Sex steroids, IGF-I, and vascular morphology from birth to adulthood in individuals born small for gestational age

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To my family

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ABSTRACT

Aim: To study whether there is an association between size at birth, sex steroids, IGF-I, and retinal vascular morphology.

Patients and methods: Two different cohorts were studied. In paper I, 25 young adult men born small for gestational age (SGA) were compared to 44 young adult men born appropriate for gestational age (AGA). In papers II–IV, participants were recruited from a cohort of 247 moderately to late preterm infants (137 boys and 110 girls). In paper II, 78 infants underwent an examination of retinal vascular morphology in the neonatal period and IGF-I was determined in umbilical cord blood. In paper III, the steroid hormone pattern in umbilical cord blood from 168 infants (99 boys and 69 girls) was determined by gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry. In paper IV, sex steroids were analyzed by GC-MS/MS and IGF-I determined from birth to 10 months corrected age in 98 boys.

Results: In paper I, young men born SGA were found to have elevated serum levels of estradiol and dihydrotestosterone (DHT), possibly due to increased activity of the enzymes aromatase and 5α-reductase, respectively. Birth weight standard deviation scores correlated inversely with estradiol-to-testosterone ratio and with DHT-to-testosterone ratio at adult age. Catch-up growth from birth to adult age also correlated with estradiol-to-testosterone ratio and with DHT-to-testosterone ratio.

In paper II, birth weight and IGF-I in umbilical cord blood were found to be the most important predictors of abnormal retinal vascularization.

In paper III, boys born SGA had lower estrone levels and girls born SGA had higher androstenedione levels than those born AGA, possibly due to decreased placental aromatase. Infants born SGA of both genders had lower cortisone levels.

In paper IV, boys born SGA had elevated testosterone levels at around the estimated date of birth. A DHT surge during minipuberty was seen, but this was less pronounced in boys born SGA. At 10 months corrected age, testosterone and androstenedione levels correlated to catch-up growth.

Conclusions: Individuals born SGA have an altered sex steroid pattern at different time-points in life. Further longitudinal studies are needed to investigate whether these changes are permanent and have a clinical impact.

Keywords: small for gestational age, preterm, sex steroid, estradiol, testosterone, dihydrotestosterone, glucocorticoid, IGF-I, retina.

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SAMMANFATTNING PÅ SVENSKA

Mål: Att undersöka om det finns ett samband mellan födelsestorlek, könshormoner, IGF-I och kärlstruktur i ögonbotten.

Patienter och metoder: Två grupper av individer studerades. I arbete ett undersöktes 25 unga vuxna män födda små för tiden. De jämfördes med 44 unga män födda normalstora för tiden. I arbete två till fyra rekryterades nyfödda från en större studie av 247 barn som var måttligt och lätt för tidigt födda (137 pojkar och 110 flickor). I arbete två genomgick 78 barn ögonundersökning för bedömning av blodkärl i ögonbotten och i navelsträngsblod analyserades tillväxtfaktorn IGF-I. I arbete tre undersöktes steroidhormoner i navelsträngsblod från 168 barn (99 pojkar och 69 flickor) med masspektrometri-baserade metoder. I arbete fyra analyserades könshormoner och IGF-I i blod från födelsen till 10 månaders korrigerad ålder hos 98 pojkar.

Resultat: I arbete ett visade sig män födda små för tiden ha ökade nivåer av östradiol och dihydrotestosteron (DHT), möjligen p.g.a. ökad aktivitet av enzymerna aromatas och 5α-reductas som katalyserar syntesen av dessa hormoner. Födelsevikt i standardavvikelser korrelerade negativt med östradiol-testosteron-kvoten och DHT-testosteron-kvoten i vuxen ålder. Även återhämtningstillväxt från födelse till vuxen ålder korrelerade med östradiol-testosteron-kvoten och DHT-testosteron-kvoten.
I arbete två fann vi att födelsevikt och IGF-I i navelsträngsblod var de viktigaste prediktorerna för förekomst av onormala kärl i ögonbotten.
I arbete tre visade vi att pojkar födda små för tiden hade lägre östron och flickor födda små för tiden hade högre androstendion i navelsträngsblod. Det skulle kunna bero på minskad aktivitet av enzymet aromatas i moderkakan. Både pojkar och flickor födda små för tiden hade lägre nivåer av kortison i navelsträngsblod.
I det fjärde arbetet fann vi att pojkar födda små för tiden hade förhöjd nivå av testosteron ungefär vid tiden för beräknat födelsedatum. Vi visade också att pojkar hade en stegring av DHT vid den så kallade minipuberteten, men att den var mindre uttalad hos pojkar födda små för tiden. Vid 10 månaders korrigerad ålder korrelerade nivåerna av testosteron och androstendion till återhämtningstillväxt.

Sammanfattning: Individer födda små för tiden har en annorlunda könshormonprofil. Det behövs longitudinella studier för att undersöka om dessa förändringar är bestående och av medicinsk betydelse.

LIST OF PAPERS

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

I. **Allvin K**, Ankarberg-Lindgren C, Fors H, Dahlgren J. Elevated serum levels of estradiol, dihydrotestosterone, and inhibin B in adult males born small for gestational age. *J Clin Endocrinol Metab. 2008 Apr; 93(4):1464-1469.*

II. **Allvin K**, Hellström A, Dahlgren J, Andersson Grönlund M. Birth weight is the most important predictor of abnormal retinal vascularisation in moderately preterm infants. *Acta Paediatr. 2014 Jun; 103(6):594-600.*

III. **Allvin K**, Ankarberg-Lindgren C, Niklasson A, Jacobsson B, Dahlgren J. Altered umbilical sex steroids in preterm infants born small for gestational age. *J Matern Fetal Neonatal Med. 2019 Apr 18:1-7.*

IV. **Allvin K**, Ankarberg-Lindgren C, Dahlgren J. Minipuberty in moderately to late preterm boys: longitudinal sex steroid and IGF-I data. *Manuscript.*

CONTENT

ABBREVIATIONS

3β-HSD	3β-hydroxysteroid dehydrogenase
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
17β-HSD	17β-hydroxysteroid dehydrogenase
AGA	Appropriate for gestational age
BMI	Body mass index
CV	Coefficient of variation
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle-stimulating hormone
GA	Gestational age
GC-MS/MS	Gas chromatography–tandem mass spectrometry
GnRH	Gonadotropin-releasing hormone
GPGRC	Gothenburg Pediatric Growth Research Center
hCG	Human chorionic gonadotropin
IGF-I	Insulin-like growth factor I
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
LGA	Large for gestational age
LH	Luteinizing hormone

LOD	Limit of detection
RIA	Radioimmunoassay
ROP	Retinopathy of prematurity
SD	Standard deviation
SDS	Standard deviation scores
SGA	Small for gestational age
SHBG	Sex hormone-binding globulin

DEFINITIONS IN SHORT

Term	Infant born at 37+0 – 41+6 weeks of gestation
Late preterm	Infant born at 34+0 – 36+6 weeks of gestation
Moderately preterm	Infant born at 32+0 – 33+6 weeks of gestation
Very preterm	Infant born at 28+0 – 31+6 weeks of gestation
Extremely preterm	Infant born at under 28 weeks of gestation

1 INTRODUCTION

Infants born small for gestational age (SGA) are at increased risk of medical complications such as hypoglycemia or jaundice even in the neonatal period. As adults, they are known to have an increased risk of developing cardiovascular morbidity and insulin resistance. We still lack knowledge about how steroid hormones, and more specifically sex steroids, may be deranged in individuals born SGA, and whether an altered hormone profile in that case contributes to the development of cardiovascular diseases. Determination of steroid hormones in infants and children is challenging. Due to low serum concentrations of many sex steroids before puberty, laboratory techniques need to be accurate and sensitive.

1.1 STEROID HORMONES

Steroid hormones include two major groups of hormones derived from cholesterol: corticosteroids and sex steroids. Corticosteroids are divided into mineralocorticoids and glucocorticoids, while sex steroids are divided into androgens, estrogens, and progestogens (1).

1.1.1 ADRENALS

The adrenal glands consist of two parts: the outer cortex and the inner medulla. The cortex synthesizes steroid hormones, while the medulla synthesizes catecholamines. The cortex consists of three layers: zona glomerulosa, zona fasciculate, and zona reticularis. The synthesis of hormones from the adrenal cortex is under control of adrenocorticotropic hormone, secreted by the anterior pituitary gland (2).

Zona glomerulosa produces mineralocorticoids, the most important one being aldosterone.

Zona fasciculata produces glucocorticoids, mainly cortisol, which is reversibly converted to biologically inactive cortisone via the enzymatic activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) (2). Serum cortisol levels show an individual circadian pattern, preserved throughout childhood (3).

Zona reticularis produces androgens such as androstenedione, dehydroepiandrosterone (DHEA), and its sulfated form dehydroepiandrosterone sulfate (DHEAS). DHEA is an adrenal precursor of sex steroids, and can be converted to androgens and estrogens in peripheral target tissues (2) (figure 1). DHEA is the precursor of androstenedione (4), which in turn is synthesized to testosterone in the testes (4,5). DHEA and DHEAS are transported bound to albumin, and DHEA is also bound by sex hormone-binding hormone (SHBG) (6). The half-life is longer and the diurnal variation is lower for DHEAS than for DHEA (7).

1.1.2 SEX STEROIDS IN MALES

The male gonads, testes, are controlled by the hypothalamus and the pituitary. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), stimulating synthesis and secretion of the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary.

FSH binds to the plasma membrane of the testicular Sertoli cells, located in the seminiferous tubules, and stimulates spermatogenesis at several levels (2). The role of the Sertoli cell is to support the multistep development of the primordial germ cells into spermatozoa. Sertoli cells also produce inhibin B, which suppresses FSH secretion (2), and has been suggested as a marker of spermatogenesis (8).

LH stimulates Leydig cells in the testes to produce testosterone, the major androgen. LH thereby indirectly enhances spermatogenesis (2). Dihydrotestosterone (DHT) is formed when testosterone is converted by 5α-reductase in peripheral tissue such as skin, hair follicles, liver, and prostate gland (9). In plasma, testosterone is transported bound to albumin (54%), SHBG (44%), or unbound as free and active testosterone (2%) (2).

Androgens are crucial for the development of male external genitalia, as shown in individuals with androgen insensitivity syndrome, who develop external female genitalia, despite being genetically male (46XY) (2). Moreover, androgens are important for growth and erythropoiesis (2).

In men, estradiol is mostly produced by aromatization of androgens in peripheral tissues (muscles and adipocytes), and only 15–25% is synthesized in the testes (2,10). Estrogens are crucial for acceleration of the pubertal growth spurt, closure of the epiphyses, and bone density. Furthermore, estrogens are important for metabolic control, as shown in men with aromatase mutations,

leading to lower estradiol levels. These men have an increased risk of impaired glucose tolerance, high low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol and triglycerides, as well as of increased visceral fat (11). An excess of estrogens in males may cause gynecomastia (2).

In prepubertal boys, estrone is the major estrogen (12). Estrone is synthesized in adipose tissue from androstenedione by the enzymatic activity of aromatase (12). Estrone is a weak estrogen and can be further transformed into estradiol by 17β-hydroxysteroid dehydrogenase (17β-HSD) (Figure 1).

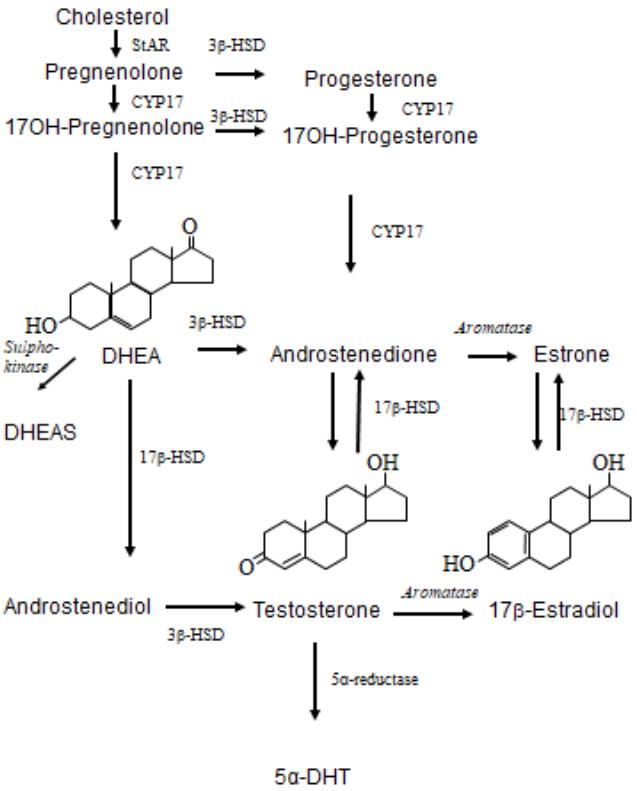

Figure 1. Pathways of testosterone and 17β-estradiol (estradiol) synthesis. Steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), 17α-hydroxylase (CYP17), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 5α-dihydrotestosterone (5α-DHT).

1.1.3 SEX STEROIDS IN FEMALES

As in males, female gonads, the ovaries, are under the control of GnRH, FSH, and LH. Due to the fertile woman's menstrual cycle, the pattern of sex hormone secretion is more complex over time than in males. The menstrual cycle is usually 26–35 days long, with menstruation starting on day one and lasting about five days (13).

When the menstruation is over, the follicular phase starts, during which pituitary FSH is secreted, stimulating granulosa cells and initiating follicular growth. FSH concentrations peak during the mid-follicular phase, around the day a dominant follicle is selected, and thereafter declines. The dominant follicle secretes estradiol for about a week before ovulation. Estradiol has a positive effect on the hypothalamus and pituitary, initiating a GnRH pulse and a LH surge (13). The luteal phase usually starts on day 14 in the menstrual cycle with a LH peak, initiating ovulation and development of the corpus luteum. The corpus luteum secretes progesterone and estradiol, reaching its maximum level 6–7 days after ovulation (13).

In women, estradiol is the major estrogen during their fertile years, and it is important for secondary sexual characteristics such as breast enlargement (2). Estradiol circulates bound to albumin (60%), to SHBG (38%), and free (2%) (6). However, during prepubertal years, estrone dominates (12), whereas estriol, together with smaller amounts of estradiol and estrone, is secreted from the placenta during pregnancy (14).

Although androgens are considered predominantly male hormones, they are also of importance in females. DHEAS is the most abundant androgen in the circulation, followed by DHEA, androstenedione, testosterone, and DHT: the latter two exert androgenic effects. DHEAS is produced only in the adrenal, the others in both the adrenal and the ovary: for instance, testosterone is synthesized from the adrenal (25%), from the ovary (25%), and from peripheral conversion of androstenedione (50%) (15).

In women, androgens in physiologic concentrations promote normal follicular development in the ovary, whereas androgen excess dysregulates follicular development. In breast cancer, androgens may both suppress or promote tumor growth, depending on which receptors for androgens and estrogens the tumor expresses (11).

1.2 INSULIN-LIKE GROWTH FACTOR I

Insulin-like growth factor I (IGF-I) is a peptide hormone with structural similarity to insulin, and it promotes growth and metabolism.

IGF-I mediates the effects of growth hormone, secreted from the anterior pituitary gland. It is mainly synthesized by the liver, but it also has autocrine/paracrine effects in peripheral tissue, such as bone and skeleton. IGF-I circulates mainly bound to insulin-like growth factor-binding protein 3 (IGFBP-3). It is produced throughout life, but serum concentrations vary depending on factors such as gender, age, levels of sex steroids, inflammation, and nutritional status, with the highest concentrations seen during the pubertal growth spurt (1,4).

1.3 PREGNANCY AND THE FETUS

1.3.1 PLACENTA

Steroid hormones are involved in pregnancy from implantation to parturition. In the pregnant woman, serum concentration of progesterone and estrogens increase dramatically during the pregnancy (14) (figure 2).

Human chorionic gonadotropin (hCG), secreted by the placenta, maintains the corpus luteum, until the placenta reaches its full steroidogenic potential (16). Progesterone is synthesized by the placenta in two steps, from cholesterol via pregnenolone, to progesterone (14) (figure 3). Furthermore, the placenta produces large amounts of estrogens, using androgen precursors. Androstenedione is synthesized from DHEA with the help of 3β-hydroxysteroid dehydrogenase (3β-HSD). Estriol is, in a multistep process, synthesized from DHEAS from the fetal adrenal glands, estrone from androstenedione, and estradiol from testosterone, with the help of aromatase (P450aro) (14) (figure 3).

Interestingly, a previous study showed that pregnant women bearing fetuses with intrauterine growth restriction had lower serum estriol levels (17). Furthermore, animal studies provide evidence that maternal androgen excess may affect fetal growth, given that the offspring of rats treated with testosterone in late pregnancy have lower birth weight (18).

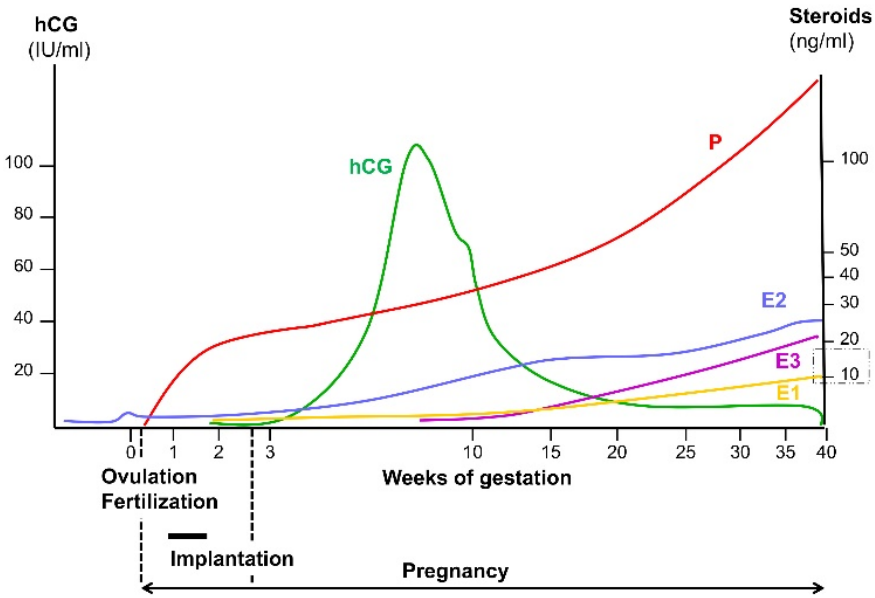

Figure 2. Plasma steroids in the mother during the pregnancy, showing human chorionic gonadotropin (hCG), progesterone (P), estrone (E1), estradiol (E2), and estriol (E3). The Y-axis on the left side shows hCG (IU/ml). The Y-axis on the right side shows steroid concentrations (ng/ml), conversion factor to SI units (nmol/L); Px3.18, E2x3.67, E3x3.47, E1x3.70. Reproduced from Morel Y, Roucher F, Plotton I, Goursaud C, Tardy V, Mallet D. Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis. Ann Endocrinol (Paris). 2016;77(2):82-89. Copyright 2016, published by Elsevier Masson SAS. All rights reserved.

1.3.2 PREECLAMPSIA

Preeclampsia, defined as blood pressure >140/90 mm Hg and proteinuria after 20 weeks' gestation, occurs in 6–10% of all pregnancies (19). Placentas from preeclamptic pregnancies have an increased expression of androgen receptors (20), and a decreased aromatase activity (21), corresponding well with increased maternal testosterone (22), and decreased maternal estradiol (23) levels in preeclampsia.

