Diplomarbeit

Fetal Programming and Risk of Cardiovascular Diseases

eingereicht von

Wolfgang Moritz Hittmann

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Assoz.-Prof. Priv.-Doz. Dr.med. Nandu Goswami, PhD

Ao.Univ.-Prof. Mag. Dr.rer.nat.Rössler Andreas

Graz, 9. November 2018

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 9. November 2018 *Wolfgang Moritz Hittmann eh.*

Acknowledgements

I want to express my sincere gratitude to Prof. Goswami for his great support during the last months. I am very thankful for his guidance and his never-ending patience, but also for his immense knowledge and his motivation.
I would also like to thank my second supervisor, Prof. Rössler, for his comments and his assistance.
Special thanks also to Mag. Haberl for proofreading this diploma thesis.

Finally, I thank my parents for their unconditional and endless support during the whole period of my studies.

Abstract

Background: Barker postulated that insults in early life would predispose an individual for several chronic diseases. Certain events in so called 'critical periods' of fetal life would lead to adaptations, which result in the development of diabetes, dyslipidemia, hypertension or cardiovascular diseases. Undernourishment is one example that leads to persisting alterations of metabolic functions of the fetus and has life long effects. Barker's hypothesis was proven to be true and the research community is working to elucidate mechanisms of how fetal programming works.

Aims and objectives: The aim of this diploma thesis is to explore the field of fetal programming and to conduct an extensive update of the current literature. Special focus in this work is given on the risk of cardiovascular diseases.

Methodology: A systemic literature search was performed in PubMed. Included were articles written in English, which were published in the last 5 years. Specific criteria were used to assess the relevance of articles. Furthermore list of references were used to obtain relevant primary literature for this diploma thesis. Information for the introduction chapter were obtained from secondary literature as well as from primary literature.

Results: 25 articles were evaluated; 12 papers were excluded, 13 papers were relevant.

Discussion: Cardiovascular diseases are still one of the leading causes of death in the world and research in fetal programming can a long way to reduce this burden. Some mechanisms are already understood and it is clear now that a variety of factors, like placental perfusion, oxidative stress and diet can lead to adverse in utero circumstances. The consequences on the cardiovascular system are enormous, but recent research offers hope for treatment approaches in the future.

German abstract - Zusammenfassung

Hintergrund: Barker postulierte erstmals, dass nachteilige Vorkommnisse in den frühen Lebensphasen eines Menschen Veranlagungen für verschiedene chronische Krankheiten schaffen würden. Bestimmte Vorfälle während kritischer Abschnitte in der Fetalperiode würden Veränderungen hervorrufen, die zur Entstehung von Diabetes, Dyslipidämie, Bluthochdruck oder Herz-Kreislauf-Erkrankungen führen. Ein Beispiel dafür ist Mangelernährung, welche den fetalen Stoffwechsel bleibend verändert, und lebenslange Auswirkungen hat. Während sich Barkers Hypothese als richtig erwies, versuchen nun Forscher weltweit die Mechanismen des ‚Fetal Programming' zu ergründen.

Ziele: Das Ziel dieser Diplomarbeit ist es, das Thema ‚Fetal Programming' zu beleuchten und eine ausführliche Literaturrecherche durchzuführen. Der Schwerpunkt dieser Arbeit liegt auf dem Thema ‚kardiovaskuläre Erkrankungen'.

Methodik: Eine systematische Literaturrecherche wurde mittels PubMed durchgeführt. Berücksichtigt wurden Artikel in englischer Sprache, welche innerhalb der letzten fünf Jahre publiziert wurden. Gezielte Kriterien wurden verwendet, um die Relevanz der Artikel zu beurteilen. Zudem wurden die Literaturverzeichnisse herangezogen, um weitere relevante Artikel für diese Arbeit zu erhalten. Für das einführende Kapitel wurde sowohl Primärliteratur, als auch Sekundärliteratur verwendet.

Ergebnisse: 25 Artikel wurden bewertet; 12 Artikel wurden ausgeschlossen, 13 Artikel waren passend.

