusted for current body size, which the authors emphasize is of importance to be able to interpret the results. This was also the conclusion in another review of children born SGA in which they found that 1 of 7 observational studies had positive association between early growth and later lipids.84
Hence, our positive association of early growth and later lipid profile, could be a type I error. However, we have not found another study exploring the early growth during different periods the first year of life, and especially not in marginally LBW children. Consequently, our findings could therefore suggest that there are critical windows during early growth that are prone to program later lipid status, an observation that should be further explored in future studies.

**Effects of early iron supplementation**

In this study, we observed a novel effect of the early iron supplementation which seem to have normalized the systolic BP at 7 years of age in the marginally LBW group. Several previous studies, of different designs, have shown a negative correlation between birth weight or gestational age, and later BP.\(^{26, 31, 131, 132}\) Lowering the average BP in the population by 5 mmHg has been estimated to reduce the risk of stroke by 34% and the risk of coronary heart disease by 21%.\(^{133}\) Hence, even small differences have great improvements on health on a population level.

**Iron and later blood pressure**

Iron is essential for many mechanisms in the human body, such as being part of the oxygen carrying molecule heme within RBCs, being part of the electron transport chain and DNA synthesis. According to our result, iron also seem to have a role in *adverse programming*, or at least being part of a way to promote normal development.

In general, documentation of metabolic long-term effects of early micronutrients are limited. Experimental studies on rats have shown that offspring to iron restricted dams have increased BP throughout life, indicating that scarce iron supply early in life leads to *adverse programming* effect of later BP.\(^{93, 95, 134}\) Although, the authors speculate that perhaps the higher BP was a result of the rapid postnatal growth observed in the iron-restricted dams, in agreement with the *accelerated postnatal growth hypothesis*, such as the correlation observed in children born SGA as well.\(^{135}\) However, in this present study, there were no effects of iron supplementation on growth and the effect is most likely not driven by different growth rates.\(^{18}\)

Epidemiological studies with regard to iron intake and later BP have been conducted. In a review by Lapice et al, the authors found associations between ID and increased risk of CVD, although concluded that data so far are inconsistent.\(^{96}\) Interestingly, treatment with intravenous iron has recently been recommended to patients with congestive heart failure and ID. In a double-blinded randomized trial, those who received iron supplements had improved 6-min-walk-test, reduced risk of hospitalizations due to heart failure, improved quality of life and...
improved NYHA (New York Heart Association) class, with an effect lasting until 52 weeks after treatment. The mechanisms behind the improvement is not entirely known, although it surely confirms that iron is important when it comes to cardiovascular risk.

Another interesting aspect is the relatively new finding of an association between ID and arterial pulmonary hypertension. Animal studies inducing ID resulted in inducing pulmonary hypertension and led to vascular remodeling with endothelial thickening as well as perivascular inflammatory infiltration. After recovering the deficiency with intravenous iron supplements, the effects normalized. Furthermore, studies have shown that one consequence of altitude induced hypoxia is an increased systolic pulmonary arterial pressure, which has been shown to be reduced when treating with intravenous iron. Hence, iron is essential for normal hemodynamic regulation and vascularization.

We can only speculate regarding possible mechanisms behind the novel findings in our study. One pathway could be through nitric oxide synthase (NOS), which synthesize NO, a regulatory molecule signaling relaxation of vascular smooth muscle cells, which leads lowered BP. Iron is essential for NOS, and in vitro studies have shown an increase in NOS in response to iron. Hence, the early iron supplementation might have induced increased production of NO, leading to lowered BP (figure 27).

![Diagram of NOS, Heme (Fe), NO, Vasodilation, Cell communication, Immune response](image)

**Figure 27.** Iron is essential for nitric oxide synthase (NOS), which produces nitric oxide (NO).

**Neurodevelopmental results**

The marginally LBW children had poorer performance in verbal comprehension, visual–motor integration and selective attention. These cognitive and behavioral differences between LBW and NBW children have been shown previously. Although to our knowledge, this has not been explored in the large group of marginally LBW children before. Of notice is that the participating children
performed well on all domains of IQ, except for working memory. This is probably due to a limitation of the Swedish version of the test that was not fully standardized according to Swedish data, but was only tested and validated on a smaller Swedish population. This limitation has been noted in other Swedish studies as well.

Neurodevelopment in children is regulated and influenced by both genetical and environmental factors, and intelligence is associated with a range of different outcomes, even mortality. Besides, lower intelligence is associated with increased risk of overweight and obesity, which might add to the cardiovascular risk observed in LBW children.

Another interesting aspect is that stunting at 1 year of age and persistently stunting at 8 years has been associated with lower school grades and poorer outcomes in math achievement, vocabulary and reading comprehension. Our marginally LBW children had larger risk of being underweight and short, with the largest risk among those born SGA, the subgroup in which we also observed the poorest cognitive results. However, adding the current height or early growth to our model did not change the differences between the groups, which suggests that current height was not an effect mediator to the lower cognitive scores.