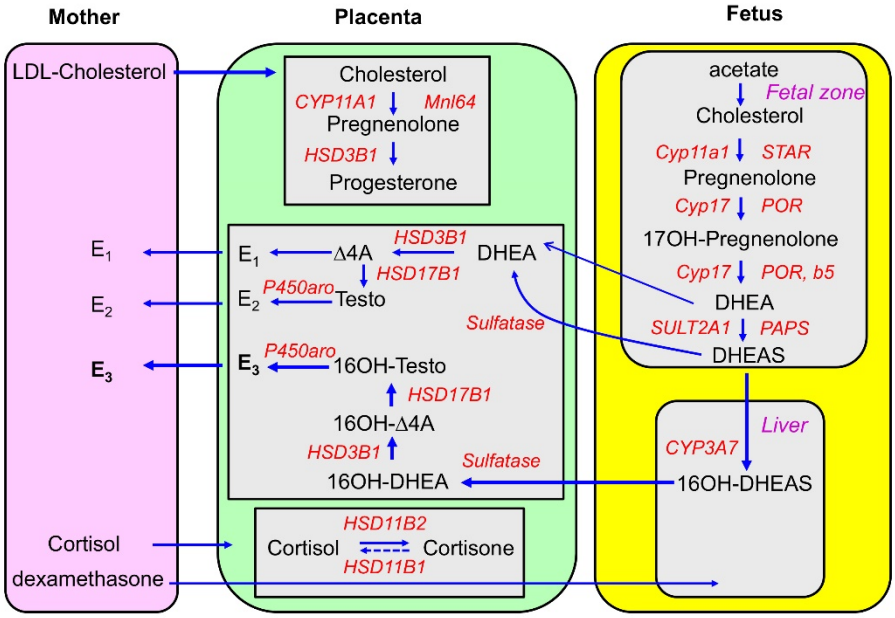

Figure 3. Pathway of biosynthesis and metabolism of steroids during pregnancy. Reproduced from Morel Y, Roucher F, Plotton I, Goursaud C, Tardy V, Mallet D. Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis. Ann Endocrinol (Paris). 2016;77(2):82-89. Androstenedione (ΔA4), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulafte (DHEAS), estrone (E1), estradiol (E2), estriol (E3), hydroxy (OH), testosterone (testo), cytochrome p450 (CYP), hydroxysteroid dehydrogenase (HSD), aromatase (p450arom), steroidogenic acute regulatory protein (STAR), sulfotransferase (SULT). Copyright 2016, published by Elsevier Masson SAS. All rights reserved.

1.3.3 FETAL GONADS AND EXTERNAL GENITALIA

Fetal gender is determined in gestational week 7. In male fetuses, Sertoli cells and Leydig cells are developed in the testes. Sertoli cells secrete Anti-Müllerian hormone, promoting development of male internal genitalia and regression of the Müllerian ducts (24). Leydig cells secrete testosterone, promoting development of the Wolffian ducts into the testes, epididymis, vas deferens, and seminal vesicles (9). In fetal life, DHT is important for differentiation of the male external genitalia.

Hypospadias is a congenital malformation with proximal displacement of the urethral opening, penile curvature, and a ventrally deficient hooded foreskin. It is likely that abnormalities in androgen synthesis may lead to hypospadias. The incidence of hypospadias is higher in boys born SGA (25) and in boys born after placental insufficiency in early gestation (26). It has been proposed that hypospadias could be caused by placental insufficiency, since DHT in the male fetus is synthesized by both testosterone (from the testis), and androsterone (from the placenta) (27).

Boys conceived by intracytoplasmic sperm injection, because of male subfertility, may have an increased risk of hypospadias (28). Furthermore, testicular Leydig cell function may be impaired in these boys, as shown by lower serum testosterone at three months of age (29).

Female fetuses lack Anti-Müllerian hormone, and the Müllerian ducts therefore develop into female internal sex organs (24).

1.3.4 INTRAUTERINE GROWTH AND IGF-I

During pregnancy, the placenta delivers nutrients and oxygen to the fetus, thereby controlling its growth (30). Placental growth hormone changes the mother's metabolism to a state of insulin resistance, facilitating transport of nutrients to the fetus (31). Prenatally, IGF-I is regulated by insulin, and these hormones, in addition to insulin-like growth factor II, are important for the regulation of fetal growth (31). Diabetes mellitus type 1 during pregnancy, with hyperglycemia, may lead to fetal hyperinsulinemia, fetal overgrowth, and an infant born large for gestational age (LGA) (32).

1.3.5 SMALL FOR GESTATIONAL AGE

Genetics and environmental factors influence size at birth (33,34). About 5% of all infants are born SGA, either in birth weight or birth length (35). No global consensus for the definition of SGA exists. The World Health Organization defines SGA as birth weight or birth length below the 10th percentile for gestational age (36), but cutoffs at the 5th or 2.3rd percentile are also used. In Sweden, the definition used for SGA is birth weight or birth length below -2 standard deviation scores (SDS) (corresponding to the 2.3rd percentile). Being born SGA has a variety of causes, such as poor nutrition (37), preeclampsia (38), infection (39), smoking (38), alcohol abuse (40), or fetal chromosomal anomalies (41). Low birth weight, on the other hand, is defined by the World Health Organization birth weight below 2500 g, regardless of gestational age (36).

Prematurity is defined as birth before 37 weeks of gestation (42). The global incidence was estimated as 11.1% of all living births in 2010 (43), but it varies around the world, and in Sweden it was 5.5% in 2017 (44). Preterm birth is classified as late preterm between 34+0 and 36+6 weeks of gestation (45), moderately preterm between 32+0 and 33+6 weeks (46,47), very preterm between 28+0 and 31+6 weeks (43), and extremely preterm at less than 28 weeks (43).

1.4 NEONATE

1.4.1 STEROID HORMONES

DISCONNECTION FROM THE PLACENTA

At birth, the fetus is disconnected from the placenta, and rapid endocrinological changes take place in the neonate, with decreasing levels of estrogens and progesterone (14), placental growth hormone (48), and hCG (49) in the circulation.

ANDROGENS

In term infants, there are known gender differences in androgens in umbilical cord blood. Boys have significantly higher testosterone and DHT concentrations than girls (50,51), but higher (52) or similar levels of DHEA and androstenedione (51).

Gestational age at birth correlates negatively with testosterone (52), but positively with DHEA (52) and DHEAS (53). Previous studies on androstenedione are inconclusive, one showing a positive correlation (52), and another no correlation (51) with gestational age.

The testes are active at birth, as shown by higher testosterone concentrations in peripheral blood than in cord blood (54).

Androgens may be of importance for fetal growth, since a previous study showed that children with partial androgen insensitivity syndrome have a birth weight between the reference for boys and girls, but those with complete androgen insensitivity have a birth weight comparable with girls (55).

ESTROGENS

Levels of estrone and estradiol in cord blood do not differ between genders (56). Cord blood estrogen levels correlate with gestational age (56), but not with size at birth (57,58), and neonates born after intrauterine growth restriction have similar umbilical estradiol levels as neonates of normal size (59).

GLUCOCORTICOIDS

During pregnancy, the placental enzyme 11β-HSD2 protects the fetus from high maternal cortisol levels, by converting cortisol to biologically inactive cortisone. Infants born after intrauterine growth restriction have an attenuated placental 11β-HSD2 activity and lower cortisone-to-cortisol ratio in cord blood (60). In preterm infants, both 11β-HSD2 activity and cortisone concentration in cord blood correlate with birth weight SDS (61), and cortisone and cortisol concentrations increase with gestational age (62). Interestingly, very preterm infants have a deficit in mineralocorticoids, glucocorticoids, and adrenal androgens at birth (62). Furthermore, placentas of preeclamptic women have a high content of cortisol, due to a reduced 11β-HSD2 activity (63).

1.4.2 IGF-I

IGF-I is a crucial growth factor during fetal and neonatal life, supported by growth retardation and reduced intellectual capacity in individuals suffering from IGF-I defects (64). Cord IGF-I concentrations correlate with birth weight and birth length (65), peripheral fat tissue accumulation (66), and gestational age in preterm neonates (67). Boys have lower cord IGF-I levels, despite being bigger at birth (68).

Neonates born after intrauterine growth restriction, diagnosed by repeated prenatal ultrasound, have reduced cord IGF-I levels (69). Neonates born after preeclamptic pregnancies have lower cord IGF-I levels than one would expect by the growth restriction alone (70). Placentas from preeclamptic pregnancies express less IGF-I than placentas from healthy pregnancies, even more pronounced if the fetus is SGA (71).

1.4.3 RETINAL VASCULAR MORPHOLOGY

In humans, the retina is fully developed and vascularized at gestational age 37 weeks. Premature infants with immature retina may develop retinopathy of prematurity (ROP), a proliferative vascular retinal disease. In industrialized countries with modern neonatal intensive care, very and extremely preterm infants are at risk of developing ROP, whereas in middle and low income countries, neonates born at higher gestational age may also risk developing ROP (72).

In Sweden, neonates born before gestational age 31 weeks are screened for ROP (73), although at the time of the study in paper II, the screening was for neonates born before gestational age 32 weeks. Since infants born moderately to late preterm do not undergo neonatal retinal examination in the industrialized world, less is known about their retinal vascular morphology. Furthermore, we lack knowledge of the impact of prenatal factors such as preeclampsia and intrauterine growth restriction on normal retinal vascularization in moderately to late preterm infants. Interestingly, animal studies have shown that lack of IGF-I in knockout mice prevents normal retinal vascular growth (74).

1.5 INFANT

1.5.1 STEROID HORMONES

ADRENALS

The neonate's adrenals undergo involution after birth, with falling serum levels of adrenal hormones such as androstenedione, DHEAS, and cortisol in the neonate's circulation (75). Girls have higher serum levels of DHEA and DHEAS until 3 months of age (76), but similar serum levels of cortisol to boys (76).

Term SGA infants have an altered adrenocortical steroid pattern in the early neonatal period. They show low glucocorticoid levels in the first 12 hours of life, in combination with elevated aldosterone levels, likely reflecting either reduced adrenocortical synthesis or a less stressful neonatal adaptation in infants born SGA compared to those born appropriate for gestational age (AGA) (77).

In preterm infants, an immature adrenal steroidogenesis is seen, since the fetal zone of the adrenal cortex persists until after term (78). They have higher DHEA, DHEAS, and androstenedione levels during their first month of life, and in preterm boys androstenedione decreases more slowly after birth than in term boys (76).

GONADS

Male minipuberty was first described in 1974 by Forest et al. (79). It is characterized by a postnatal transient activation of the hypothalamic–pituitary–testicular axis, and is believed to be important for future fertility. Levels of LH increase (80), followed by a rise in testosterone, peaking at 1–3 months of age and thereafter declining to 6 months of age (81,82). Minipuberty leads to an increased number of Sertoli cells (83), Leydig cells (84,85), and germ cells (86,87).

The minipuberty in term boys born SGA is more prolonged, with a later decline in testosterone (76).

Preterm boys have an increased postnatal hypothalamic–pituitary–testicular axis activation, with increased levels of gonadotropins FSH and LH, as well as testosterone in urine during the first six months of life compared to term boys. Furthermore, preterm boys have a faster testicular and penile growth compared to full-term boys (88).

Female minipuberty has not been as well described as male minipuberty. However, there is evidence of a postnatal activation of the hypothalamus–pituitary–ovarian axis in girls (89,90). Estrogen effects on peripheral organs can be seen in 3-month-old infants of both genders, but more frequently in girls (91).

1.5.2 GROWTH

Infancy is a period of intensive growth. By the age of two years, 87% of children born SGA have completed catch-up growth in height (92). Growth velocity is significantly faster from birth to 6 months of age in boys than in girls, with the greatest difference at one month of age, at the time of the peak of postnatal gonadal activation in boys (93). There is also a correlation between neonatal sex steroids and size in preschool children, as seen by a positive relation between cord progesterone and testosterone and weight-to-height ratio at age 4 years in girls. For boys, there was an association between cord estrogens and height at 4 years of age (94).

1.6 CHILD AND ADOLESCENT

1.6.1 STEROID HORMONES

ADRENARCHE

An increase in adrenal androgens, adrenarche, is seen several years before the onset of puberty. Serum concentrations of DHEA and DHEAS increase in boys at 7–8 years and in girls at 6–7 years of age (12).

Size at birth may impact the adrenals in childhood. A study of prepubertal children found that those with low birth weight had a higher excretion of urinary adrenal metabolites (from DHEAS, cortisol, and cortisone) (95). Furthermore, in children born SGA, there is evidence of a more pronounced adrenarche (96-98) and elevated serum DHEAS concentrations in adolescence if they have no catch-up growth (99).

In girls, birth weight is inversely related to morning peak cortisol, whereas in boys and young adult males, birth weight is inversely related to cortisol responses to stress (100).

PUBERTY

In boys, puberty starts with testis enlargement, usually between 9 and 14 years of age (101). For girls, puberty usually starts with breast enlargement somewhat earlier, at 8 to 12 years of age (101).

Being born SGA does not seem to affect the onset of puberty in boys (102), whereas girls born SGA may have an earlier onset of puberty and menarche (102), a low responsiveness to FSH, and a reduced ovulation rate (103,104).

Preterm birth, on the other hand, does not seem to affect the onset of puberty in either gender (105).

1.6.2 CARDIOVASCULAR FACTORS, GROWTH, AND IGF-I

Even in childhood, the associations between size at birth, postnatal growth, blood pressure, and IGF-I are seen. For instance, at 7 years of age, children with marginally low birth weight born SGA may present with early signs of glucose imbalance (106). The impact of early growth is evident as early as at 8 years of age, as seen by correlations between weight gain from birth to 3 years of age and decreased insulin sensitivity, current body mass index (BMI), and waist circumference (107). The importance of the prenatal and neonatal environment is revealed in early childhood, since preterm infants with low IGF-I in the neonatal period may have increased blood pressure at 4 years of age (108).

1.6.3 RETINAL VASCULAR MORPHOLOGY

In a previous study of preschool children, weight, length, and head circumference at birth were associated with narrower retinal arteriolar caliber at 6 years of age (109). Birth size also has an impact in adolescence, since there is an association between low birth weight and narrower retinal arterioles in 12-year-old children. Furthermore, those born with smaller head circumference have an increased risk of having reduced complexity of their retinal microvasculature (110).

1.7 ADULT

1.7.1 STEROID HORMONES

ADRENALS

Low birth weight is associated with increased fasting cortisol levels in adult life (111), which has been suggested as a factor in the link between low birth weight and hypertension. Cortisol-to-cortisone ratio is an indirect marker of 11β-HSD2 enzyme activity. Suboptimal activity of 11β-HSD2 may have negative metabolic consequences (112).

GONADS

In adult males, low birth weight is associated with increased risk of testicular cancer, especially seminomas (113). It is still unclear whether low birth weight or intrauterine growth restriction affect adult sex hormone status and fertility in men. Previous studies show contradictory results, with either no effect of being born preterm, SGA or intrauterine growth-restricted on hormone status and testicular size in young adult men (114,115), or a tendency towards hypogonadism with smaller testes, increased LH, and lower serum testosterone and inhibin B in young men born SGA without complete catch-up growth (116).

In young women who had very low birth weight, there is no evidence of disturbed gonadal function, since they have levels of reproductive hormones (LH, FSH, estradiol, testosterone, and SHBG) that are similar to healthy controls (117).

1.7.2 CARDIOVASCULAR FACTORS, GROWTH, AND IGF-I

In 1990, David Barker, a British epidemiologist, launched his theory, later called the Barker hypothesis, of a fetal origin of diseases in adulthood. Size at birth is the result of gestational length, genetics, intrauterine life and maternal environment, nutrition and diseases such as preeclampsia, and diabetes mellitus type 2.

Low birth weight increases the risk of developing insulin resistance, hypertension, and hyperlipidemia in adulthood (118-120), but also mortality due to increased risk of cardiovascular disease, such as cardiac-related death, hypertension, stroke, and diabetes mellitus type 2 (121). Moreover, one study showed that adults born preterm and now in their twenties had increased systolic blood pressure (122). Furthermore, in boys, low weight gain during infancy is correlated with increased risk of coronary heart disease in adulthood, regardless of size at birth (123).

In men, steroid hormones seem to be of importance for cardiovascular health, since low serum levels of DHEA and DHEAS predict an increased risk of major coronary heart disease (124). Furthermore, low serum testosterone and high serum estradiol are associated with lower-extremity peripheral artery disease (125).

Cardiovascular disease is also linked to IGF-I, as low serum IGF-I levels increase the risk of ischemic heart disease within the following 15 years in healthy middle-aged individuals (126) and also correlate with impaired glucose tolerance and diabetes mellitus type 2 (127).

The adiponectin-to-leptin ratio is a marker of cardiovascular fitness (128). Adiponectin and leptin are two adipocytokines secreted by adipose tissue. Low serum adiponectin levels are seen in obesity and diabetes mellitus type 2 (129), while serum leptin levels correlate with body fat (130).

1.7.3 RETINAL VASCULAR MORPHOLOGY

Even in adult age, the influence of growth restriction or prematurity at birth is seen in the microvasculature structure of the retina. In adults, both those born with low birth weight (131) and those born after intrauterine growth restriction (132) have abnormal retinal vascular morphology. In very, but not extremely, preterm women, a higher length index for retinal arterioles, fewer branching points, and high casual blood pressure compared to women born at term have been reported (133).

2 AIM

GENERAL AIM

The principal aim of this thesis was to study whether there is an association between size at birth and sex steroids, IGF-I, and retinal vascular morphology.

SPECIFIC AIMS

- To evaluate the sex hormone levels and indirectly the different enzyme activities in adult males born SGA (paper I)
- To find predictors of abnormal retinal vascularization in moderately to late preterm newborn infants considered to have no risk of developing ROP (paper II)
- To investigate whether infants born SGA have an altered steroid profile at birth (paper III)
- To evaluate the influence of size at birth on changes in sex steroids and IGF-I during minipuberty in boys (paper IV)
- To investigate the association between androgen secretion, IGF-I, and growth during infancy in boys (paper IV)

3 PATIENTS

3.1 YOUNG ADULT MEN COHORT (PAPER I)

The study was perfomed as a case-control study, where the study group comprised of 25 adult men born SGA, defined as a birth weight and/or birth length below -2 SDS, according to the Swedish reference for newborns (134). All these men had a spontaneous catch-up growth and were of normal height, defined as a final height above -2 SDS according to the Swedish reference (135). Twenty-two of these men were recruited from a previously described cohort of children born SGA (136), and three men were recruited from a population-based cohort (137). Five were born preterm (gestational age 33–36 weeks), and the rest at term. Auxological data were available at 2 years of age for 20 of the men. They had a median age of 23.1 years and median final height of -0.5 SDS at examination.

The control group comprised of 44 healthy adult men of normal stature, recruited from the same population-based cohort (137). Thirty-nine of the controls were born AGA and five were born LGA, defined as a birth weight or birth length above 2 SDS, according to the Swedish reference for newborns (134). Forty-two were born term, and two were born preterm (gestational age 33 and 36 weeks). They had a median age of 20.5 years and median final height of 0.4 SDS at examination.

All of the participants in the study had reached their final height and had a testicular volume of at least 20 ml. None of them had hypospadias or cryptorchidism. All of the blood samples were collected between 8 a.m. and 10 a.m. after a 12-h fasting period. The blood samples in both groups studied were collected over a period of three months. Sera were frozen and stored at –80 ºC until hormone determinations were made.

3.2 MODERATE-TO-LATE PRETERM COHORT (PAPERS II–IV)

The study population was recruited prospectively as a population-based cohort. All the study participants were born during a period of 1 year and 9 months at the two delivery wards at Sahlgrenska University Hospital in Gothenburg. Two hundred forty-seven neonates (137 boys, 110 girls), born between gestational age 32+0 and 36+6, were included in the moderate-to-late preterm cohort. Of the 247 neonates, 195 were singletons (113 boys, 82 girls), and 52 were twins (24 boys, 28 girls). Fifty-two neonates (27 boys, 25 girls) were born SGA, defined as a birth weight and/or birth length below -2 SDS, according to the Swedish reference for newborns established by Niklasson et al. (138). Three singleton boys had cryptorchidism on one side, and one twin boy had cryptorchidism on one side in combination with hypospadias. For further details about birth characteristics and maternal medical background, see table 1.