Diskussion: Herz-Kreislauf-Erkrankungen stellen immer noch eine der häufigsten Todesursachen weltweit dar und Forschung in ‚Fetal programming' kann ihren Teil dazu beitragen dies zu verändern. Einige Mechanismen werden bereits verstanden und es ist nun evident, dass verschiedenste Faktoren, wie die Durchblutung der Plazenta, oxidativer Stress oder Ernährung zu nachteiligen Lebensverhältnissen des Fötus führen. Die Auswirkungen auf das Herz-Kreislauf-System sind einerseits drastisch, andererseits lassen neueste Forschungsergebnisse auf künftige Therapien hoffen.

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Abbreviations

11β – HSD1 - 11β-Hydroxysteroid-Dehydrogenase 1

25OHD - 25 hydroxy vitamin D_3

APGRAR score - Appearance, Pulse, Grimace, Activity, Respiration score

ART - Assisted reproductive technologies

CRP – C-reactive protein

DNA - Deoxyribonucleic acid

eNOS - Nitric oxide synthase

F1 generation – filial generation

H_2S - Hydrogen sulfide

hCG - Human chorionic gonadotropin

HDL - High density lipoprotein

IGF – Insulin-like growth factors

IGF-1 - Insulinlike growth factor 1

L-NAME - N^G-Nitro-Larginine-methyl ester

LAD - Left anterior descending

LDL - Low density lipoprotein

NO – Nitric oxide

RCX - Left circumflex

RNA - Ribonucleic acid

RNS - Nitrogen oxygen species

ROS - Reactive oxygen species

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1. Introduction

1.1 Cardiovascular system

The cardiovascular system is an organ system and consists of the heart, the artery system, the venous system and circulating blood. The blood circuit contains the pulmonary circulation, and the systemic circulation. Its purpose includes transport, temperature stabilization and it participates in the immune system and in homeostasis.

The heart, a hollow muscular organ, which is divided into four chambers, is located in the mediastinum, and drives the circulation. It is surrounded by the pericardium, a protective double-walled sac. Pericardial fluid enables the heart's movement in the pericardial cavity by reducing friction between the pericardium and the heart.

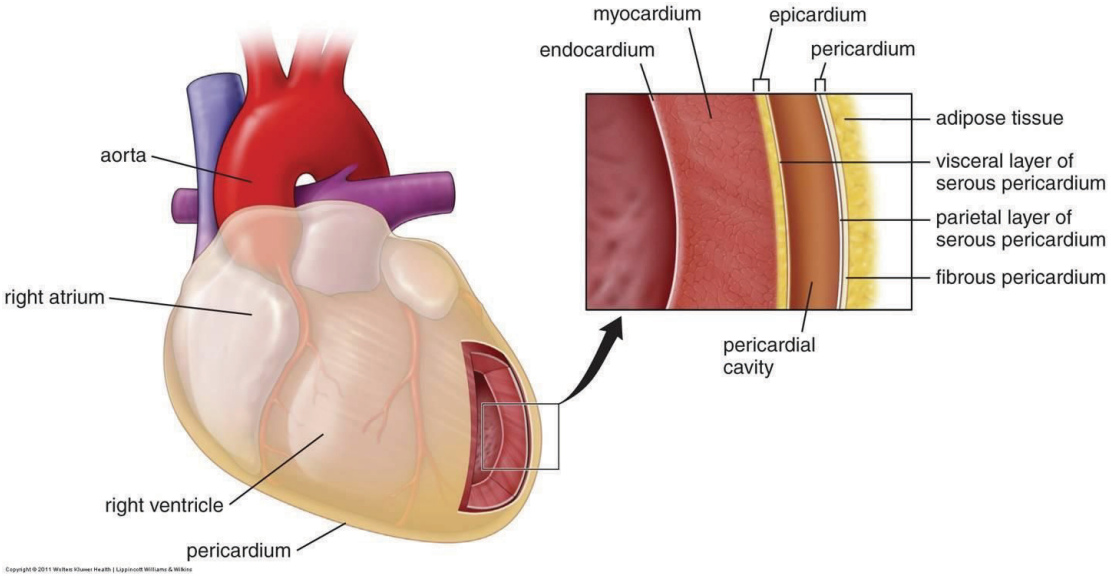

Fig. 1: The heart with the pericardium in the overview and its layer in profile https://i.pinimg.com/originals/49/f9/df/49f9df1c15f79ec3878f514a201b012d.jpg (July, 2018)