**Mechanisms**
The lower cognitive performance observed in preterms have been associated with morphological alteration in the brain, lasting until adulthood. We can speculate that the extrauterine brain maturation does not result in the same morphology and functions as in normal healthy intrauterine maturation. Baron et al reported that children born late preterm have reduced cortical grey matter volumes and reorganized white matter compared to those born at term, a structural alteration lasting until adulthood. Also, adolescents born late preterm have thinner parietal and temporal cortex, areas involved in for instance verbal and motor function (figure 8).

Furthermore, imaging studies by Peterson and colleagues showed that 8-year old children born preterm have thinner cortex in premotor, sensorimotor, midtemporal and parietooccipital regions, compared to term born peers. The authors also correlated the volumes of these regions with intelligence and found a positive association with verbal, performance (from earlier versions of the WISC test) and full scale IQ. However, their study sample of preterms were born with a mean gestational age of 28 weeks, including children with perinatal injuries such as intraventricular hemorrhage, and are therefore not quite applicable to our group. Nevertheless, the results do confirm an altered morphologic brain development, with the strongest associations to the cortical regions crucial for verbal comprehension and visual-motor integration. Other imaging studies have
found similar results. For instance, Nagy and colleagues found thinner cortex in adolescents born preterm, which they proposed was caused by elevated corticosteroids due to stress in connection with preterm birth. This theory is supported by some other studies that have shown altered brain development after corticosteroid treatment in preterms.

Another interesting study have looked at healthy preterms (22-35 gestational weeks at birth, mean birth weight of 1700 g) at 6-8 years of age, and compared their upper-limb movement to term born peers. They found that during a goal-directed task, there was an inverse relationship between gestational age at birth and extended duration as well as increased movement segmentation. In addition, these kinetic performances predicted full scale IQ in those born preterm, an association not observed in full term children. This indicates that connections in the brain could be altered, and affect for instance hand-finger movement, crucial for visual-motor integration.

This has been further supported by recent studies of neural tracts and microstructure within the brain of preterm children and adults. According to these studies, core connections between cortical regions are prioritized over peripheral short-range connections during development, leading to a reduced microstructural connectivity in preterms compared to those born at term. These tracts have been associated with cognition, emotional regulation, attention, behavior and verbal performance and could explain some of the neurocognitive results in our cohort.

These studies above suggest that children born preterm or with LBW are at risk for altered brain development. However, most of the studies are performed on extremely or very preterm born subjects, and further data with regard to other subgroups of LBW and preterm children are warranted. More studies are needed to explore the cause behind the deficits in marginally LBW preterms and those born SGA, to further on be able to give these subgroups the optimal care and follow-up.

**Early growth and neurodevelopment**

**Verbal comprehension**

According to our analyses, growth in length the first 6 weeks of life was a positive predictor to later verbal comprehension. When adding preterm birth, being born SGA or current body size to the model, the significant contribution of early growth remained. In fact, being born preterm or SGA did not have any significant role in the model, neither when analyzing verbal comprehension IQ as a continuous or as a dichotomized variable (<85).
A previous RCT comparing preterm or term formula given to preterm born infants (<1850 g) found that those who had been fed preterm formula had the greatest weight gain and better performance on WISC at 8 years of age, especially verbal IQ. This effect was only observed in boys. The authors also found an association of slow early growth and later risk of having a verbal IQ <85. Another study explored brain volumes with MRI scans in adolescents born preterm. They found that those who had been randomized to receive high-nutrient formula postnatally had better verbal IQ and larger caudate nuclei. When stratifying according to sex, this correlation was also only seen in boys. Nevertheless, it suggests that early nutrition can program the volume of the caudate nuclei, which in turn could affect verbal comprehension IQ.

In addition, other neuroimaging studies have found more matured gray and white matter areas after promoting enhanced nutrient intake in children born with very LBW and preterm. This indicates that both neural bodies (gray matter) and neural connectivity (white matter) are affected, perhaps by increased intake of essential fats that have been shown to improve brain development. Still, not all clinical trials promoting early weight gain found associations to later cognitive performance, either in children born preterm or in those born SGA, and conclusions are so far indefinite.

According to a trial of rats who were growth restricted postnatally and then fed different diets leading to different degrees of catch-up growth, those with the earliest catch-up growth performed better on cognitive tests. This is in agreement with our results, that the early growth is most crucial. It also emphasizes the need to further investigate different time range of early postnatal growth, rather than growth from birth to 2 or 5 years.

**Visual-motor integration**

In our regression analyses of early growth and nutrition and later visual-motor integration, we found a negative association with early growth in weight. This is in contrast to our hypothesis, that early growth would have a protective effect on neurodevelopment. We could not find a possible mediator to the effect, for instance being born preterm or SGA or current body size.