From the moderate-to-late preterm cohort, 68 neonates (44 boys, 34 girls) were included in paper II, 168 neonates (99 boys, 69 girls) in paper III, and 98 boys in paper IV. In paper II, twins were also included, but not in paper III and IV, where sex steroids were determined. The decision to exclude twins was based on the twin testosterone transfer hypothesis, which states that twin girls with a male co-twin are exposed to androgen excess in utero (139), leading to reduced fecundity (140).

Table 1. Birth characteristics of the moderate-to-late preterm cohort.

	All n=247	Boys n=137	Girls n=110	P value
Gestational age (week)	35.6 (32.0 – 36.9)	35.6 (32.1 – 36.9)	35.4 (32.0 – 36.7)	0.367
Birth weight (g)	2495 (550 – 3885)	2500 (1015 – 3885)	2398 (550 – 3815)	0.057
Birth length (cm)	46.0 (29.0 – 54.0)[a]	47.0 (36.0 – 54.0)[b]	46.0 (29.0 – 51.0)	0.037
Head circumference (cm)	32.5 (23.4 – 36.5)[a]	33.0 (26.0 – 36.5)[b]	32.0 (23.4 – 35.0)	0.001
Birth weight (SDS)	-0.55 (-10.1 – 2.63)	-0.61 (-5.46 – 2.63)	-0.48 (-10.1 – 2.44)	0.999
Birth length (SDS)	-0.55 (-11.0 – 4.85)[a]	-0.58 (-5.72 – 4.85)[b]	-0.47 (-11.0 – 2.85)	0.964
Head circumference (SDS)	-0.05 (-5.48 – 2.25)[a]	0.02 (-3.39 – 2.25)[b]	-0.13 (-5.48 – 1.87)	0.403
Small for gestational age (n)	52 (21.1%)	27 (19.7%)	25 (22.7%)	0.563
Placenta weight (g)	536 (145 – 1160)[c]	530 (185 – 1160)[d]	550 (145 – 1160)[e]	0.324
Twin (n)	52 (21.1%)	24 (17.5%)	28 (25.5%)	0.128
Cesarean delivery (n)	90 (36.4%)	43 (31.4%)	47 (42.7%)	0.066
Maternal age (year)	30.5 (19.5 – 42.8)	30.3 (19.5 – 42.8)	31.1 (21.9 – 41.5)	0.206
Preeclampsia /hypertension (n)	58 (23.5%)	30 (21.9%)	28 (25.5%)	0.512
Diabetes mellitus (n)	13 (5.3%)	8 (5.8%)	5 (4.5%)	0.651
Assisted reproduction technologies (n)	27 (10.9%)	11 (8.0%)	16 (14.5%)	0.103
Antenatal betamethasone	56 (22.7%)	29 (21.2%)	27 (24.5%)	0.529

For continuous variables, data are expressed as median with range in parenthesis, and P values were calculated with the Mann–Whitney U test. For dichotomous values, data are expressed as number with percentage in parentheses, and P values were calculated with Pearson chi-square test. [a]n=246, [b]n=136, [c]n=234, [d]n=129, [e]n=105. The stated placenta weight may be for a mutual placenta for monozygotic twins.

For further details about the neonates included in papers II, III, and IV, respectively, please see figure 4.

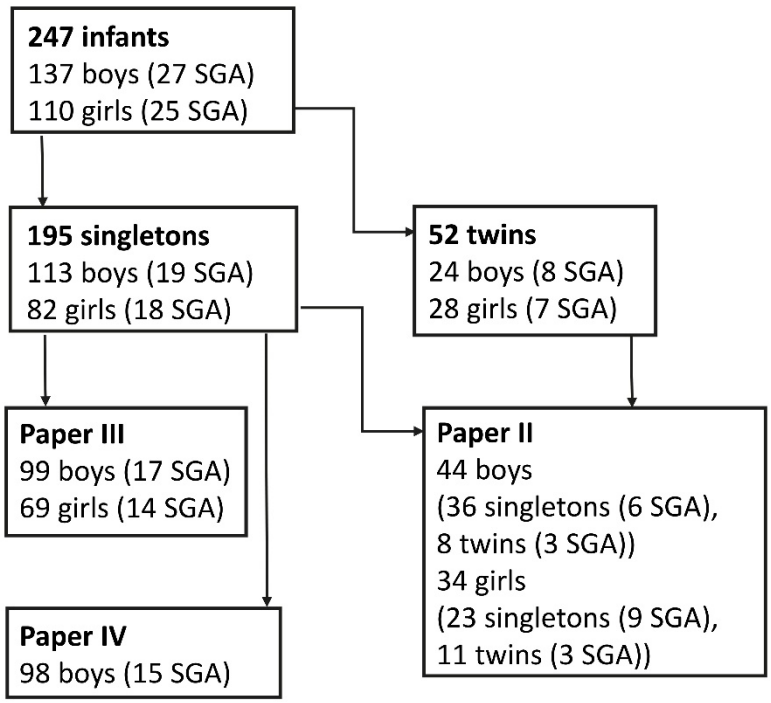

Figure 4. Flow chart infants included in papers II–IV.

Several infants were included in all three papers, II–IV, but some were only included in one or two of the studies. For details, see figure 5.

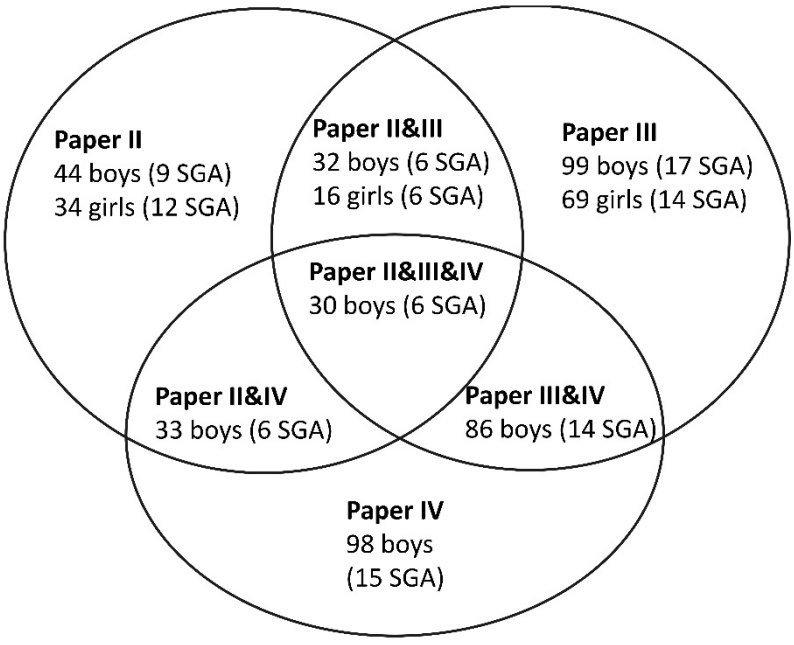

Figure 5. Overview of which infants were included in papers II, III, and IV.

3.2.1 MATERNAL DATA

Maternal data were collected from maternity centers and hospital charts. The median maternal age at delivery was 30.5 years.

Fifty-eight mothers developed hypertensive disorders of pregnancy. Of these, six had hypertension (blood pressure > 140/90); five mild, 12 moderate, and 27 severe preeclampsia (blood pressure >140/90 and proteinuria after 20 weeks of gestation); eight had HELLP syndrome (preeclampsia with hemolysis, elevated liver enzymes, and low platelet count).

Four mothers had diabetes mellitus type 1, and nine had a pathological oral glucose tolerance test during pregnancy (the latter defined as plasma glucose > 10.0 mmol/L at 2 h); of these, one needed insulin.

Twenty-seven neonates were conceived by assisted reproductive technologies: 24 with in vitro fertilization, two with induced ovulation, and one with sperm insemination.

To help improve the outcome for the preterm infants, antenatal betamethasone was given as a routine medication to pregnant women at risk of delivering before gestational week 35+0. In the moderate-to-late preterm cohort, antenatal betamethasone was given to 50 mothers before delivery. Of these women, six were pregnant with twins, which meant that a total of 56 neonates were exposed to antenatal betamethasone.

3.2.2 PAPER II

Of the 247 infants included in the moderate-to-late preterm cohort described above, the first 162 infants were considered for participation in a substudy examining retinal vascular morphology. Eighty-two of these were not enrolled in the study and did not undergo ophthalmological examinations due to early dropout in seven cases and early discharge in 75 cases. Parents of one twin boy were unwilling to let the boy undergo examination. Finally, one extremely growth-restricted girl who had been examined but later died, was excluded from the analysis (figure 6).

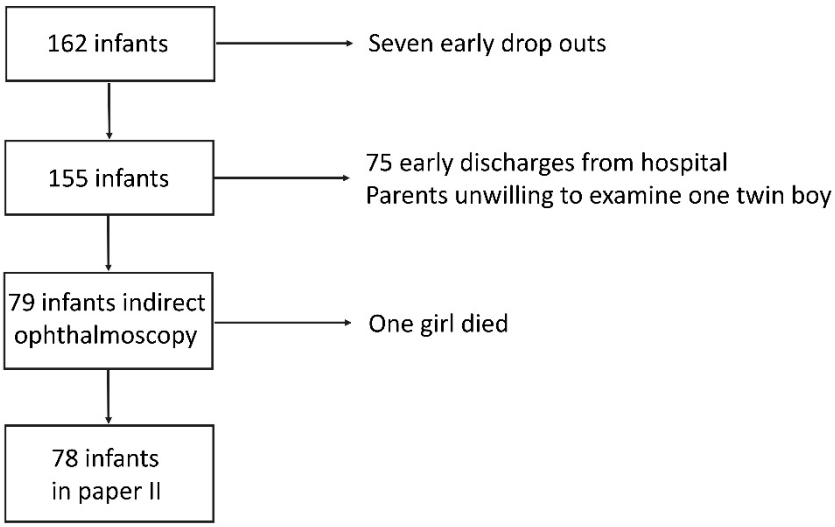

Figure 6. Flow chart of infants examined in paper II.

There was no difference in maternal illness, such as hypertension, preeclampsia and diabetes mellitus, in mothers of infants in the study group and those not examined. However, since 75 infants did not undergo an ophthalmological examination due to early discharge from the hospital, these were probably healthier at birth, and therefore gestational age and size at birth differed between the groups (table 2).

Table 2. Birth characteristics, showing differences between neonates who had an ophthalmological examination (the study group) and those who did not.

	Study group n=78	Not examined n=75	P value
Boys (n)	44 (56%)	42 (56%)	1.000
Small for gestational age (n)	21 (27%)	8 (11%)	0.018
Gestational age (week)	34.6 (32.3 – 36.9)	36.3 (32.3 – 36.9)	0.0001
Birth weight (g)	2250 (1190 – 3575)	2660 (1585 – 3420)	0.0001
Birth length (cm)	45.0 (38.0 – 50.0)[a]	47.0 (42.0 – 51.0)	0.0001
Head circumference (cm)	31.8 (27.5 – 36.5)	33.0 (29.5 – 35.0)	0.0001

For continuous variables, data are expressed as median with range in parenthesis, and P values were calculated with the Mann–Whitney U test. For dichotomous values, data are expressed as number with percentage in parentheses, and P values were calculated with Fisher's exact test.
[a]n=77

Umbilical venous blood was collected directly after birth and immediately chilled to 4 °C. The blood was centrifuged within 24 h, and thereafter sera were frozen and stored at -80 °C until IGF-I determinations were made.

3.2.3 PAPER III

The moderate-to-late preterm cohort included 195 singletons. Twenty-seven of these were excluded from the substudy in paper III due to maternal diabetes mellitus (n=8), conception by assisted reproduction technologies (n=11), or lack of an umbilical blood sample (n=8). In total, 168 singletons (99 boys, 69 girls) were included in paper III. They had a median gestational age of 35.6 weeks, median weight of 2510 g, and median length of 47 cm at birth. Three boys had cryptorchidism on one side, and two of these underwent orchidopexy after the end of the study. No boys had hypospadias.

Thirty-one infants (17 boys, 14 girls) included in the study were born SGA, defined as birth weight or birth length below -2 SDS, according to the Swedish reference for newborns (138). Twenty-seven of these (14 boys, 13 girls) were born to mothers who had developed preeclampsia during pregnancy.

Umbilical venous blood was collected directly after birth and immediately chilled to 4 °C. The blood was centrifuged within 24 h, and thereafter sera were frozen and stored at -80 °C until hormone determinations were made.

3.2.4 PAPER IV

Ninety-eight singleton boys from the moderate-to-late preterm cohort were included in paper IV. They had a median gestational age of 35.5 weeks, median weight of 2502 g, and median length of 47 cm at birth. These boys all had serial serum hormone determinations and auxological measurements made from birth to 10 months corrected age. Venous blood was drawn at 3– 7 days of age (at the time of routine screening for metabolic diseases), once a week if the baby was admitted to a neonatal ward, at around the estimated date of birth, 2, 5, and 10 months thereafter (figure 7). Blood sampling was performed at different time points during the day, and since the participants were infants, they were not fasting. Sera were frozen and stored at –80 ºC until hormone determinations were made.

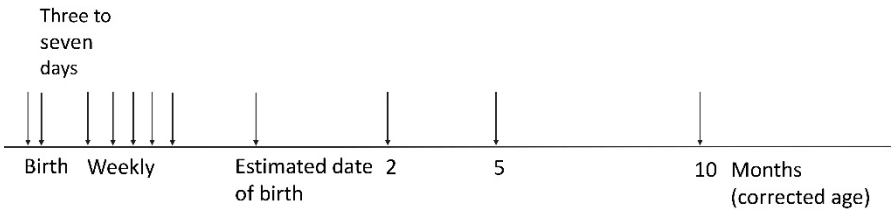

Figure 7. Auxological measurements and blood sampling in the neonatal cohort from birth to 10 months corrected age.

4 METHODS

4.1 AUXOLOGICAL MEASUREMENTS

WEIGHT

In the young adult men cohort (paper I), the participants' birth weight was collected from the Swedish Medical Birth Register. SDS for birth weight were calculated according to the Swedish reference for newborns (134). At 2 years of age and at adult age, weight was measured using electronic step scales, and weight SDS were calculated according to the Swedish growth reference from birth to 18 years by Wikland et al. (135).

In the moderate-to-late preterm cohort (papers II–IV), birth weight and weight during infancy were measured with the infant in the supine position using baby scales or electronic step scales. Gender-specific weight SDS were calculated according to the Swedish growth reference up to 24 months by Niklasson et al. (138).

HEIGHT

In the young adult men cohort (paper I), the participants' birth length was collected from the Swedish Medical Birth Register. Birth length SDS were calculated according to the Swedish reference for newborns (134). At two years of age, height was measured using a mechanical length board or a Harpenden stadiometer. At adult age, height was measured using an Ulmer stadiometer attached to the wall. Height was measured three times and the mean value calculated. SDS height at 2 years of age and at adult age were calculated according to the Swedish growth reference from birth to 18 years (135). Body Mass Index (BMI) was calculated as weight (kg)/length $(m)^2$.

In the moderate-to-late preterm cohort (papers II–IV), birth length and length during infancy were measured with the infant in the supine position, using electronic infant length boards. Length was measured three times and the mean value calculated. Gender-specific SDS were calculated according to the the Swedish growth reference up to 24 months (138).

HEAD CIRCUMFERENCE

In the moderate-to-late preterm cohort (papers II–IV), head circumference was measured from birth to approximately 10 months corrected age using measuring tape. Head circumference was measured twice and the mean value calculated. Gender-specific SDS were calculated using a reference by Niklasson et al. (manuscript in preparation).

4.2 HORMONE DETERMINATIONS

When the work with this thesis started, radioimmunoassays (RIAs) were used for determination of DHEAS, testosterone, DHT, and estradiol concentrations. Our laboratory at Gothenburg Pediatric Growth Research Center (GPGRC) has developed sensitive extraction RIAs for the determination of estradiol (141,142) and testosterone (143) in prepubertal and pubertal children. We have previously shown that RIA and tandem mass spectrometry are comparable when analyzing testosterone concentrations in prepubertal and pubertal children (144).

The use of accurate laboratory methods with high specificity and sensitivity for determination of hormones is of utmost importance, especially when studying sex steroid levels in the lower ranges in a pediatric population. For instance, there is a concern for cross-reactivity, giving falsely elevated testosterone, when using RIA in neonatal samples, emphasizing the importance of using mass spectrometry methods for testosterone determinations in neonates (145). Furthermore, previous studies measuring testosterone in cord blood with mass spectrometry also report lower concentrations than those using RIA (146). Comparisons of liquid chromatography–tandem mass spectrometry (LC-MS/MS) with direct immunoassays revealed the latter to be incorrect, with interferences when determining low concentrations of estrone and estradiol (147).

Figure 8. Equipment for gas chromatography–tandem mass spectrometry (left) and liquid chromatography–tandem mass spectrometry (right).

Ankarberg-Lindgren and co-workers have developed mass spectrometry-based methods for determination of sex steroids, especially useful in pediatric research and in clinical practice. In 2018, the gas chromatography–tandem mass spectrometry (GC-MS/MS) method for determination of androstenedione, testosterone, DHT, estrone, and estradiol concentrations in children was published (148). Ankarberg-Lindgren and co-workers also developed a LC-MS/MS method for determination of DHEAS, androstenedione, testosterone, cortisone, and cortisol concentrations in children (Ankarberg-Lindgren et al., manuscript in preparation). The serum samples in the studies presented in this thesis had been stored frozen for more than a decade the before analyses, but as has been shown in previous studies, sex steroids are not affected by long-term storage or repeated thaw/freeze cycles (149,150). In papers III and IV, not all blood sample volumes reached the prerequisite of 200 µL for GC-MS/MS. We were therefore forced to adjust the limit of detection (LOD) accordingly, the consequence of which was a larger number of samples resulting in hormone concentrations below LOD, and accordingly lower precision at low levels. Presumably, potential differences between study groups may have been masked and would have been more pronounced with larger serum volumes.

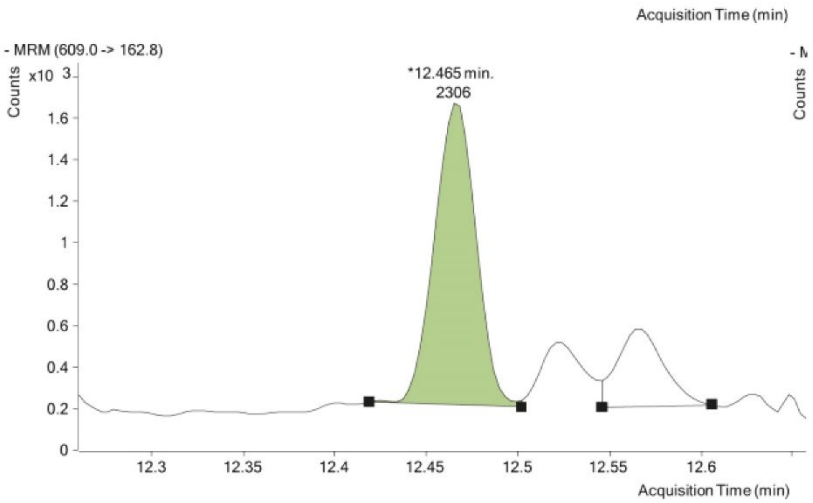

Figure 9. Representative chromatogram for testosterone analyzed by gas chromatography–tandem mass spectrometry. (MRM = multiple reaction monitoring.)