Three layers may be distinguished in the heart muscle:

- Epicardium: the inner layer of the double-walled pericardium is the outer layer of the heart muscle, the epicardium, and consists of epithelium and a connective layer
- Myocardium: the thickest layer is made up of striated muscle tissue and is responsible for the contraction of the heart

- Endocardium: inner most layer of endothelial cells lining the surface of the heart

Functionally the heart is divided into a right heart and a left heart. Each part consists of an atrium and a chamber.
The right heart receives deoxygenated blood from the venae cavae, two large vessels of the venous system. The inferior vena cava contains blood from the lower part of the body, while the superior vena cava transports blood from the arms and the head. The right atrium opens in the right ventricle via the tricuspid valve, from where blood is ejected into the pulmonary circulation.
The pulmonary circulation supplies the lung with blood, venous blood gets oxygenated, and returns as arterial blood through the pulmonary vein into the left heart, the left atrium.

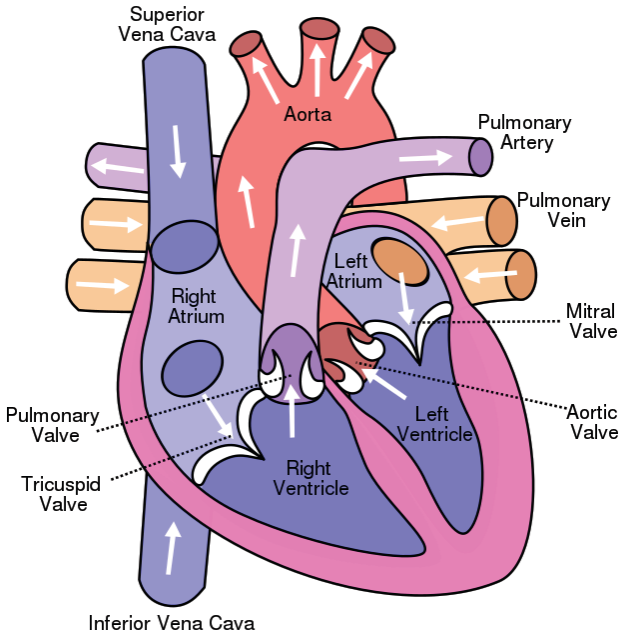

Fig. 2: The heart with its chambers, valves and the great vessels
https://upload.wikimedia.org/wikipedia/commons/thumb/e/e5/Diagram_of_the_human_heart_%28cropped%29.svg/650px-Diagram_of_the_human_heart_%28cropped%29.svg.png (June 2018)

The left atrium and the left ventricle are connected by the mitral valve. The left ventricle, which is much stronger than the right ventricle, opens through the aortic valve in the systemic circulation.

The valves are positioned in the cardiac skeleton, a structure of connecting tissue, which separates the atria from the ventricles. The cardiac skeleton stabilizes the anatomical structure of the heart and serves as an insulating layer between the atria and the ventricles.

The heart muscle itself is supplied with blood by the coronary circulation system, which is composed of the right coronary artery and the left coronary artery.

The left coronary artery originates from Sinus aortae, above the left cusp of the aortic valve, and branches into the LAD (left anterior descending) and the RCX (left circumflex). The right coronary artery originates from the Sinus aortae, above the right cusp of the aortic valve. Both arteries have several branches, which are varying individually. (1)

Coronary Artery	Cardiac Muscle Supplied	Conducting Tissue Supplied
Left Main Coronary Artery		
Left anterior descending	Anterior ventricular septum Anterior left ventricle The apex	Bundle branches
Left circumflex	Left atrium Left ventricular lateral wall Left ventricular posterior wall	SA node in 45% of hearts AV node in 10% of hearts
Right Coronary Artery		
	Right atrium Right ventricle Posterior ventricular septum Inferior wall of left ventricle	SA node in 55% of hearts AV node in 90% of hearts