In contrast with our results, a study of early weight gain in term born children found that slower growth postnatally was associated with poorer visual-motor integration at 4.5 years of age. This would be more logical, since early growth faltering have been associated with poorer neurocognitive results later in life.

We cannot support these results with any mechanism or theory, and despite the correlation being strong, we believe it is a type I error, a false positive finding.
Strength and limitations
The foremost strengths of this study were its longitudinal design, the long-term follow-up, and the well-defined study population, which together enabled well supported and clinically relevant conclusions compared to many other non-experimental study designs. The carefully matched controls and the low drop-out rate considering the study duration of 7 years, added additional strength.

The study participants have been followed by responsible research nurses and have been tested by only two psychologists at each study center, limiting the risk of measuring bias. The use of well validated psychological tests and the objectivity of the examination with DXA for assessing body composition, strengthened the validity of those results. In addition, the analyses of the laboratory tests were performed with similar techniques at both study centers.

Nonetheless, this study is limited in several manners. First, except for when analyzing effects of the early iron intervention (paper III), we used an observational approach. In those, the risk of un-identified confounding is always present. Furthermore, there was a lack of data regarding those who declined participation, and there might have been a selection bias of both LBW infants and control children, i.e. with regard to mothers' birth country. Another limitation is the lack of postnatal growth and nutritional data from the control group. The children were recruited at two Swedish university cities, with high socioeconomic status limiting the external validity of the results, even though we have tried to adjust for this in the analyses.

Even though the study sample was powered to detect clinically relevant differences between the groups, the size of the study population is still small compared to large national cohorts or register studies, designs that could be useful to support the findings in this current study.

The Nordic Health Questionnaire used was problematic to evaluate. Mostly since the constellation of families today often are complex, with the child sometimes living in two different households. This made is difficult to evaluate socioeconomic status and even size of the family, from a single questionnaire.

Ethical considerations
This study was approved by the regional ethical boards in Stockholm and in Umeå. The parents received oral and written information about the study and were asked to sign a written consent form before entering the study. The participants could drop out of the study at any time. There were no profits involved in entering the study, the only advantage was that parents were
promised information regarding abnormal test results and follow-up concerning these.

Before blood samples were collected, each child was offered a local anesthesia patch to minimize the discomfort. If the child declined any tests, his or her request was accepted.

Regarding possibility of exposure to radiation during the DXA examination, a local radiation committee reviewed the study protocol and stated that we did not have to apply for approval for this study, since the radiation levels were very low.

The aim of this study was to explore possible cardiovascular risk and neurocognitive outcomes for children born with marginally LBW, which is a large group of children being born every year. The results can support future guidelines so that these children will get the best possible care. Therefore, the benefits of the study were considered greater than the risks.
Conclusions and clinical implication

Cardiovascular risk
In this foremost observational follow-up study, we explored cardiovascular risk, including anthropometrics and laboratory markers, in 7-year-old children born with marginally LBW and compared those to peers born with NBW.

The marginally LBW children were thinner, shorter and had a higher prevalence of underweight at 7 years of age, suggesting no anthropometric signs of increased cardiovascular risk, in contrast with the theory of metabolic programming. The largest risk of being underweight were among those born SGA.

- The observed high rates of underweight and short stature suggest that strengthened focus regarding such outcomes are needed in health care follow-up programs of the large group of children born in this weight interval.
- High rates of overweight or obesity should not be expected at early school age. However, this does not exclude that the risk of overweight might be increased later in life, which is why longer follow-up studies, including this and other subgroups of LBW and SGA children, are recommended.

In agreement with the theory of adverse metabolic programming, the LBW children showed an increased risk of high fasting insulin levels and the children born SGA had a significantly higher level of mean fasting glucose. The findings require confirmative studies but may suggest an increased risk of T2DM.

- The clinical relevance of the observed signs of glucose and insulin imbalance in the marginally LBW children is still unclear, although considering the high rates of CVD in adult populations, the findings are likely to be relevant. Before we can recommend any clinical interventions, we suggest larger studies with even longer follow-up schedules, to further investigate this risk in those born with marginally LBW.

Early growth and nutrition
According to our results, weight gain during the first year of life has a significant impact on several outcomes at 7 years of age including positive correlation to BMI and fat mass, as well as fasting glucose and ApoB/ApoA1. Another significant predictor of long-term risk was breastfeeding that correlated negatively to fasting
glucose and adverse lipid profile, supporting previous studies suggesting that breastfeeding lowers the risk of hyperglycemia, T2DM as well as hyperlipidemia.