FOLLICLE-STIMULATING HORMONE

In paper I, serum FSH concentrations were determined in duplicate using time-resolved fluoroimmunoassay (AutoDELFIA FSH, Wallac, Turku, Finland). LOD was 0.05 U/liter. The intraassay coefficient of variation (CV) was less than 1% and the interassay CV was less than 3%.

LUTEINIZING HORMONE

In paper I, serum LH concentrations were determined in duplicate using time-resolved fluoroimmunoassay (AutoDELPHIA hLH Spec; Wallac, Turku, Finland). LOD was 0.05 U/liter. The intraassay CV was less than 9% and the interassay CV was less than 3%.

DEHYDROEPIANDROSTERONE SULFATE

In paper I, serum DHEAS concentrations were determined in duplicate using a RIA (Coat-A-Count DHEA-SO_4; Diagnostic Products Corp., Los Angeles, CA, USA). LOD was 0.03 μmol/L (11 ng/mL). The intraassay CV was less than 8% for values over 1.0 μmol/L (369 ng/mL). The interassay CV was 14% at 4.0 μmol/L (1476 ng/mL) and 11% at 14 μmol/L (5166 ng/mL).

In paper III, serum DHEAS concentrations were determined by LC-MS/MS (Ankarberg-Lindgren et al., manuscript in preparation). LOD was 0.1 μmol/L. Total CV was less than 7% at ≥1.0 μmol/L.

The conversion factor for DHEAS was: ng/ml x 0.00271= μmol/L.

ANDROSTENEDIONE

In paper III, serum androstenedione concentrations were determined by LC-MS/MS (Ankarberg-Lindgren et al. manuscript in preparation). LOD was 0.1 nmol/L. Total CV was 13% at ≥2.0 nmol/L.

In paper IV, serum androstenedione concentrations were determined by GC-MS/MS (148). LOD was 0.1 nmol/L. Total CV was 17% at 0.5 nmol/L.

TESTOSTERONE

In paper I, testosterone concentrations were determined using a modified RIA (Spectria testosterone; Orion Diagnostica, Espoo, Finland) (143). LOD was 0.03 nmol/L (0.01 ng/mL). The intraassay CV was less than 6% and the interassay CV was less than 8%. Free testosterone was calculated from testosterone, SHBG and albumin using the method described by Vermeulen et al. (151) and Dunn et al. (6), where the association constant for testosterone binding to albumin is $3.6x10^4$ L/mol for an average albumin concentration of 43 g/L (151), and the association constant for testosterone binding to SHBG is $1.6x10^9$ L/mol.

In paper III and IV, serum testosterone was determined by GC-MS/MS (148). LOD was 0.1 nmol/L. Total CV was 16% at 0.3 nmol/L and less than 10% at >1.5 nmol/L.

The conversion factor for testosterone was: ng/ml x 3.467= nmol/L.

DIHYDROTESTOSTERONE

In paper I, serum DHT concentrations were determined using an extraction RIA (active dihydrotestosterone RIA; Diagnostic Systems Laboratories, Webster, Texas, USA). LOD was 10 pmol/L (0.003 ng/mL). The intraassay CV was less than 6% and the interassay CV was less than 9%.

In paper III and IV, serum DHT concentrations were determined by GC-MS/MS (148). LOD was 27 pmol/L. Total CV was 15% at 55 pmol/L and 10% at 200 pmol/L.

The conversion factor for DHT was: ng/ml x 3440=pmol/L.

ESTRONE

In paper III and IV, serum concentrations of estrone were determined by GC-MS/MS (148). LOD was 9 pmol/L. Total CV was 14% at 38 pmol/L and 11% at ≥ 100 pmol/L.

ESTRADIOL

In paper I, serum estradiol concentrations were determined using an extraction-RIA (Spectria estradiol; Orion diagnostic, Espoo, Finland) (141). LOD was 6 pmol/L (1.6 pg/mL). The intraassay CV was 17% at 10 pmol/L (2.7 pg/mL) and less than 8% for levels above 44 pmol/L (12 pg/mL). The interassay CV was 29% at 11 pmol/L (3.0 pg/mL), 16% at 40 pmol/L (11 pg/mL), and 10% at 114 pmol/L (31 pg/mL).

In paper III and IV, serum concentrations of estradiol were determined by GC-MS/MS (148). LOD was 2 pmol/L. Total CV was 19% at 8 pmol/L and 6% at ≥ 36 pmol/L.

The conversion factor for estradiol was: pg/ml x 3.67= pmol/L.

CORTISOL

In paper III, serum cortisol concentrations were determined by LC-MS/MS (Ankarberg-Lindgren et al. manuscript in preparation). LOD was 11 nmol/L. Total CV was 7% at 240 nmol/L and 19% at 680 nmol/L.

CORTISONE

In paper III, serum cortisone concentrations were determined by LC-MS/MS (Ankarberg-Lindgren et al. manuscript in preparation). LOD was 1.8 nmol/L. Total CV was 15% at 60 nmol/L.

SEX HORMONE-BINDING GLOBULIN

In paper I, serum SHBG concentrations were determined using an immunoradiometric assay (Spectria SHBG immunoradiometric assay; Orion Diagnostica, Espoo, Finland). LOD was 1.3 nmol/L (0.15 µg/ml). The intraassay CV was less than 5% and the interassay CV was less than 6%.

The conversion factor for SHBG was: µg/ml x 8.6 =nmol/L.

INHIBIN B

In paper I, serum inhibin B concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratories, Webster, Texas, USA). The detection limit was 7 pg/mL. The intraassay CV was less than 12% and the interassay CV was 7% at 61 pg/mL and 6% at 121 pg/mL.

ADIPONECTIN

In paper I, serum adiponectin concentrations were determined by ELISA (Mediagnost, Reutlingen, Germany). LOD was 0.5 µg/mL. The intra- and interassay CVs were less than 10%.

LEPTIN

In paper I, serum leptin concentrations were determined by RIA (Linco Research, Inc., Charles, MO, USA). LOD was 0.3 ng/mL. The intraassay CV was less than 7% and the interassay CV was less than 10%.

INSULIN-LIKE GROWTH FACTOR I

In paper II and IV, serum IGF-I concentrations were determined using a specific RIA (Mediagnost GmbH, Tűbingen, Germany) (152). Samples were diluted 1:50. The measurement range was 3.9–250 µg/L. The intraassay CVs were 18%, 11% and 7% at concentrations of 9, 33, and 179 µg/L, respectively. The corresponding interassay CVs were 29%, 11%, and 6%. The total CV was 18% at 40 µg/L and 11% at 225µg/L.

4.3 METHODOLOGICAL CONSIDERATIONS

In the research reported in paper III, we initially used RIA for determinations of estradiol and testosterone concentrations in cord blood. We later discovered that RIA is not a reliable method for measurements of androgens in cord blood (146). Since the GPGRC laboratory had in the meantime developed the methods based on GC-MS/MS (148) and LC-MS/MS (Ankarberg-Lindgren et al., manuscript in preparation) described above, we decided to also analyze the umbilical cord blood samples with GC-MS/MS for 133 boys and 103 girls. Table 3 presents a comparison between our RIA and GC-MS/MS results for estradiol and testosterone, illustrating the importance of using accurate laboratory methods when determining sex steroids in cord blood.

Table 3. Cord blood concentrations of estradiol and testosterone in 133 boys and 103 girls, measured with RIA and GC-MS/MS.

	Estradiol RIA (pmol/L)	Estradiol GC-MS/MS (pmol/L)	Testosterone RIA (nmol/L)	Testosterone GC-MS/MS (nmol/L)
Boys (n=133; 109 singletons, 24 twins)	14770 (939 – 71765)	16660 (588 – 64987)	3.6 (0.9 – 11.2)	0.5 (0.2 – 8.1)
Girls (n=103; 77 singletons, 26 twins)	12442 (413 – 64348)	14457 (499 – 68825)	3.1 (0.7 – 15.0)*	0.2 (0.1 – 0.9)*

Data are expressed as medians with range in parentheses. *n=101

4.4 EXAMINATION OF RETINAL VASCULAR MORPHOLOGY

Seventy-eight neonates in the moderate-to-late preterm cohort underwent an ophthalmological examination at a mean postnatal age of 7 days (range 1– 28 days). Retinal vascular morphology (figure 10) was examined under cycloplegia (cyclopentolate 0.2% + phenylephrine 1%) by indirect ophthalmoscopy.

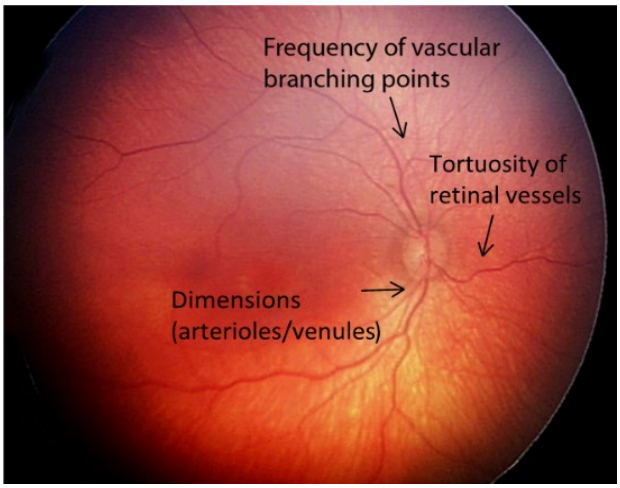

Figure 10. Retina of a preterm infant.

A specific, standardized study protocol (table 4) was used to evaluate retinal vascular morphology. Tortuosity of the retinal vessels (normal, moderately increased, significantly increased) was examined. The number of vascular branching points (decreased, normal, increased) were calculated. Retinal vessel caliber (narrow, normal, increased) was noted. The same pediatric ophthalmologist performed all the examinations, blinded to the neonate's gestational age, birth weight, or presence of growth retardation.

Table 4. Standardized study protocol for examination of retinal vascular morphology

Retinal vascular morphology	Left side	Right side
Tortuosity of retinal vessels (normal, moderately increased, significantly increased) Vascular branching points (decreased, normal, increased) Central retinal vessel caliber (narrow, normal, increased)		

4.5 STATISTICAL METHODS

Continuous data was presented as median and interquartile range (paper I), or median and range (paper II–IV). Since the data were not normally distributed, non-parametric statistical methods were used for the analyses: the Mann-Whitney U test for independent samples (papers I–III), and Wilcoxon matched-pairs signed-rank test for matched samples (paper IV).

Dichotomous data were presented as number and percentage (paper II-IV), and Fisher's exact test (paper II) or Pearson chi-square test (paper III and IV) was used for comparisons between groups.

All correlation analyses were made with Spearman non-parametric rank correlation (paper I, III, and IV).

In paper I, when comparing the concentrations of estradiol and DHT between SGA and AGA, we adjusted for the differences in adult age and height between groups using analysis of covariance with adult age and height as covariates.

In paper II, a stepwise logistic regression was conducted to find the variable, or set of variables, that best predicted abnormal retinal vascularization.

All tests were two-tailed and conducted at the 5% significance level.

Hormone concentrations below LOD were set to LOD/2 (paper III and IV).

The software IBM SPSS Statistics for Windows (IBM Corp, Armonk, NY, USA) was used for statistical analyses.

4.6 ETHICAL APPROVAL AND INFORMED CONSENT

The Ethics Committee of the Medical Faculty of the University of Gothenburg approved the studies. For paper I, written informed consent was obtained from the participants (approval number Ö 350-99), and for papers II–IV it was obtained from the parents of the participants (approval numbers Ö 562-01 and 1067-18).

5 RESULTS

5.1 YOUNG ADULT MEN BORN SGA

5.1.1 ANDROGENS

Adult men born SGA had similar median testosterone concentrations to controls, 14.9 nmol/L (4.29 ng/ml) vs. 15.6 nmol/L (4.50 ng/ml), but higher DHT concentrations, 1867 pmol/L (0.543 ng/ml) vs. 1456 pmol/L (0.423 ng/ml) ($p<0.05$). After adjusting for the differences in adult age and height between SGA and controls, in an analysis of covariance there was still a significant mean difference between SGA and controls for DHT of 313 pmol/L (0.091 ng/ml, 95% confidence interval 48 – 578 pmol/L (0.014 – 0.168 ng/ml), $p<0.05$). DHT-to-testosterone ratio, a proxy of 5α-reductase activity, was higher in the SGA group than in controls, median 0.11 vs. 0.09 (in SI units: median 11.1 % vs. 9.0%) ($p<0.01$). DHT-to-testosterone ratio showed an inverse correlation with birth weight SDS ($r= -0.51$, $p<0.001$) (figure 11B) and birth length SDS ($r=-0.38$, $p<0.01$). On the other hand, there was a positive correlation between DHT-to-testosterone ratio and catch-up growth from birth to adult age, Δ weight (adult–birth) SDS ($r=0.39$, $p<0.01$) (figure 12B), and Δ height (adult–birth) SDS ($r=0.30$, $p<0.05$).

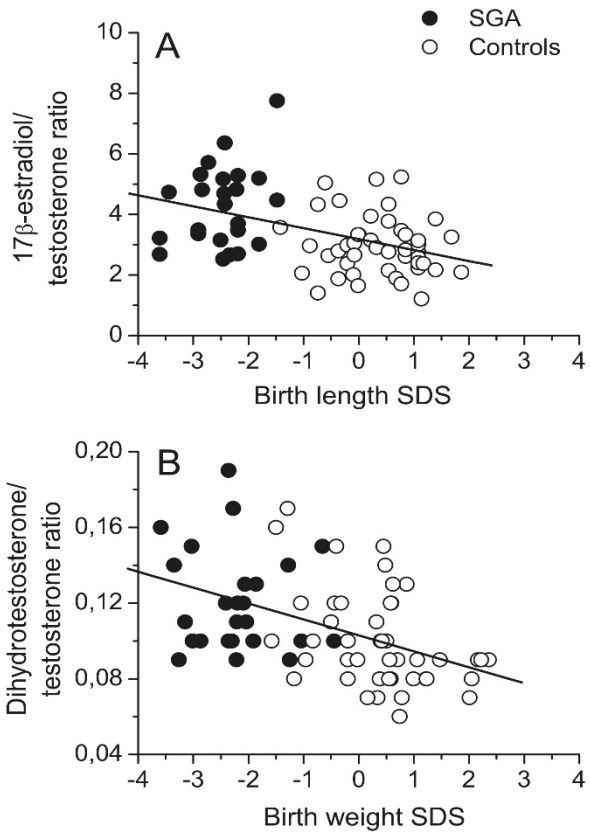

Figure 11. (A) Estradiol-to-testosterone ratio vs. birth length SDS, showing an inverse correlation (r=-0.44, p<0.001). (B) Dihydrotestoterone-to-testosterone ratio vs. birth weight SDS, also showing an inverse correlation. (r=-0.51, p<0.001). (Reprinted with permission from Oxford University Press: Allvin et al. J Clin Endocrinol Metab. 2008 Apr; 93(4):1464-1469.)

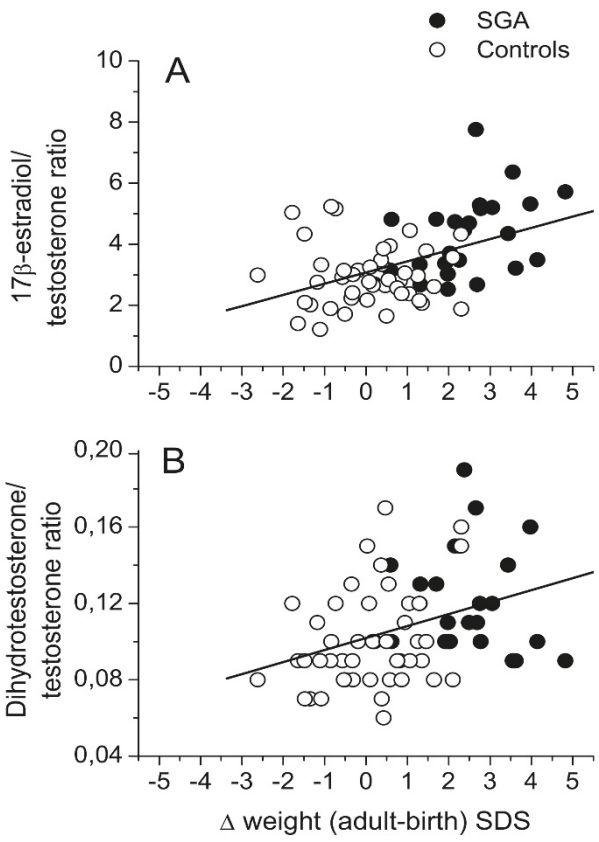

Figure 12. (A) Estradiol-to-testosterone ratio vs. Δ weight (adult–birth) SDS, showing a positive correlation (r=0.45, p<0.001). (B) Dihydrotestoterone-to-testosterone ratio vs. Δ weight (adult–birth) SDS, also showing a positive correlation. (r=0.39, p<0.01). Δ weight (adult–birth) = catch-up in weight from birth to adulthood. (Reprinted with permission from Oxford University Press: Allvin et al. J Clin Endocrinol Metab. 2008 Apr; 93(4):1464-1469.)

Auxological data was available at two years of age only for 20 men born SGA and for them catch-up in height from birth to two years of age correlated inversely with DHT-to-testosterone ratio at adult age (r=-0.61, p<0.01) (figure 13).

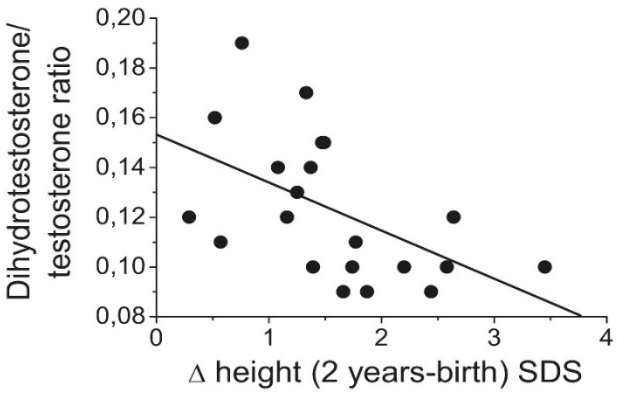

Figure 13. Dihydrotestosterone-to-testosterone ratio vs. Δ height (2 yr–birth) SDS, for SGA men only, showing an inverse correlation (r=-0.61, p<0.01). Δ height (2 yr–birth) = catch-up in height from birth to two years of age. (Reprinted with permission from Oxford University Press: Allvin et al. J Clin Endocrinol Metab. 2008 Apr; 93(4):1464-1469.)

5.1.2 ESTRADIOL

Adult men born SGA had higher serum estradiol concentrations than controls, median 66 pmol/L (17.9 pg/ml) vs. 46 pmol/L (12.6 pg/ml), p<0.001. After adjusting for the differences in adult age and height between SGA and controls, using analysis of covariance, there was still a significant mean difference between SGA and controls for estradiol of 18.7 pmol/L (5.1 pg/ml) (95% confidence interval 7.3 – 30.1 pmol/L (2.0 – 8.2 pg/ml), p<0.01).

Estradiol-to-testosterone ratio, a proxy of aromatase activity, was higher in men born SGA than in controls, median 4.34×10^{-3} vs. 2.81×10^{-3} (in SI units: median 0.46% vs. 0.30%), p<0.001. Estradiol-to-testosterone ratio showed an inverse correlation with birth weight SDS (r= -0.40, p<0.01) and birth length

SDS (r=-0.44, p<0.001) (figure 11A), and a positive correlation with catch-up growth from birth to adult age, Δ weight (adult-birth) SDS (r=0.45, p<0.001) (figure 12A), and Δ height (adult-birth) SDS (r=0.37, p<0.01). Estradiol and estradiol-to-testosterone ratio did not correlate with adult BMI.

5.1.3 INHIBIN B

Serum inhibin B concentrations were higher in adult men born SGA than controls, median 164 pg/ml vs. 137 pg/ml, p<0.05, and correlated inversely with birth length SDS (r=-0.34, p<0.01) (figure 14).

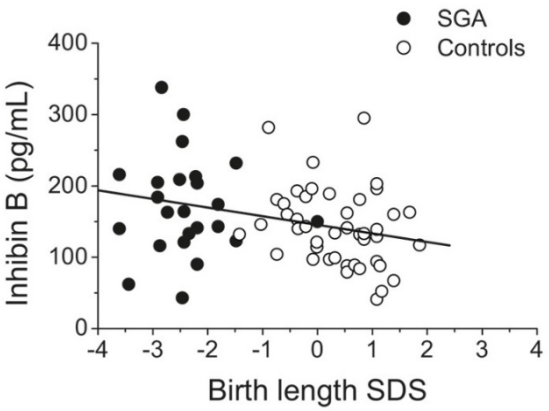

Figure 14. Serum inhibin B vs. birth length SDS, showing an inverse correlation (r=-0.34, p<0.01). (Reprinted with permission from Oxford University Press: Allvin et al. J Clin Endocrinol Metab. 2008 Apr; 93(4):1464-1469.)

5.1.4 ADIPOCYTOKINES

Men born SGA and controls had similar BMI, median 22.9 kg/m^2 vs. 22.7 kg/m^2, and leptin concentrations, median 3.00 ng/ml vs. 2.23 ng/ml. However, compared to controls, men born SGA had lower adiponectin concentrations, median 5.40 µg/ml vs. 6.35 µg/ml, $p<0.05$, and higher leptin-to-adiponectin ratio, $0.66x10^{-3}$ vs. $0.38x10^{-3}$, $p<0.05$. As expected, BMI correlated positively with leptin ($r=0.42$, $p<0.01$) and leptin-to-adiponectin ratio ($r=0.56$, $p<0.001$), and inversely with adiponectin ($r=-0.43$, $p<0.001$). Catch-up in weight from birth to adulthood, calculated as Δ weight (adult–birth) SDS, correlated with leptin-to-adiponectin ratio ($r=0.47$, $p<0.001$).

5.2 NEONATES AND INFANTS

5.2.1 ANDROGENS

The neonates in the moderate-to-late preterm cohort had similar DHEAS concentrations in umbilical cord blood, regardless of gender or being born SGA, median 3.2 µmol/L for both boys and girls. DHEAS concentration in umbilical cord blood correlated with gestational age at birth ($r=0.44$, $p<0.001$) (figure 15). DHEAS was not determined during infancy in this study.

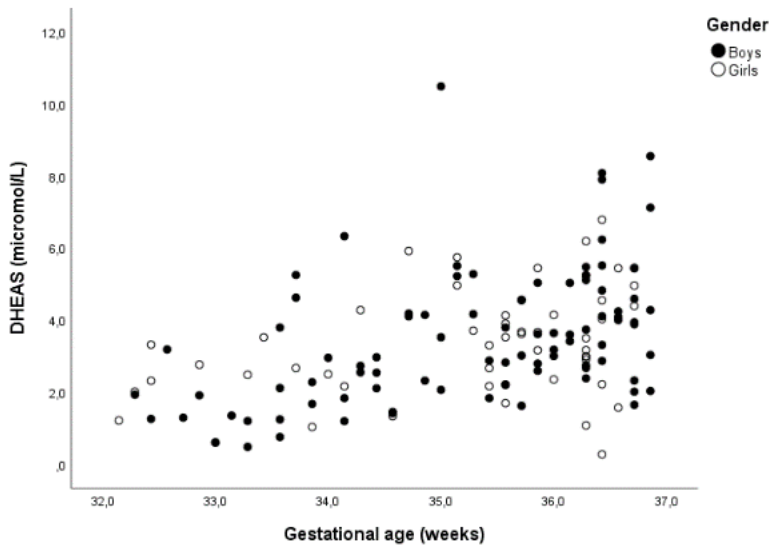

Figure 15. DHEAS vs. gestational age at birth in moderate-to-late preterm infants. For both boys and girls, DHEAS correlated with gestational age at birth ($r=0.44$, $p<0.001$).

In umbilical cord blood, androstenedione concentrations were similar between genders: for boys median 2.8 nmol/L, for girls 2.9 nmol/L. Girls born SGA had higher androstenedione concentrations than girls born AGA, median 4.0 nmol/L vs. 2.6 nmol/L, $p<0.01$ (figure 16 upper panel). Serum concentrations in boys, both SGA and AGA, declined to a median of 0.2 nmol/L at 10 months corrected age.

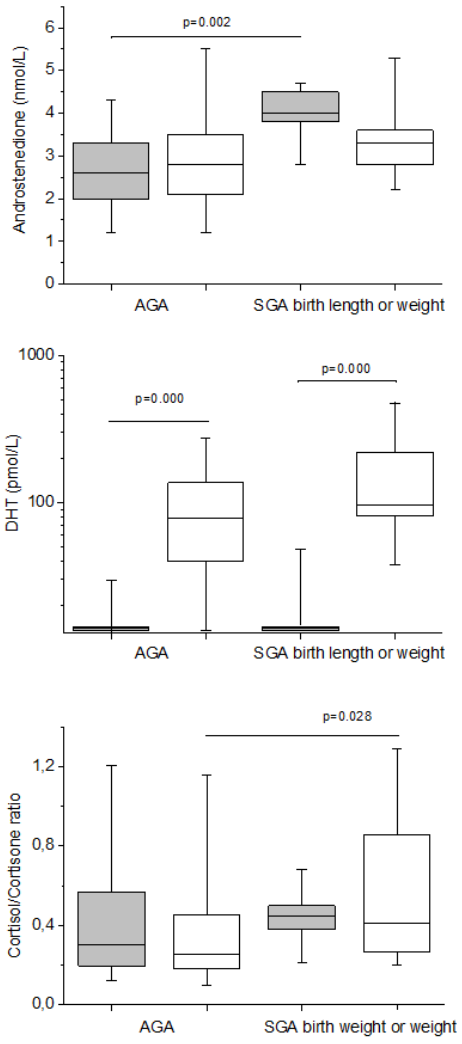

Figure 16. Hormone concentrations for androstenedione (panel A), dihydrotestosterone (DHT) (panel B), and cortisol-to-cortisone ratio (panel C), in umbilical cord blood in moderate-to-late preterm infants. Boys and girls born small for gestational age (SGA) are compared with those born appropriate for gestational age (AGA). Box plots show 5th, 25th, 50th, 75th, and 95th percentiles. Gray boxes represent girls, and white boxes represent boys. (Reprinted with permission from Taylor & Francis: Allvin et al. J Matern Fetal Neonatal Med. 2019 Apr 18:1-7.)

Testosterone concentrations in umbilical cord blood were higher in boys than in girls, median 0.5 nmol/L vs. 0.2 nmol/L, $p<0.001$. In boys, we found the previously described minipuberty, as testosterone increased from birth to median 4.7 nmol/L at 0 months corrected age, $p<0.001$, peaking at 2 months corrected age at a median 6.5 nmol/L, $p<0.001$, thereafter declining. Boys born SGA had higher testosterone concentrations than boys born AGA at 0 months corrected age, median 6.0 nmol/L vs. 4.4 nmol/L, $p<0.05$ (figure 17 upper panel). At 10 months corrected age, catch-up in weight, calculated as the difference in weight SDS from birth to 10 months corrected age, correlated with testosterone ($r=0.32$, $p=0.014$), and with androstenedione ($r=0.33$, $p=0.013$).

Boys had higher DHT concentrations in umbilical cord blood than girls, median 89 pmol/L vs. <27 pmol/L, $p<0.001$ (figure 16 middle panel). There was no difference in DHT concentrations in boys and girls born SGA vs. AGA, respectively (figure 16 middle panel). For all boys, DHT concentrations increased to median 1166 pmol/L at 0 months corrected age, $p<0.001$, and were unchanged to 2 months corrected age (figure 17 lower panel). In boys born AGA only, and with DHT determinations at both 0 and 2 months corrected age ($n=44$), there was a significant rise in DHT from 0 to 2 months corrected age, from median 996 pmol/L to 1161 pmol/l, $p<0.05$. In the first months of life, during minipuberty, DHT concentrations in moderate-to-late preterm boys (paper IV) are almost in the same range as in young adult men (paper I) (figure 17 lower panel).

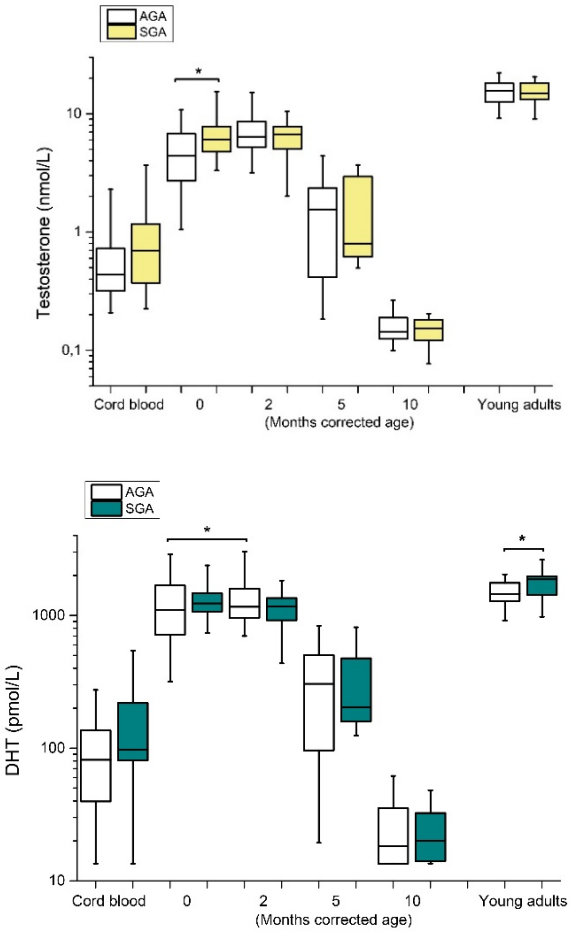

*Figure 17. Summary of the findings in paper I, III, and IV. Serum testosterone (upper panel) and DHT concentrations (lower panel) in males. Box whisker plots present hormone levels determined in umbilical cord blood (n=98 for testosterone vs. 96 for DHT) (paper III), at 0 (n=81 for both testosterone and DHT), 2 (n=69 for both testosterone and DHT), 5 (n=65 for testosterone vs. 64 for DHT), and 10 (n=69 for testosterone vs. 68 for DHT) months corrected age (paper IV) and in young adulthood (n=69 for both testosterone and DHT) (paper I). White boxes depict hormone levels in males born AGA and colored boxes depict hormone levels in males born SGA. Boxes represent the 25th, median, and 75th percentile, and whiskers represent the 5th and 95th percentile. *p< 0.05.*

5.2.2 ESTROGENS

Boys and girls had similar concentrations of estrone (median 57543 pmol/L vs. 59649 pmol/L) and estradiol (16885 pmol/L vs. 16751 pmol/L) in umbilical cord blood. Boys born SGA had lower estrone levels in umbilical cord blood than boys born AGA, 33822 pmol/L vs. 62471 pmol/L, $p<0.05$. During the time studied, from birth to 10 months corrected age, estrone and estradiol concentrations gradually declined in boys, with similar levels between boys born SGA and AGA.

5.2.3 GLUCOCORTICOIDS

Boys and girls had similar concentrations of glucocorticoids in umbilical cord blood. Neonates born SGA had lower cortisone concentrations that those born AGA, for boys 308 nmol/L vs. 521 nmol/L, $p<0.05$, and for girls 340 nmol/L vs. 579 nmol/L, $p<0.05$. Furthermore, boys born SGA had an elevated cortisol-to-cortisone ratio compared to boys born AGA, 0.41 vs. 0.25, $p<0.05$ (figure 16 lower panel). Glucocorticoids were not determined during infancy in this study.

5.2.4 IGF-I

There were no gender differences in IGF-I levels in umbilical cord blood in the SGA and AGA group (table 5). In both boys and girls, IGF-I correlated with gestational age and size at birth (data not shown). Infants of both genders born SGA had significantly lower IGF-I concentrations compared to those born AGA (table 5).

Table 5. Serum concentrations of IGF-I in umbilical cord for 99 boys and 69 girls born moderately to late preterm

	Boys SGA (n=17)	Boys AGA (n=82)	P value (boys SGA vs. AGA)	Girls SGA (n=14)	Girls AGA (n=55)	P value (girls SGA vs. AGA)	P value (boys vs. girls SGA)	P value (boys vs. girls AGA)
IGF-I (µg/L)	18 (3–79)	50 (9–97)	0.000	14 (6–96)	55 (11–99)	0.001	0.438	0.051

Data are presented as median with range in parentheses. P values were calculated with the Mann–Whitney U test.

In 86 moderate-to-late preterm boys, serum concentrations of IGF-I were repeatedly measured up to 10 months corrected age (figure 18). Fourteen boys were born SGA, and in these, a rise in IGF-I was seen from birth to 0 months corrected age (median 15 µg/L vs. 40 µg/L, $p<0.05$) and a decrease from 5 to 10 months (45 µg/L vs. 38 µg/L, $p<0.001$). AGA boys showed a different pattern, in which IGF-I decreased from 2 to 5 months corrected age (median 55 µg/L vs. 42 µg/L, $p<0.001$). At around estimated time of birth, there was a

negative correlation between testosterone and IGF-I (r=-0.42, p<0001), which was more pronounced for the SGA group (r=-0.60, p<0.05).

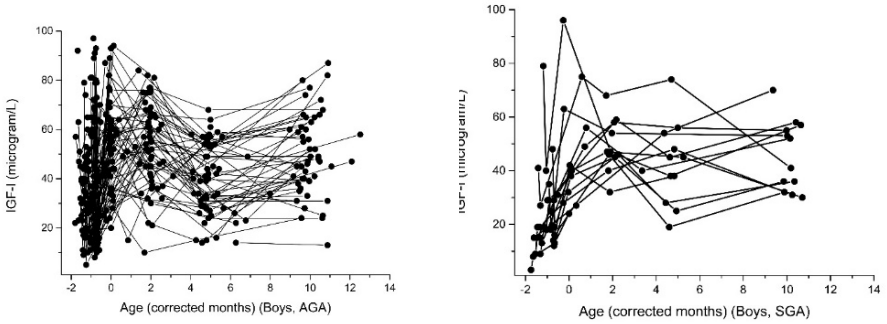

Figure 18. Longitudinal IGF-I versus age in moderate-to-late preterm boys included in both paper III and IV. Left panel boys born AGA (n=72), right panel boys born SGA (n=14). In AGA boys IGF-I decreased from 2 to 5 months corrected age (median 55 µg/L vs. 42 µg/L, p<0.001). In SGA boys a rise in IGF-I was seen from birth to 0 months corrected age (median 15 µg/L vs. 40 µg/L, p<0.05), and a decrease from 5 to 10 months (45 µg/L vs. 38 µg/L, p<0.001).

5.2.5 RETINAL VASCULAR MORPHOLOGY

In moderate-to-late preterm infants, retinal vascular morphology was examined at a mean postnatal age of 7 days. Twenty-one of 78 infant had abnormal retinal vascularization (increased vascular tortuosity (n=3), decreased number of branching points (n=12), increased number of vascular branching points (n=1), and narrow arterioles and/or venules (n=13)). They were to a higher extent born after pregnancies complicated by preeclampsia or hypertension (47.6% vs. 19.3%, p<0.05), and were more often born SGA (57.1% vs. 15.8%, p<0.01), compared to those with normal retinal vascular morphology (figure 19). Infants with abnormal retinal vascularization had lower IGF-I concentration in umbilical cords blood, 22 µg/L vs. 46 µg/L, p≤0.0001 (figure 20).

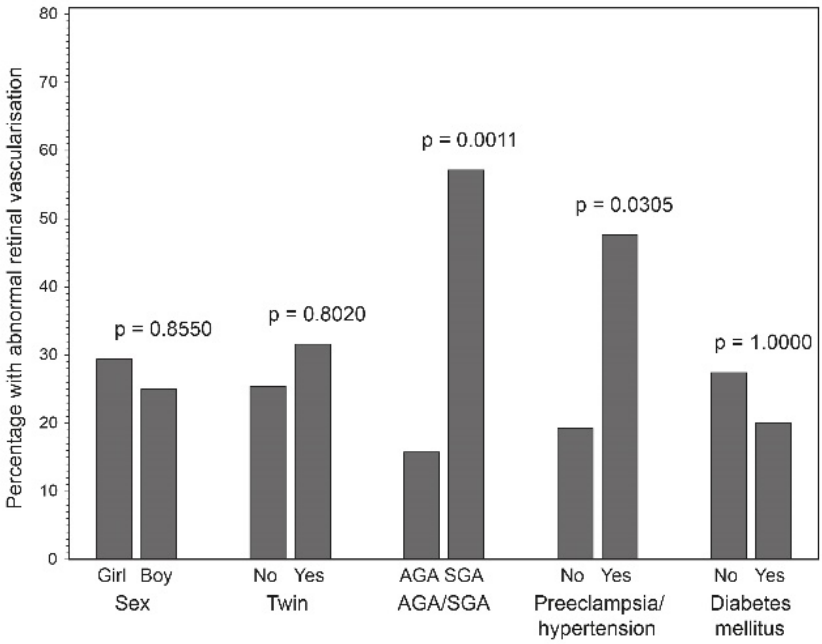

Figure 19. Percentage of infants with abnormal retinal vascularization vs. the following categories: sex, twin vs. singleton, SGA vs. AGA, preeclampsia and hypertension vs. normotensive, diabetes mellitus vs. no diabetes mellitus. (Reprinted with permission from John Wiley and Sons: Allvin et al. Acta Paediatr. 2014 Jun; 103(6):594-600.)

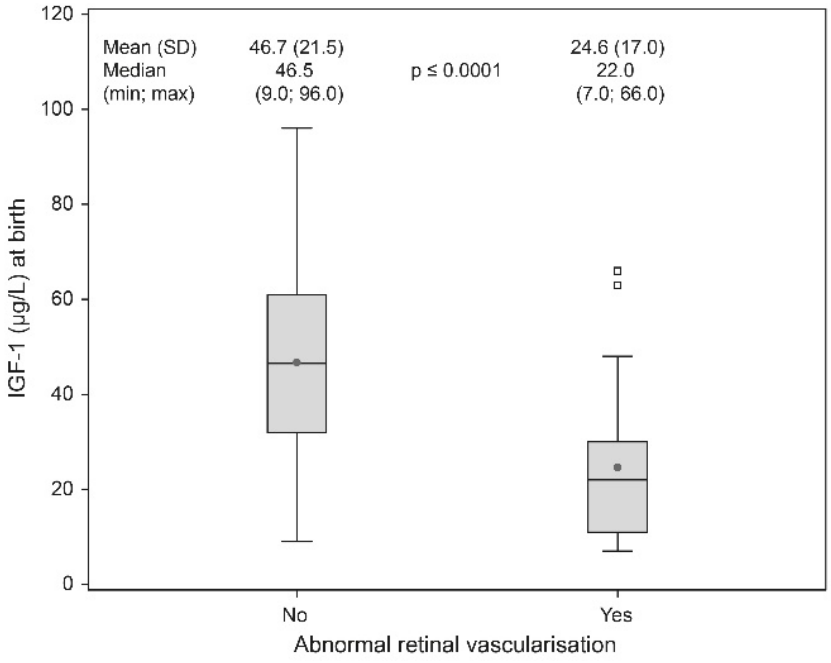

Figure 20. IGF-I in umbilical cord blood vs. abnormal retinal vascularization. Infants with abnormal retinal vascularization had lower median IGF-I, 22.0 vs. 46.5 µg/L, p≤0.0001. (Reprinted with permission from John Wiley and Sons: Allvin et al. Acta Paediatr. 2014 Jun; 103(6):594-600.)