- The correlation between early growth and later BMI, fat mass, fasting glucose and lipid profile, give further support to the hypothesis that weight gain during infancy may have long term consequences. However, since the overall cohort was underweight, rather than overweight, the association to BMI might not be harmful and the ideal growth rate in marginally LBW children should be further explored, optimally in intervention studies.

- Breastfeeding have protective effect against glucose intolerance and hyperlipidemia in LBW infants, and should be recommended as their optimal diet.

**Iron supplementation**

While the anthropometric outcomes and the laboratory risk markers did not depend on the early iron intervention, this study showed that the children who received iron supplementation between 6 weeks and 6 months of age had approximately 2 mmHg lower systolic BP at 7 years of age, and their odds of having a high systolic BP was reduced by 68 %. This suggests that marginally LBW children may be at risk of high BP but that this risk is reversed with iron supplements. Furthermore, it suggests that iron might have a mechanistic role in the *adverse metabolic programming*, in this and other settings.

Early iron supplementation had no effect on the herein studied neurocognitive tests (WISC, Beery VMI and TEA- Ch) but in previously published work from the same study cohort, a decreased risk of iron deficiency and behavioral problems was observed.

- 2 mg/kg/day of iron supplements should be recommended to marginally LBW children between 6 weeks and 6 months of age, since it gives long-term beneficial effects including improved behavioral outcomes and a reduced risk of having a high BP.

- Iron availability should be included as a possible mediator in future mechanistic studies of long-term programming effects on BP.
Neurocognitive results

The marginally LBW children had lower scores in verbal comprehension IQ, visual-motor integration and selective attention. This indicates that being born a little bit too small increase the risk of several cognitive abilities being inadequate, which might result in poorer school achievement compared to NBW born peers.

The marginally LBW children born at term had the lowest scores for verbal comprehension and visual-motor integration, whereas poor attention results were prominent in both the term and the preterm born children. Consequently, there might be different mechanisms behind affected verbal comprehension and integration of hand-finger movements, compared the ability of attention.

- Clinicians and teachers should be aware of the possible neurocognitive deficits these otherwise healthy children might encounter, to be able to act on time for those children that might be in need of special support.
- Special attention should be given to these children at school entry.
Acknowledgements

My supervisor, Staffan Berglund – thank you for your patience, for always taking your time and for teaching me that doing research is interesting, fun and unwinding. If you and Magnus had not been so insistent and if you had not taken me under you wing, I would never had managed to do a PhD.

My co-supervisor, Magnus Domellöf – thank you for the encouragements, for all the feedback and for arranging lovely conference dinners.

My co-authors - Mikael Norman, for always reviewing our manuscripts with an open mind and coming up with new ideas. Björn Westrup, for your interesting opinions and thorough reviewing. Tove Öhrman, for doing a detailed job with the DXA and assisting us with the manuscript.

Our research nurses, Åsa Sundström and Kerstin Andersson – for your gentle and professional work with the children and their parents throughout the years.

Carina Lagerqvist, Catarina Lundell and all the other ladies in the lab – thank you for you attentive and thorough work analyzing our blood samples.

The pediatric psychologists Lea Forsman, Stephanie Sundén-Cullberg, Anna Crüsell and Marie Adamsson Johansson – for a great job testing and administrating the neurocognitive assessments of the children.

My fellow PhD students – thank you for helpful feedback, for interesting discussions and for making me feel like part of the team despite the distance. A special thank you to Frida, Cornelia, Itay, Ulrica, Tove and Marie, that I have had the pleasure to get to know and work with a little extra.

Leif Gothefors – for letting me interview you in med school and for mediating contact with Magnus and Staffan.

Karin Moström – for always assisting me, my fellow PhD students and our supervisors regarding the administrative work and planning.

Elina, Elisabeth, Ania, Tove, Torbjörn, Niklas, Marlene, Anna, Christina and all other staff at the Pediatric department, thank you for being a lovely company at the department as well as at conferences, for always being helpful and for the encouragements.
Agneta Brunzell – for your support during my medical practice and your flexibility arranging my complex schedules.

Nils – my partner in crime. Thank you for all your support, your endless love and for reminding me of what matters in life. You are going to be a wonderful dad.

Mum, dad and Emma – thank you for all the hysterical laughers, the cheering on and the unconditional love.

My grandparents Kerstin, Sune, Carina and Per-Erik, who always acknowledge my efforts and supports me in any ways possible.

My friends, and especially Madde, Linda and Mia – for cheering me on, for always being there, and for being you.

Danne and Tyra – for all the times you let me crash at your place when in Umeå, there are no better hosts than you guys.

Sara and Hanna, and your lovely families, thank you for all the get-togethers, the baby cuddle and all the fika. Looking forward to more of everything.

Vera – your feet are gorgeous.
References

74


122. Bennet L, Groop L, Franks PW. Ethnic differences in the contribution of insulin action and secretion to type 2 diabetes in immigrants from the