To establish which variable, or sets of variables, best explained abnormal retinal vascularization, a stepwise logistic regression was run with the following variables: SGA (yes/no), preeclampsia or hypertension (yes/no), gestational age (week), head circumference (cm), birth weight (g), birth weight (SDS). The regression showed that birth weight (g) was the strongest predictor ($p<0.0001$, odds ratio 0.040, 95% confidence interval 0.007–0.216, area under the curve 0.849) of abnormal retinal vascularization.

6 DISCUSSION

The research presented in this thesis enhances our knowledge on endocrinological disturbances in individuals born SGA.

We found elevated serum levels of estradiol, DHT, and inhibin B, with normal SHBG, testosterone, and gonadotropins in adult males of normal stature born SGA.

In moderate-to-late preterm infants, we found an altered steroid hormone profile in umbilical cord blood. Boys born SGA had lower levels of estrone and elevated cortisol-to-cortisone ratio, and girls born SGA had elevated levels of androstenedione.

Boys were followed longitudinally during infancy, and were found to have a peak in testosterone and DHT at 2 months corrected age. Furthermore, boys born SGA had higher testosterone levels at around estimated date of birth and a flatter DHT peak. At 10 months corrected age, boys with good catch-up in weight from birth had higher testosterone and androstenedione levels.

Ophthalmological examinations were performed in both boys and girls in the neonatal period, and low birth weight was found to be a predictor of abnormal retinal vascularization, with increased vascular tortuosity, narrowing of the retinal arterioles or venules and few vascular branching points.

6.1 ANDROGENS

DHEAS

Gender or size at birth did not influence cord blood concentrations of DHEAS. DHEAS correlated to gestational age, as previously seen (53). There was no impact of size at birth or catch-up growth on the DHEAS levels in young adult men either, in line with another study (153).

ANDROSTENEDIONE

The placenta synthesizes androstenedione from fetal DHEA, and umbilical cord levels of androstenedione probably reflect placental production. Girls born SGA had higher umbilical cord androstenedione levels than girls born AGA, likely reflecting impaired aromatase activity, which affects the conversion of androstenedione to estrone. Half of the girls born SGA were born after preeclamptic pregnancies, and it is established that preeclampsia (21) as well as intrauterine growth restriction decreases aromatase activity in the placenta (154). In the boys studied, serum androstenedione concentrations gradually declined until 10 months corrected age, as seen in previous studies (75,155), reflecting decreasing adrenal production during prepubertal years.

TESTOSTERONE

As previously shown (51), and due to the testicular contribution of testosterone (54), boys had higher umbilical cord testosterone levels than girls. Testosterone is synthesized mainly by the testes in the first four months of life, and thereafter mainly by the adrenals (155). The well-established minipuberty was seen as a testosterone peak at 2 months corrected age. The elevated testosterone seen even at 0 months corrected age in boys born SGA may be partly in line with a previous study showing altered postnatal testosterone peaks in boys born preterm or SGA (76). The finding of elevated testosterone during early postnatal life in boys born SGA in our study is further supported by a study in rats born with intrauterine growth restriction, showing increased intratesticular testosterone concentrations 20 days postpartum (156).

At 10 months corrected age, there was a correlation between catch-up in weight from birth and serum testosterone and androstenedione concentrations,

possibly emphasizing the importance of androgens for growth, or weight gain for androgen secretion. A positive correlation has been found between growth velocity and urinary testosterone during infancy in boys (93), indicating the importance of testosterone for growth. Another study found serum testosterone levels at 8 weeks of life to correlate negatively with BMI at 3 years and body weight at 6 years, possibly reflecting a higher postnatal testosterone peak in boys of already smaller size (157).

Given that adult males born SGA had similar serum testosterone levels as males born AGA, the findings during infancy may not persist throughout life.

DIHYDROTESTOSTERONE

As shown in a previous study (51), boys had higher umbilical cord DHT concentrations than girls. Boys were followed during infancy and were found to have a DHT peak during minipuberty, to our knowledge never described before. DHT followed the same pattern as testosterone, peaking at 2 months corrected age and thereafter declining. In boys born SGA, we could not statistically show a DHT peak at 2 months corrected age, although DHT values did rise after birth. The lack of a DHT peak at 2 months corrected age in boys born SGA may be true, or merely reflect difficulties in attaining statistical significance due to the small number of SGA boys and infrequent blood sampling. A previous study did not capture the postnatal DHT peak, likely due to the use of a less sensitive DHT laboratory method, with a LOD of 100 pmol/L, compared to 27 pmol/L in our study (158).

As with infant boys born SGA, adult men born SGA seem to have altered DHT levels. DHT is mainly synthesized in peripheral tissue from testosterone by the enzyme 5α-reductase expressed in genital skin, hair follicles, and liver. The DHT-to-testosterone ratio may be seen as a proxy of 5α-reductase activity. Adult males born SGA had higher DHT and DHT-to-testosterone ratio than men born AGA. Furthermore, DHT-to-testosterone ratio correlated inversely with size at birth and positively with catch-up in weight and length from birth to adulthood.

To give a mechanistic explanation for the altered DHT levels seen in boys and men born SGA is difficult. Speculatively, the changes could be caused by increased 5α-reductase activity or decreased androgen receptor sensitivity, possibly due to an unfavorable prenatal environment and growth restriction, and the clinical implication remains to be elucidated.

6.2 ESTROGENS

With the help of aromatase, androgens are synthesized to estrogens: androstenedione to estrone and testosterone to estradiol (14). In a previous *in vitro* study, the placentas of infants born SGA were found to have a likely decreased aromatase activity (154). Furthermore, placentas from preeclamptic pregnancies have been shown to have a lower aromatase activity (21). Since estrogens measured in cord blood derive from the placenta, it is not surprising that estrone levels were lower in boys born SGA. These boys were to a greater extent born after pregnancies complicated by preeclampsia than boys born AGA. Other studies have shown either similar (159) or lower (56) estrogen concentrations in cord blood after preeclamptic pregnancies. What possible long-term impact decreased estrogen concentrations during fetal life may have on an individual is currently not known. Estrone and estradiol levels gradually declined in boys from birth to 10 months corrected age and did not differ between boys born SGA or AGA.

Young men born SGA had higher estradiol levels and estradiol-to-testosterone ratio than men born AGA. Furthermore, estradiol-to-testosterone ratio correlated negatively with birth weight SDS and birth length SDS, but positively with catch-up in weight SDS and length SDS from birth to adulthood.

In adult men, the main part of estradiol comes from aromatization of androgens in peripheral tissues such as muscles and adipocytes (2). A normal weight and BMI in SGA males does not reveal the distribution of fat between visceral and subcutaneous components. SGA males had a catch-up growth after birth, and the subsequent weight gain, presumably with increased visceral fat, could contribute to an increased aromatase activity. Interestingly, a previous study in male monozygotic twin pairs found increased estrone and estradiol levels in the heavier twin, compared to the leaner twin brother, due to increased adiposity-related expression of aromatase in adipose tissue (160).

Furthermore, there is evidence for a future disturbance in sex steroid metabolism after a stressful prenatal environment. In a previous study, endotoxin was given to pregnant rats, which resulted in increased estradiol levels in male offspring (161).

The clinical importance of elevated estradiol levels in males born SGA remains to be clarified. A study in middle-aged men showed that circulating estradiol is a predictor of carotid artery intima-media thickness (162). The potential role of estradiol in cardiovascular disease in men is further supported by a study

showing overexpression of a gene encoding for aromatase in the development of atherosclerosis (163).

6.3 INHIBIN B

Young men born SGA had higher serum inhibin B levels than those born AGA, and furthermore, inhibin B correlated negatively with birth length SDS. All the men in our study were of normal height, had a testicular volume ≥ 20 ml, and none of them had a history of hypospadias or cryptorchidism. Inhibin B is secreted by Sertoli cells in the testis and correlates to testicular volume and sperm count (8).

Cicognani et al. showed that young men born SGA, without complete catch-up growth in height, had smaller testes and lower inhibin B values than those born AGA (116). Furthermore, there was a correlation between testis size and inhibin B in the SGA group (116). In contrast, Jensen at al. found no difference in testicular volume and inhibin B values between adult men born SGA and those born AGA (115). The men born SGA in their study had had a catch-up growth in height and, although shorter than men born AGA, they were still in the normal height rage of ±2 SDS (115). In our study, the men born SGA had higher inhibin B than those born AGA. However, one inclusion criterion was normal final height for the men born SGA, which indicates that there may be a selection bias in the SGA group. Furthermore, we excluded men who in their infancy had hypospadias or cryptorchidism, with or without orchidopexy, and these were included in the study by Jensen et al. so these two studies may not be completely comparable.

We lack data on sperm quality, and consequently we do not know if elevated inhibin B levels in the men born SGA reflect gonadal dysfunction. There is a controversy about whether or not intrauterine growth restriction or being born SGA affect the individual's future fertility.

6.4 GLUCOCORTICOIDS

Infants born after intrauterine growth restriction have been described as having a reduced placental 11β-HSD2 (60), the enzyme that protects the fetus from high cortisol levels by converting cortisol to biologically inactive cortisone. Previous studies have shown a positive relation between birth weight SDS and placental 11β-HSD2 activity (61).

These previous studies are in line with our results in paper III, where cortisone levels were lower in neonates born SGA, and the cortisol-to-cortisone ratio was higher in umbilical cord blood in boys born SGA, compared to those born AGA. Both of these findings may be explained by an attenuated 11β-HSD2 activity, probably to some extent caused by, or a consequence of, intrauterine growth restriction.

In the research described in this thesis, glucocorticoids were not measured during infancy. However, a previous study found that low markers of 11β-HSD2 activity in urine was associated with both lower absolute weight and decreased weight gain over the first year of life (164).

In 12-year-old children born SGA, a high cortisol-to-cortisone ratio has been associated with high low-density lipoprotein cholesterol, high insulin resistance, poor gain in height from birth, and early pubertal stage (112).

Low birth weight is a risk factor for development of diabetes mellitus type 2, hypertension, and hyperlipidemia as an adult (120). It has been suggested that the prenatal environment, with exposure to a low placental 11β-HSD2 activity, and high cortisol levels during pregnancy, is part of the prenatal developmental programming of adult disease leading to these metabolic changes (165).

Retrospectively, with the results from glucocorticoids in umbilical cord blood, it would have been interesting to measure cortisol and cortisone levels, as well as blood pressure in the young adult men cohort. However, that was not the aim of the study, and mass spectrometry-based methods for determination of cortisol and cortisone had not been developed at GPGRC at that time.

6.5 IGF-I

In the moderate-to-late preterm cohort, we found no gender differences in IGF-I levels in umbilical cord blood. Previous studies show conflicting results, some finding gender differences (65,68), and one finding no gender differences (166). IGF-I levels were lower in neonates born SGA, and correlated with gestational age and size at birth, in line with previous studies (65,67,167).

In boys, serum IGF-I was determined during infancy. In boys born SGA, a rise in IGF-I was seen between birth and the time around estimated date of birth, probably reflecting catch-up growth and sufficient nutrition. Whether the rise in IGF-I level is beneficial or not for the individual's future health remains unknown.

As previously mentioned, low birth weight is related to cardiovascular disease and diabetes mellitus type 2 in adulthood (120), and both low and high IGF-I levels are risk markers for cardiovascular events (168) and cancer mortality (169).

6.6 ADIPOCYTOKINES

In paper I, young adult men born SGA had lower adiponectin, but similar leptin levels as those born AGA. This result is supported by a previous study, which found reduced adiponectin levels in children born SGA with catch-up growth (170).

Men born SGA had similar leptin levels and BMI as those born AGA. Leptin levels may serve as an indirect marker of total body fat (130), but they give no information on the distribution of fat.

Adiponectin acts anti-inflammatory whereas leptin has proinflammatory effects (128), and leptin-to-adiponectin ratio is suggested as an atherosclerotic index (171). In our study, leptin-to-adiponectin ratio was higher in the groups of men born SGA, in line with their well-established increased risk of hypertension (119).

6.7 RETINAL VASCULAR MORPHOLOGY

In the moderate-to-late preterm cohort, 78 neonates underwent ophthalmological examination in the neonatal period, and 21 of these had abnormal retinal vascularization, assessed as increased vascular tortuosity, decreased or increased number of vascular branching points, and narrow arterioles and/or venules.

Low birth weight was the strongest predictor for abnormal retinal vascularization, but the group with retinal vascular changes also had lower IGF-I concentrations in umbilical cord blood, and were more often born SGA or after a pregnancy complicated by hypertension or preeclampsia.

IGF-I is an important growth factor for the fetus (64), and cord IGF-I concentrations correlate with size at birth (65). Neonates born after intrauterine growth restriction have lower IGF-I (69), which is even more pronounced if the neonate was born after a preeclamptic pregnancy (70). IGF-I is important for normal retinal vascularization (74), and it may be the total load of risk factors for low IGF-I that contributes to the low IGF-I and abnormal retinal vascularization seen in this group of preterm neonates.

Previous studies are contradictory as to whether low birth weight and growth restriction at birth leads to permanent changes in retinal vascularization. In childhood, low birth weight has been associated with narrower retinal arteriolar caliber at 6 years of age (109), but not associated with retinal vascular structure at 7 to 9 years of age (172); low birth weight is then associated again with narrower retinal arterioles at 12 years of age (110). In adulthood, low birth weight has been associated with narrower retinal arterioles (131), and being born after intrauterine growth restriction has been associated with a lower number of vascular branching points (132).

Low birth weight is a known risk factor for hypertension, diabetes mellitus type 2, and hyperlipidemia (173). It is therefore tempting to speculate that the abnormal retinal vascularization we found is an indirect marker of an increased risk of developing cardiovascular disease in adulthood, and that intrauterine growth restriction permanently changes blood vessels. A study performed with ultrasound in newborns showed that poor intrauterine growth did not affect all internal organs uniformly; instead, the fetal growth of the liver and kidneys was more impaired than that of the body as a whole (174). The unfavorable fetal environment and its associated hypoxia leads to a redistribution of blood flow and to metabolic and endocrine changes in the fetus, with increased cortisol and reduced insulin and IGF-I levels (175). Furthermore, it has been

suggested that the vascular adaptations *in utero*, with persisting impaired synthesis of elastin and endothelial dysfunction, are the link between prenatal growth restriction and cardiovascular disease in adulthood (176).

6.8 STRENGTHS AND WEAKNESSES

STRENGTHS

This research project has several strengths. The most important is the use of accurate laboratory techniques for the determination of steroid hormones. In paper I, RIA was used (estradiol, testosterone), in paper III, GC-MS/MS (testosterone, DHT, estrone, estradiol), and LC-MS/MS (androstenedione, DHEAS, cortisone, cortisol), and in paper IV, GC-MS/MS (all the hormones mentioned above and androstenedione). These methods are ultrasensitive and were developed and validated at the laboratory at GPGRC in the Department of Pediatrics at the University of Gothenburg. Furthermore, we studied individuals born SGA at different ages, to investigate whether an altered steroid hormone profile could be present from the neonatal period to adulthood.

In paper I, we had access to auxological data from birth for all the young adult men in the study, both those born SGA and AGA.

Individuals studied in papers II–IV come from the same longitudinally followed population-based cohort. All pregnancies were dated using ultrasound. We had complete data on maternal health during pregnancy, birth characteristics, and, for the boys, on cryptorchidism and hypospadias.

In paper II, a single trained ophthalmologist, using the same study protocol, examined all neonates. She was blinded to the data on maternal health, the neonates' gestational age, and birth weight.

In paper III, umbilical venous blood was used for hormone determinations, of importance because levels of unconjugated steroids differ between umbilical arterial and venous blood (177).

In paper IV, longitudinal data is presented up to 10 months corrected age.

WEAKNESSES

No study design is perfect; you always find weaknesses, things you retrospectively would have done differently. I would have wanted to examine both SGA girls and boys at birth, prepubertally, during puberty, and as young adults, but that project would have been too extensive for my thesis.

In paper I, all the young adult men born SGA had spontaneous catch-up growth and were of normal height. They may therefore not be representative of those who remain short or catch up with the help of growth hormone therapy. We lack data on causality for being SGA, so in the SGA group there may be an over-representation of men who are genetically of normal height or even tall and who reached their genetic final height, being SGA at birth for no other reason than that their mother had preeclampsia or placenta dysfunction during pregnancy.

Since the cohort studied in papers II–IV was population based, there was an unequal distribution between neonates born SGA and those born AGA, which may have affected the results of the studies, likely diminishing the differences found between the groups. All neonates were born moderate-to-late preterm, which is why we could not compare preterm with term neonates; furthermore, the analyses are not necessarily applicable to term neonates.

In paper II, the same trained ophthalmologist performed all the retinal examinations. This is an advantage but could also be a disadvantage. If the examiner's assessments were to deviate over time, there could arise a need to calibrate against a standard. The assessment of retinal vascular morphology was subjective, since we did not have access to Retcam, an imaging technique for neonatal fundus examinations. The Retcam equipment was on the market when the article was published, but was not available when the examinations were carried out. The neonates who did not undergo an eye examination had a higher gestational age and were larger at birth than those examined, which may have resulted in an overestimation of the incidence of abnormal retinal vascularization in neonates born moderately to late preterm.

In paper III, we lacked complete data on smoking during pregnancy, and could therefore not adjust for smoking, potentially affecting steroidogenesis in the placenta and fetus (178).

In paper IV, more frequent blood sampling during minipuberty would have been preferable, to more precisely catch the exact time for the postnatal surge in testosterone and DHT concentrations during minipuberty in boys. Most

likely, ethical approval for such frequent blood sampling in otherwise healthy infants would have been difficult to obtain.

For papers III and IV, not all blood sample volumes reached the prerequisite of 200 µL for analysis with GC-MS/MS, which forced us to adjust the LOD accordingly. The consequence was that a larger number of samples had hormone concentrations below LOD, and therefore lower precision at low levels. It is likely that potential differences between groups were never seen, differences that would have been more pronounced with larger serum volumes.

For boys and men in papers I, III, and IV, we lack data on testicular volume and penile length.

7 CONCLUSION

Boys born SGA had decreased levels of estrone and girls born SGA had increased levels of androstenedione in cord serum. Neonates born SGA of both genders had decreased cortisone levels, and furthermore, boys born SGA had an increased cortisol-to-cortisone ratio.

Low birth weight was the strongest predictor of abnormal retinal vascularization, as assessed by a trained ophthalmologist, in moderately to late preterm infants.

Longitudinal data on five sex steroids were analyzed with GC-MS/MS during infancy in moderately to late preterm boys. A postnatal rise in DHT during minipuberty was seen, although the increase in DHT levels from 0 to 2 months corrected age was less pronounced in boys born SGA than in boys born AGA. Boys born SGA had elevated serum testosterone at around estimated time of birth. Catch-up growth in weight from birth to 10 months corrected age correlated with levels of androstenedione and testosterone.

In young adult men, those born SGA who had reached normal stature had elevated serum levels of estradiol, DHT, and inhibin B, with normal serum levels of SHBG, testosterone, and gonadotropins, compared to those born AGA.

Even if the results and conclusions in this thesis do not change the medical care of individuals born SGA, my hope is that the thesis will raise awareness and concern about changes in steroids hormones and IGF-I in individuals born SGA. These changes may be a sign of immaturity in the moderate-to-late preterm cohort, or be lifelong changes with a negative effect on the development of cardiovascular disease, glucose resistance, and infertility later in life.

8 FUTURE PERSPECTIVES

The question as to whether and in that case how intrauterine growth restriction causes changes in the steroid hormone profile remains. We found an altered steroid hormone profile in individuals born SGA, both in umbilical cord blood, in serum in boys during infancy, and in young adult men. To investigate whether these changes continue during childhood and through puberty requires further studies in other cohorts. As it would be of scientific value to investigate whether our results are also applicable to term neonates born SGA, we would like to include and study such a cohort. However, since term neonates would most likely be quite healthy, we might face problems with ethical approval for frequent, ideally monthly, blood sampling.

Our research on the moderate-to-late preterm cohort continues. The recently developed ultrasensitive GC-MS/MS and LC-MS/MS methods at the laboratory at GPGRC, University of Gothenburg, have provided us with new opportunities for the determination of steroid hormones in serum.

In the moderate-to-late preterm cohort, we intend to analyze data on the girls as well as on the twins during infancy. All the children in the cohort have been examined annually between 5 and 10 years of age. Their steroid hormones will be analyzed, hopefully elucidating whether individuals born SGA have disturbances in their sex hormone profile as early as during their prepubertal years. Data on adipocytokines, blood pressure, and other metabolic parameters have also been collected and will be analyzed.

Ophthalmological research on the moderate-to-late preterm cohort continues. The children have undergone examinations of visual function, refraction, and fundus morphology, and will be followed until adulthood at the Department of Ophthalmology, Sahlgrenska University Hospital.

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REFERENCES

1. Norman AW, Litwack G. Hormones [Elektronisk resurs]. San Diego: Academic Press. 1997.
2. Larsen PR, Williams RH. Williams textbook of endocrinology. Philadelphia, Pa.: W. B. Saunders. 2003.
3. Knutsson U, Dahlgren J, Marcus C, Rosberg S, Bronnegard M, Stierna P, Albertsson-Wikland K. Circadian cortisol rhythms in healthy boys and girls: relationship with age, growth, body composition, and pubertal development. *The Journal of clinical endocrinology and metabolism*. 1997;82(2):536-540.
4. Griffin JE, Ojeda SR. Textbook of endocrine physiology. New York: Oxford Univ. Press. 1996.
5. Bacila IA, Elder C, Krone N. Update on adrenal steroid hormone biosynthesis and clinical implications. *Arch Dis Child*. 2019;104(12):1223-1228.
6. Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *The Journal of clinical endocrinology and metabolism*. 1981;53(1):58-68.
7. Rosenfeld RS, Rosenberg BJ, Fukushima DK, Hellman L. 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *The Journal of clinical endocrinology and metabolism*. 1975;40(5):850-855.
8. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertil Steril*. 2006;86(2):332-338.
9. Gurung P, Jialal I. Physiology, Male Reproductive System. StatPearls. Treasure Island (FL)2019.
10. Akingbemi BT. Estrogen regulation of testicular function. *Reprod Biol Endocrinol*. 2005;3:51.
11. Hammes SR, Levin ER. Impact of estrogens in males and androgens in females. *J Clin Invest*. 2019;129(5):1818-1826.
12. Sperling MA. Pediatric endocrinology. Philadelphia: Saunders. 1996.
13. Mihm M, Gangooly S, Muttukrishna S. The normal menstrual cycle in women. *Anim Reprod Sci*. 2011;124(3-4):229-236.
14. Morel Y, Roucher F, Plotton I, Goursaud C, Tardy V, Mallet D. Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis. *Ann Endocrinol (Paris)*. 2016;77(2):82-89.
15. Burger HG. Androgen production in women. *Fertil Steril*. 2002;77 Suppl 4:S3-5.
16. Pasqualini JR. Enzymes involved in the formation and transformation of steroid hormones in the fetal and placental compartments. *The*

Journal of steroid biochemistry and molecular biology. 2005;97(5):401-415.
17. Tayama C, Ichimaru S, Ito M, Nakayana M, Maeyama M, Miyakawa I. Unconjugated estradiol, estriol and total estriol in maternal peripheral vein, cord vein, and cord artery serum at delivery in pregnancies with intrauterine growth retardation. *Endocrinol Jpn*. 1983;30(2):155-162.
18. Sun M, Maliqueo M, Benrick A, Johansson J, Shao R, Hou L, Jansson T, Wu X, Stener-Victorin E. Maternal androgen excess reduces placental and fetal weights, increases placental steroidogenesis, and leads to long-term health effects in their female offspring. *Am J Physiol Endocrinol Metab*. 2012;303(11):E1373-1385.
19. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA, Giannone PJ. Maternal preeclampsia and neonatal outcomes. *Journal of pregnancy*. 2011;2011:214365.
20. Sathishkumar K, Balakrishnan M, Chinnathambi V, Chauhan M, Hankins GD, Yallampalli C. Fetal sex-related dysregulation in testosterone production and their receptor expression in the human placenta with preeclampsia. *Journal of perinatology : official journal of the California Perinatal Association*. 2012;32(5):328-335.
21. Perez-Sepulveda A, Monteiro LJ, Dobierzewska A, Espana-Perrot PP, Venegas-Araneda P, Guzman-Rojas AM, Gonzalez MI, Palominos-Rivera M, Irarrazabal CE, Figueroa-Diesel H, Varas-Godoy M, Illanes SE. Placental Aromatase Is Deficient in Placental Ischemia and Preeclampsia. *PLoS One*. 2015;10(10):e0139682.
22. Acromite MT, Mantzoros CS, Leach RE, Hurwitz J, Dorey LG. Androgens in preeclampsia. *American journal of obstetrics and gynecology*. 1999;180(1 Pt 1):60-63.
23. Hertig A, Liere P, Chabbert-Buffet N, Fort J, Pianos A, Eychenne B, Cambourg A, Schumacher M, Berkane N, Lefevre G, Uzan S, Rondeau E, Rozenberg P, Rafestin-Oblin ME. Steroid profiling in preeclamptic women: evidence for aromatase deficiency. *American journal of obstetrics and gynecology*. 2010;203(5):477 e471-479.
24. Xu HY, Zhang HX, Xiao Z, Qiao J, Li R. Regulation of anti-Mullerian hormone (AMH) in males and the associations of serum AMH with the disorders of male fertility. *Asian J Androl*. 2019;21(2):109-114.
25. Akre O, Boyd HA, Ahlgren M, Wilbrand K, Westergaard T, Hjalgrim H, Nordenskjold A, Ekbom A, Melbye M. Maternal and gestational risk factors for hypospadias. *Environ Health Perspect*. 2008;116(8):1071-1076.
26. Fujimoto T, Suwa T, Kabe K, Adachi T, Nakabayashi M, Amamiya T. Placental insufficiency in early gestation is associated with hypospadias. *J Pediatr Surg*. 2008;43(2):358-361.
27. O'Shaughnessy PJ, Antignac JP, Le Bizec B, Morvan ML, Svechnikov K, Soder O, Savchuk I, Monteiro A, Soffientini U, Johnston ZC,

Bellingham M, Hough D, Walker N, Filis P, Fowler PA. Alternative (backdoor) androgen production and masculinization in the human fetus. *PLoS Biol*. 2019;17(2):e3000002.
28. Jwa SC, Jwa J, Kuwahara A, Irahara M, Ishihara O, Saito H. Male subfertility and the risk of major birth defects in children born after in vitro fertilization and intracytoplasmic sperm injection: a retrospective cohort study. *BMC Pregnancy Childbirth*. 2019;19(1):192.
29. Mau Kai C, Main KM, Andersen AN, Loft A, Skakkebaek NE, Juul A. Reduced serum testosterone levels in infant boys conceived by intracytoplasmic sperm injection. *The Journal of clinical endocrinology and metabolism*. 2007;92(7):2598-2603.
30. Freemark M. Placental hormones and the control of fetal growth. *The Journal of clinical endocrinology and metabolism*. 2010;95(5):2054-2057.
31. Mullis PE, Tonella P. Regulation of fetal growth: consequences and impact of being born small. *Best Pract Res Clin Endocrinol Metab*. 2008;22(1):173-190.
32. McGrath RT, Glastras SJ, Hocking SL, Fulcher GR. Large-for-Gestational-Age Neonates in Type 1 Diabetes and Pregnancy: Contribution of Factors Beyond Hyperglycemia. *Diabetes Care*. 2018;41(8):1821-1828.
33. Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. *American journal of epidemiology*. 2007;165(7):734-741.
34. Griffiths LJ, Dezateux C, Cole TJ. Differential parental weight and height contributions to offspring birthweight and weight gain in infancy. *International journal of epidemiology*. 2007;36(1):104-107.
35. Albertsson-Wikland K, Boguszewski M, Karlberg J. Children born small-for-gestational age: postnatal growth and hormonal status. *Hormone research*. 1998;49 Suppl 2:7-13.
36. WHO. International Statistical Classification of Diseases and Health Related Problems. *World Health Organization*. 2011.
37. Ramakrishnan U, Grant F, Goldenberg T, Zongrone A, Martorell R. Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: a systematic review. *Paediatric and perinatal epidemiology*. 2012;26 Suppl 1:285-301.
38. Spracklen CN, Ryckman KK, Harland K, Saftlas AF. Effects of smoking and preeclampsia on birth weight for gestational age. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet*. 2014:1-6.

39. Adams Waldorf KM, McAdams RM. Influence of infection during pregnancy on fetal development. *Reproduction*. 2013;146(5):R151-162.
40. Carter RC, Jacobson JL, Sokol RJ, Avison MJ, Jacobson SW. Fetal alcohol-related growth restriction from birth through young adulthood and moderating effects of maternal prepregnancy weight. *Alcohol Clin Exp Res*. 2013;37(3):452-462.
41. de Wit MC, Srebniak MI, Joosten M, Govaerts LC, Kornelisse RF, Papatsonis DN, de Graaff K, Knapen MF, Bruggenwirth HT, de Vries FA, Van Veen S, Van Opstal D, Galjaard RJ, Go AT. Prenatal and postnatal findings in small-for-gestational-age fetuses without structural ultrasound anomalies at 18-24 weeks. *Ultrasound Obstet Gynecol*. 2017;49(3):342-348.
42. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. *Acta Obstet Gynecol Scand*. 1977;56(3):247-253.
43. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet*. 2012;379(9832):2162-2172.
44. Socialstyrelsen. The Swedish Medical Birth Register. 2017.
45. Engle WA, Tomashek KM, Wallman C, Committee on F, Newborn AAoP. "Late-preterm" infants: a population at risk. *Pediatrics*. 2007;120(6):1390-1401.
46. de Jong M, Verhoeven M, van Baar AL. School outcome, cognitive functioning, and behaviour problems in moderate and late preterm children and adults: a review. *Seminars in fetal & neonatal medicine*. 2012;17(3):163-169.
47. Shapiro-Mendoza CK, Lackritz EM. Epidemiology of late and moderate preterm birth. *Seminars in fetal & neonatal medicine*. 2012;17(3):120-125.
48. Velegrakis A, Sfakiotaki M, Sifakis S. Human placental growth hormone in normal and abnormal fetal growth. *Biomed Rep*. 2017;7(2):115-122.
49. Nwabuobi C, Arlier S, Schatz F, Guzeloglu-Kayisli O, Lockwood CJ, Kayisli UA. hCG: Biological Functions and Clinical Applications. *Int J Mol Sci*. 2017;18(10).
50. Morisset AS, Dube MC, Drolet R, Pelletier M, Labrie F, Luu-The V, Tremblay Y, Robitaille J, John Weisnagel S, Tchernof A. Androgens in the maternal and fetal circulation: association with insulin resistance. *The journal of maternal-fetal & neonatal medicine : the*

official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2013;26(5):513-519.

51. Lundell AC, Ryberg H, Vandenput L, Rudin A, Ohlsson C, Tivesten A. Umbilical cord blood androgen levels in girls and boys assessed by gas chromatography-tandem mass spectrometry. *The Journal of steroid biochemistry and molecular biology*. 2017;171:195-200.
52. Keelan JA, Mattes E, Tan H, Dinan A, Newnham JP, Whitehouse AJ, Jacoby P, Hickey M. Androgen concentrations in umbilical cord blood and their association with maternal, fetal and obstetric factors. *PLoS One*. 2012;7(8):e42827.
53. Kari MA, Raivio KO, Stenman UH, Voutilainen R. Serum cortisol, dehydroepiandrosterone sulfate, and steroid-binding globulins in preterm neonates: effect of gestational age and dexamethasone therapy. *Pediatric research*. 1996;40(2):319-324.
54. Forest MG, Cathiard AM. Pattern of plasma testosterone and delta4-androstenedione in normal newborns: Evidence for testicular activity at birth. *The Journal of clinical endocrinology and metabolism*. 1975;41(5):977-980.
55. de Zegher F, Francois I, Boehmer AL, Saggese G, Muller J, Hiort O, Sultan C, Clayton P, Brauner R, Cacciari E, Ibanez L, Van Vliet G, Tiulpakov A, Saka N, Ritzen M, Sippell WG. Androgens and fetal growth. *Hormone research*. 1998;50(4):243-244.
56. Hickey M, Hart R, Keelan JA. The relationship between umbilical cord estrogens and perinatal characteristics. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2014;23(6):946-952.
57. Nagata C, Iwasa S, Shiraki M, Shimizu H. Estrogen and alpha-fetoprotein levels in maternal and umbilical cord blood samples in relation to birth weight. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2006;15(8):1469-1472.
58. Troisi R, Potischman N, Roberts J, Siiteri P, Daftary A, Sims C, Hoover RN. Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States). *Cancer Causes Control*. 2003;14(4):347-355.
59. Reynolds JW, Barnhart BJ, Carlson CV. Feto-placental steroid metabolism in growth retarded human fetuses. *Pediatric research*. 1986;20(2):166-168.
60. Dy J, Guan H, Sampath-Kumar R, Richardson BS, Yang K. Placental 11beta-hydroxysteroid dehydrogenase type 2 is reduced in pregnancies complicated with idiopathic intrauterine growth Restriction: evidence that this is associated with an attenuated ratio of

cortisone to cortisol in the umbilical artery. *Placenta*. 2008;29(2):193-200.
61. Kajantie E, Dunkel L, Turpeinen U, Stenman UH, Wood PJ, Nuutila M, Andersson S. Placental 11 beta-hydroxysteroid dehydrogenase-2 and fetal cortisol/cortisone shuttle in small preterm infants. *The Journal of clinical endocrinology and metabolism*. 2003;88(1):493-500.
62. Travers S, Martinerie L, Boileau P, Lombes M, Pussard E. Alterations of adrenal steroidomic profiles in preterm infants at birth. *Archives of disease in childhood Fetal and neonatal edition*. 2017.
63. Aufdenblatten M, Baumann M, Raio L, Dick B, Frey BM, Schneider H, Surbek D, Hocher B, Mohaupt MG. Prematurity is related to high placental cortisol in preeclampsia. *Pediatric research*. 2009;65(2):198-202.
64. Netchine I, Azzi S, Le Bouc Y, Savage MO. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. *Best Pract Res Clin Endocrinol Metab*. 2011;25(1):181-190.
65. Vatten LJ, Nilsen ST, Odegard RA, Romundstad PR, Austgulen R. Insulin-like growth factor I and leptin in umbilical cord plasma and infant birth size at term. *Pediatrics*. 2002;109(6):1131-1135.
66. Carlsen EM, Renault KM, Jensen RB, Norgaard K, Jensen JE, Nilas L, Cortes D, Michaelsen KF, Pryds O. The Association between Newborn Regional Body Composition and Cord Blood Concentrations of C-Peptide and Insulin-Like Growth Factor I. *PLoS One*. 2015;10(7):e0121350.
67. Verhaeghe J, Van Herck E, Billen J, Moerman P, Van Assche FA, Giudice LC. Regulation of insulin-like growth factor-I and insulin-like growth factor binding protein-1 concentrations in preterm fetuses. *American journal of obstetrics and gynecology*. 2003;188(2):485-491.
68. Geary MP, Pringle PJ, Rodeck CH, Kingdom JC, Hindmarsh PC. Sexual dimorphism in the growth hormone and insulin-like growth factor axis at birth. *The Journal of clinical endocrinology and metabolism*. 2003;88(8):3708-3714.
69. Verkauskiene R, Beltrand J, Claris O, Chevenne D, Deghmoun S, Dorgeret S, Alison M, Gaucherand P, Sibony O, Levy-Marchal C. Impact of fetal growth restriction on body composition and hormonal status at birth in infants of small and appropriate weight for gestational age. *European journal of endocrinology / European Federation of Endocrine Societies*. 2007;157(5):605-612.
70. Vatten LJ, Odegard RA, Nilsen ST, Salvesen KA, Austgulen R. Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight. *Obstet Gynecol*. 2002;99(1):85-90.

71. Dubova EA, Pavlov KA, Lyapin VM, Kulikova GV, Shchyogolev AI, Sukhikh GT. Expression of insulin-like growth factors in the placenta in preeclampsia. *Bull Exp Biol Med*. 2014;157(1):103-107.
72. Gilbert C. Retinopathy of prematurity: a global perspective of the epidemics, population of babies at risk and implications for control. *Early human development*. 2008;84(2):77-82.
73. Gerd H. Retinopathy of Prematurity - State of the art dokument. *Svenska Neonatalföreningens nationella vårdprogram*. 2012.
74. Hellstrom A, Perruzzi C, Ju M, Engstrom E, Hard AL, Liu JL, Albertsson-Wikland K, Carlsson B, Niklasson A, Sjodell L, LeRoith D, Senger DR, Smith LE. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc Natl Acad Sci U S A*. 2001;98(10):5804-5808.
75. Ben-David S, Zuckerman-Levin N, Epelman M, Shen-Orr Z, Levin M, Sujov P, Hochberg Z. Parturition itself is the basis for fetal adrenal involution. *The Journal of clinical endocrinology and metabolism*. 2007;92(1):93-97.
76. Forest MG, de Peretti E, Bertrand J. Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared with that of full-term infants. *J Steroid Biochem*. 1980;12:25-36.
77. Doerr HG, Versmold HT, Bidlingmaier F, Sippell WG. Adrenocortical steroids in small-for-gestational-age term infants during the early neonatal period. *Pediatric research*. 1989;25(2):115-118.
78. Nomura S. Immature adrenal steroidogenesis in preterm infants. *Early human development*. 1997;49(3):225-233.
79. Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *J Clin Invest*. 1974;53(3):819-828.
80. Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *The Journal of clinical endocrinology and metabolism*. 1975;40(4):545-551.
81. Kuiri-Hanninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Hormone research in paediatrics*. 2014;82(2):73-80.
82. Winter JS, Hughes IA, Reyes FI, Faiman C. Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *The Journal of clinical endocrinology and metabolism*. 1976;42(4):679-686.

83. Zivkovic D, Hadziselimovic F. Development of Sertoli cells during mini-puberty in normal and cryptorchid testes. *Urol Int*. 2009;82(1):89-91.
84. Svechnikov K, Landreh L, Weisser J, Izzo G, Colon E, Svechnikova I, Soder O. Origin, development and regulation of human Leydig cells. *Hormone research in paediatrics*. 2010;73(2):93-101.
85. Prince FP. The triphasic nature of Leydig cell development in humans, and comments on nomenclature. *J Endocrinol*. 2001;168(2):213-216.
86. Zivkovic D, Fratric I. Disturbances of sperm maturation and minipuberty: is there a connection? *BioMed research international*. 2014;2014:912746.
87. Stukenborg JB, Kjartansdottir KR, Reda A, Colon E, Albersmeier JP, Soder O. Male germ cell development in humans. *Hormone research in paediatrics*. 2014;81(1):2-12.
88. Kuiri-Hanninen T, Seuri R, Tyrvainen E, Turpeinen U, Hamalainen E, Stenman UH, Dunkel L, Sankilampi U. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *The Journal of clinical endocrinology and metabolism*. 2011;96(1):98-105.
89. Forest MG, Cathiard AM. Ontogenic study of plasma 17alpha-hydroxyprogesterone in the human. I. Postnatal period: evidence for a transient ovarian activity in infancy. *Pediatric research*. 1978;12(1):6-11.
90. Bidlingmaier F, Strom TM, Dorr HG, Eisenmenger W, Knorr D. Estrone and estradiol concentrations in human ovaries, testes, and adrenals during the first two years of life. *The Journal of clinical endocrinology and metabolism*. 1987;65(5):862-867.
91. Schmidt IM, Chellakooty M, Haavisto AM, Boisen KA, Damgaard IN, Steendahl U, Toppari J, Skakkebaek NE, Main KM. Gender difference in breast tissue size in infancy: correlation with serum estradiol. *Pediatric research*. 2002;52(5):682-686.
92. Albertsson-Wikland K, Karlberg J. Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl*. 1994;399:64-70; discussion 71.
93. Kiviranta P, Kuiri-Hanninen T, Saari A, Lamidi ML, Dunkel L, Sankilampi U. Transient Postnatal Gonadal Activation and Growth Velocity in Infancy. *Pediatrics*. 2016;138(1).
94. Jacklin CN, McBride C, McCrory P, Gallahan L. Neonatal sex-steroid hormones and physical size at four years. *J Pediatr Endocrinol*. 1994;7(3):253-259.
95. Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ. Size at birth and adrenocortical function in childhood. *Clinical endocrinology*. 1996;45(6):721-726.
96. Francois I, de Zegher F. Adrenarche and fetal growth. *Pediatric research*. 1997;41(3):440-442.

97. Dahlgren J, Boguszewski M, Rosberg S, Albertsson-Wikland K. Adrenal steroid hormones in short children born small for gestational age. *Clinical endocrinology*. 1998;49(3):353-361.
98. Ibanez L, Potau N, Marcos MV, de Zegher F. Exaggerated adrenarche and hyperinsulinism in adolescent girls born small for gestational age. *The Journal of clinical endocrinology and metabolism*. 1999;84(12):4739-4741.
99. Petraitiene I, Valuniene M, Albertsson-Wikland K, Verkauskiene R. Adrenal Function in Adolescence is Related to Intrauterine and Postnatal Growth. *Medicina (Kaunas)*. 2019;55(5).
100. Jones A, Godfrey KM, Wood P, Osmond C, Goulden P, Phillips DI. Fetal growth and the adrenocortical response to psychological stress. *The Journal of clinical endocrinology and metabolism*. 2006;91(5):1868-1871.
101. Abreu AP, Kaiser UB. Pubertal development and regulation. *Lancet Diabetes Endocrinol*. 2016;4(3):254-264.
102. Persson I, Ahlsson F, Ewald U, Tuvemo T, Qingyuan M, von Rosen D, Proos L. Influence of perinatal factors on the onset of puberty in boys and girls: implications for interpretation of link with risk of long term diseases. *American journal of epidemiology*. 1999;150(7):747-755.
103. Ibanez L, Potau N, de Zegher F. Ovarian hyporesponsiveness to follicle stimulating hormone in adolescent girls born small for gestational age. *The Journal of clinical endocrinology and metabolism*. 2000;85(7):2624-2626.
104. Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, de Zegher F. Reduced ovulation rate in adolescent girls born small for gestational age. *The Journal of clinical endocrinology and metabolism*. 2002;87(7):3391-3393.
105. James E, Wood CL, Nair H, Williams TC. Preterm birth and the timing of puberty: a systematic review. *BMC pediatrics*. 2018;18(1):3.
106. Starnberg J, Norman M, Westrup B, Domellof M, Berglund SK. Cardiometabolic risk factors in children born with marginally low birth weight: A longitudinal cohort study up to 7 years-of-age. *PLoS One*. 2019;14(4):e0215866.
107. Ong KK, Petry CJ, Emmett PM, Sandhu MS, Kiess W, Hales CN, Ness AR, Dunger DB, team As. Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. *Diabetologia*. 2004;47(6):1064-1070.
108. Kistner A, Sigurdsson J, Niklasson A, Lofqvist C, Hall K, Hellstrom A. Neonatal IGF-1/IGFBP-1 axis and retinopathy of prematurity are associated with increased blood pressure in preterm children. *Acta paediatrica*. 2014;103(2):149-156.

109. Mitchell P, Liew G, Rochtchina E, Wang JJ, Robaei D, Cheung N, Wong TY. Evidence of arteriolar narrowing in low-birth-weight children. *Circulation*. 2008;118(5):518-524.
110. Gopinath B, Baur LA, Wang JJ, Teber E, Liew G, Cheung N, Wong TY, Mitchell P. Smaller birth size is associated with narrower retinal arterioles in early adolescence. *Microcirculation*. 2010;17(8):660-668.
111. Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C, Barker DJ, Whorwood CB. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension*. 2000;35(6):1301-1306.
112. Tenhola S, Turpeinen U, Halonen P, Hamalainen E, Voutilainen R. Association of serum lipid concentrations, insulin resistance index and catch-up growth with serum cortisol/cortisone ratio by liquid chromatography tandem mass spectrometry in children born small for gestational age. *Pediatric research*. 2005;58(3):467-471.
113. Michos A, Xue F, Michels KB. Birth weight and the risk of testicular cancer: a meta-analysis. *Int J Cancer*. 2007;121(5):1123-1131.
114. Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC. Influence of preterm birth and birth size on gonadal function in young men. *The Journal of clinical endocrinology and metabolism*. 2009;94(11):4243-4250.
115. Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *The Journal of clinical endocrinology and metabolism*. 2007;92(4):1353-1357.
116. Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E. Low birth weight for gestational age and subsequent male gonadal function. *The Journal of pediatrics*. 2002;141(3):376-379.
117. Sydsjo G, Tornblom P, Gaddlin PO, Finnstrom O, Leijon I, Nelson N, Theodorsson E, Hammar M. Women born with very low birth weight have similar menstrual cycle pattern, pregnancy rates and hormone profiles compared with women born at term. *BMC Womens Health*. 2019;19(1):56.
118. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;303(6809):1019-1022.
119. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ*. 1990;301(6746):259-262.
120. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*. 1993;36(1):62-67.

121. Class QA, Rickert ME, Lichtenstein P, D'Onofrio BM. Birth weight, physical morbidity, and mortality: a population-based sibling-comparison study. *American journal of epidemiology*. 2014;179(5):550-558.
122. Kerkhof GF, Breukhoven PE, Leunissen RW, Willemsen RH, Hokken-Koelega AC. Does preterm birth influence cardiovascular risk in early adulthood? *The Journal of pediatrics*. 2012;161(3):390-396.e391.
123. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. *BMJ*. 2001;322(7292):949-953.
124. Tivesten A, Vandenput L, Carlzon D, Nilsson M, Karlsson MK, Ljunggren O, Barrett-Connor E, Mellstrom D, Ohlsson C. Dehydroepiandrosterone and its sulfate predict the 5-year risk of coronary heart disease events in elderly men. *J Am Coll Cardiol*. 2014;64(17):1801-1810.
125. Tivesten A, Vandenput L, Labrie F, Karlsson MK, Ljunggren O, Mellstrom D, Ohlsson C. Low serum testosterone and estradiol predict mortality in elderly men. *The Journal of clinical endocrinology and metabolism*. 2009;94(7):2482-2488.
126. Juul A, Scheike T, Davidsen M, Gyllenborg J, Jorgensen T. Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation*. 2002;106(8):939-944.
127. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet*. 2002;359(9319):1740-1745.
128. Lopez-Jaramillo P, Gomez-Arbelaez D, Lopez-Lopez J, Lopez-Lopez C, Martinez-Ortega J, Gomez-Rodriguez A, Triana-Cubillos S. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Investig*. 2014;18(1):37-45.
129. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *The Journal of clinical endocrinology and metabolism*. 2001;86(5):1930-1935.
130. Fors H, Matsuoka H, Bosaeus I, Rosberg S, Wikland KA, Bjarnason R. Serum leptin levels correlate with growth hormone secretion and body fat in children. *The Journal of clinical endocrinology and metabolism*. 1999;84(10):3586-3590.
131. Liew G, Wang JJ, Duncan BB, Klein R, Sharrett AR, Brancati F, Yeh HC, Mitchell P, Wong TY, Atherosclerosis Risk in Communities S. Low birthweight is associated with narrower arterioles in adults. *Hypertension*. 2008;51(4):933-938.

132. Hellstrom A, Dahlgren J, Marsal K, Ley D. Abnormal retinal vascular morphology in young adults following intrauterine growth restriction. *Pediatrics*. 2004;113(2):e77-80.
133. Kistner A, Jacobson L, Jacobson SH, Svensson E, Hellstrom A. Low gestational age associated with abnormal retinal vascularization and increased blood pressure in adult women. *Pediatric research*. 2002;51(6):675-680.
134. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand*. 1991;80(8-9):756-762.
135. Wikland KA, Luo ZC, Niklasson A, Karlberg J. Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. *Acta paediatrica*. 2002;91(7):739-754.
136. Hesselmar B, Dahlgren J, Wennergren G, Aberg N, Albertsson-Wiklan K. Born small for gestational age: relation to future allergy and asthma. *Acta paediatrica*. 2002;91(9):992-994.
137. Samuelson G, Bratteby LE, Enghardt H, Hedgren M. Food habits and energy and nutrient intake in Swedish adolescents approaching the year 2000. *Acta Paediatr Suppl*. 1996;415:1-19.
138. Niklasson A, Albertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. *BMC pediatrics*. 2008;8:8.
139. Tapp AL, Maybery MT, Whitehouse AJ. Evaluating the twin testosterone transfer hypothesis: a review of the empirical evidence. *Horm Behav*. 2011;60(5):713-722.
140. Lummaa V, Pettay JE, Russell AF. Male twins reduce fitness of female co-twins in humans. *Proc Natl Acad Sci U S A*. 2007;104(26):10915-10920.
141. Norjavaara E, Ankarberg C, Albertsson-Wikland K. Diurnal rhythm of 17 beta-estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay. *The Journal of clinical endocrinology and metabolism*. 1996;81(11):4095-4102.
142. Ankarberg-Lindgren C, Norjavaara E. A purification step prior to commercial sensitive immunoassay is necessary to achieve clinical usefulness when quantifying serum 17beta-estradiol in prepubertal children. *European journal of endocrinology / European Federation of Endocrine Societies*. 2008;158(1):117-124.
143. Ankarberg C, Norjavaara E. Diurnal rhythm of testosterone secretion before and throughout puberty in healthy girls: correlation with 17beta-estradiol and dehydroepiandrosterone sulfate. *The Journal of clinical endocrinology and metabolism*. 1999;84(3):975-984.

144. Ankarberg-Lindgren C, Norjavaara E. Sensitive RIA measures testosterone concentrations in prepubertal and pubertal children comparable to tandem mass spectrometry. *Scandinavian journal of clinical and laboratory investigation*. 2015;75(4):341-344.
145. Hamer HM, Finken MJJ, van Herwaarden AE, du Toit T, Swart AC, Heijboer AC. Falsely elevated plasma testosterone concentrations in neonates: importance of LC-MS/MS measurements. *Clin Chem Lab Med*. 2018;56(6):e141-e143.
146. Hollier LP, Keelan JA, Hickey M, Maybery MT, Whitehouse AJ. Measurement of androgen and estrogen concentrations in cord blood: accuracy, biological interpretation, and applications to understanding human behavioral development. *Front Endocrinol (Lausanne)*. 2014;5:64.
147. Nelson RE, Grebe SK, DJ OK, Singh RJ. Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma. *Clin Chem*. 2004;50(2):373-384.
148. Ankarberg-Lindgren C, Dahlgren J, Andersson MX. High-sensitivity quantification of serum androstenedione, testosterone, dihydrotestosterone, estrone and estradiol by gas chromatography-tandem mass spectrometry with sex- and puberty-specific reference intervals. *The Journal of steroid biochemistry and molecular biology*. 2018;183:116-124.
149. Ankarberg-Lindgren C, Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. *European journal of endocrinology / European Federation of Endocrine Societies*. 2004;151(6):747-757.
150. Ankarberg-Lindgren C, Norjavaara E. Twenty-four hours secretion pattern of serum estradiol in healthy prepubertal and pubertal boys as determined by a validated ultra-sensitive extraction RIA. *BMC endocrine disorders*. 2008;8:10.
151. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *The Journal of clinical endocrinology and metabolism*. 1999;84(10):3666-3672.
152. Blum WF, Breier BH. Radioimmunoassays for IGFs and IGFBPs. *Growth regulation*. 1994;4 Suppl 1:11-19.
153. Todorova B, Salonen M, Jaaskelainen J, Tapio A, Jaaskelainen T, Palvimo J, Turpeinen U, Hamalainen E, Rasanen M, Tenhola S, Voutilainen R. Adrenocortical hormonal activity in 20-year-old subjects born small or appropriate for gestational age. *Hormone research in paediatrics*. 2012;77(5):298-304.

154. Thoumsin HJ, Alsat E, Cedard L. In vitro aromatization of androgens into estrogens in placental insufficiency. *Gynecol Obstet Invest*. 1982;13(1):37-43.
155. Bidlingmaier F, Dorr HG, Eisenmenger W, Kuhnle U, Knorr D. Contribution of the adrenal gland to the production of androstenedione and testosterone during the first two years of life. *The Journal of clinical endocrinology and metabolism*. 1986;62(2):331-335.
156. Pampanini V, Germani D, Puglianiello A, Stukenborg JB, Reda A, Savchuk I, Kjartansdottir KR, Cianfarani S, Soder O. Impact of uteroplacental insufficiency on postnatal rat male gonad. *J Endocrinol*. 2017;232(2):247-257.
157. Becker M, Oehler K, Partsch CJ, Ulmen U, Schmutzler R, Cammann H, Hesse V. Hormonal 'minipuberty' influences the somatic development of boys but not of girls up to the age of 6 years. *Clinical endocrinology*. 2015;83(5):694-701.
158. Kulle AE, Riepe FG, Melchior D, Hiort O, Holterhus PM. A novel ultrapressure liquid chromatography tandem mass spectrometry method for the simultaneous determination of androstenedione, testosterone, and dihydrotestosterone in pediatric blood samples: age- and sex-specific reference data. *The Journal of clinical endocrinology and metabolism*. 2010;95(5):2399-2409.
159. Troisi R, Potischman N, Johnson CN, Roberts JM, Lykins D, Harger G, Markovic N, Siiteri P, Hoover RN. Estrogen and androgen concentrations are not lower in the umbilical cord serum of pre-eclamptic pregnancies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2003;12(11 Pt 1):1268-1270.
160. Vihma V, Naukkarinen J, Turpeinen U, Hamalainen E, Kaprio J, Rissanen A, Heinonen S, Hakkarainen A, Lundbom J, Lundbom N, Mikkola TS, Tikkanen MJ, Pietilainen KH. Metabolism of sex steroids is influenced by acquired adiposity-A study of young adult male monozygotic twin pairs. *The Journal of steroid biochemistry and molecular biology*. 2017;172:98-105.
161. Nilsson C, Larsson BM, Jennische E, Eriksson E, Bjorntorp P, York DA, Holmang A. Maternal endotoxemia results in obesity and insulin resistance in adult male offspring. *Endocrinology*. 2001;142(6):2622-2630.
162. Tivesten A, Hulthe J, Wallenfeldt K, Wikstrand J, Ohlsson C, Fagerberg B. Circulating estradiol is an independent predictor of progression of carotid artery intima-media thickness in middle-aged men. *The Journal of clinical endocrinology and metabolism*. 2006;91(11):4433-4437.

163. Murakami H, Harada N, Sasano H. Aromatase in atherosclerotic lesions of human aorta. *The Journal of steroid biochemistry and molecular biology*. 2001;79(1-5):67-74.
164. Rogers SL, Hughes BA, Jones CA, Freedman L, Smart K, Taylor N, Stewart PM, Shackleton CH, Krone NP, Blissett J, Tomlinson JW. Diminished 11beta-hydroxysteroid dehydrogenase type 2 activity is associated with decreased weight and weight gain across the first year of life. *The Journal of clinical endocrinology and metabolism*. 2014;99(5):E821-831.
165. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci*. 2009;3:19.
166. Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, Korner A, Obermayer-Pietsch B, Hubener C, Dahlgren J, Frystyk J, Pfeiffer AF, Doering A, Bielohuby M, Wallaschofski H, Arafat AM. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *The Journal of clinical endocrinology and metabolism*. 2014;99(5):1712-1721.
167. Nagano N, Okada T, Fukamachi R, Yoshikawa K, Munakata S, Usukura Y, Hosono S, Takahashi S, Mugishima H, Matsuura M, Yamamoto T. Insulin-like growth factor-1 and lipoprotein profile in cord blood of preterm small for gestational age infants. *J Dev Orig Health Dis*. 2013;4(6):507-512.
168. Carlzon D, Svensson J, Petzold M, Karlsson MK, Ljunggren O, Tivesten A, Mellstrom D, Ohlsson C. Both low and high serum IGF-1 levels associate with increased risk of cardiovascular events in elderly men. *The Journal of clinical endocrinology and metabolism*. 2014;99(11):E2308-2316.
169. Svensson J, Carlzon D, Petzold M, Karlsson MK, Ljunggren O, Tivesten A, Mellstrom D, Ohlsson C. Both low and high serum IGF-I levels associate with cancer mortality in older men. *The Journal of clinical endocrinology and metabolism*. 2012;97(12):4623-4630.
170. Cianfarani S, Martinez C, Maiorana A, Scire G, Spadoni GL, Boemi S. Adiponectin levels are reduced in children born small for gestational age and are inversely related to postnatal catch-up growth. *The Journal of clinical endocrinology and metabolism*. 2004;89(3):1346-1351.
171. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin : adiponectin ratio as an atherosclerotic index in patients with type 2 diabetes : relationship of the index to carotid intima-media thickness. *Diabetologia*. 2005;48(12):2684-2686.
172. Cheung N, Islam FM, Saw SM, Shankar A, de Haseth K, Mitchell P, Wong TY. Distribution and associations of retinal vascular caliber with ethnicity, gender, and birth parameters in young children. *Invest Ophthalmol Vis Sci*. 2007;48(3):1018-1024.

173. Barker DJ, Fall CH. Fetal and infant origins of cardiovascular disease. *Arch Dis Child*. 1993;68(6):797-799.
174. Latini G, De Mitri B, Del Vecchio A, Chitano G, De Felice C, Zetterstrom R. Foetal growth of kidneys, liver and spleen in intrauterine growth restriction: "programming" causing "metabolic syndrome" in adult age. *Acta paediatrica*. 2004;93(12):1635-1639.
175. Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of in utero health. *Obstet Gynecol Sci*. 2017;60(6):506-519.
176. Sasongko MB, Wong TY, Wang JJ. Retinal arteriolar changes: intermediate pathways linking early life exposures to cardiovascular disease? *Microcirculation*. 2010;17(1):21-31.
177. Paskova A, Parizek A, Hill M, Velikova M, Kubatova J, Duskova M, Adamcova K, Koucky M, Simjak P, Cerny A, Starka L. Steroid metabolome in the umbilical cord: is it necessary to differentiate between arterial and venous blood? *Physiol Res*. 2014;63(1):115-126.
178. Adamcova K, Kolatorova L, Chlupacova T, Simkova M, Jandikova H, Parizek A, Starka L, Duskova M. Changes to fetal steroidogenesis caused by maternal smoking. *Physiol Res*. 2017;66(Supplementum 3):S375-S386.