Fig. 3: Coronary Artery Blood Supply for cardiac muscle and conducting system

https://soperedi.files.wordpress.com/2013/07/b5a91-1.jpg (July 2018)

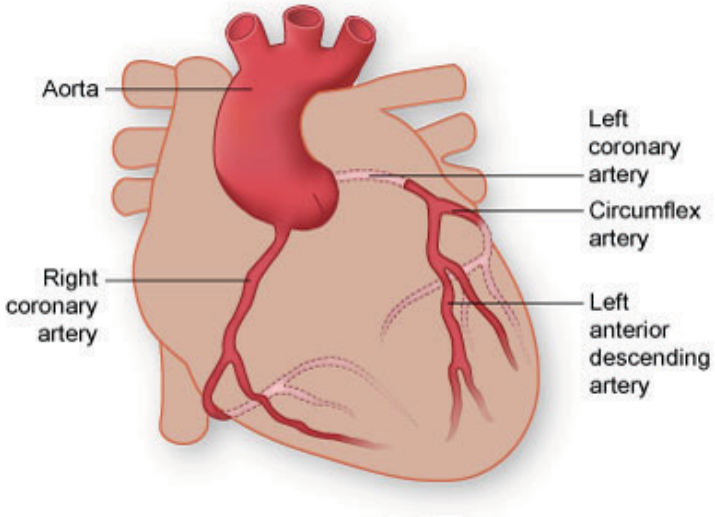

Fig. 4: The right and the left coronary artery originate from the Aorta https://www.texasheart.org/wp-content/uploads/2017/12/thi-coronary-arteries.jpg (July 2018)

Generally blood vessels have three layers, whose function and position in the body can vary:

- Tunica intima: innermost layer of the blood vessel, consists of endothelium cells and is in contact with the blood
- Tunica media: this layer is composed of smooth muscle and collagen fibers
- Tunica adventitia: is made up of connective tissue

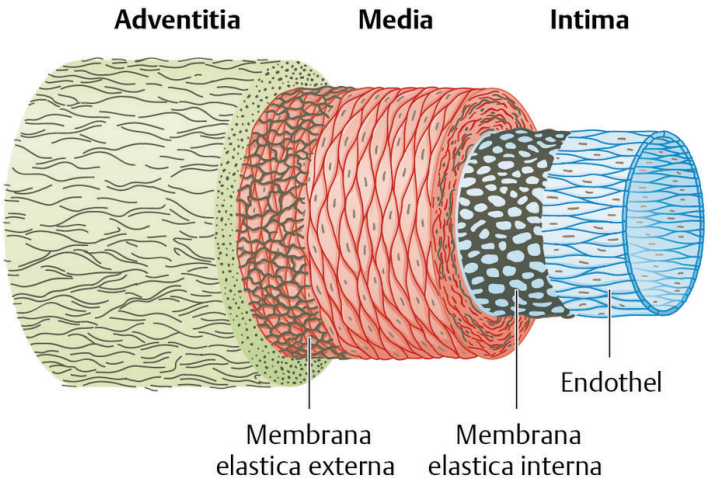

Fig. 5: Different layers of blood vessels https://viamedici.thieme.de/api/images/l/c/h/e/m/a/histo_004800_arterie_wand_schema.png (July 2018)

Oxygenated blood is leaving the left ventricle in arteries to the periphery tissues (only two arteries are carrying deoxygenated blood: the pulmonary artery brings venous blood from the right heart to the lungs, and the umbilical artery brings deoxygenated blood back from the fetus to the mother).
Arteries can be divided into two groups:

- Elastic arteries are the closest arteries to the heart and very dilative. They are responsible for converting the pulsatile blood flow coming from the heart into a constant blood pressure.
- Muscular arteries can be found more distal and involve arteries as well as smaller arterioles. Due to the muscular layer they are able to help maintain the blood pressure.

The exchange of gas and nutrients happens in the capillaries. Capillaries are the smallest vessels of the circulatory system; they are made up of one layer of endothelial cells and have a diameter of 7 µm. The capillaries connect the arteries and the veins that bring blood back to the right heart. (2)

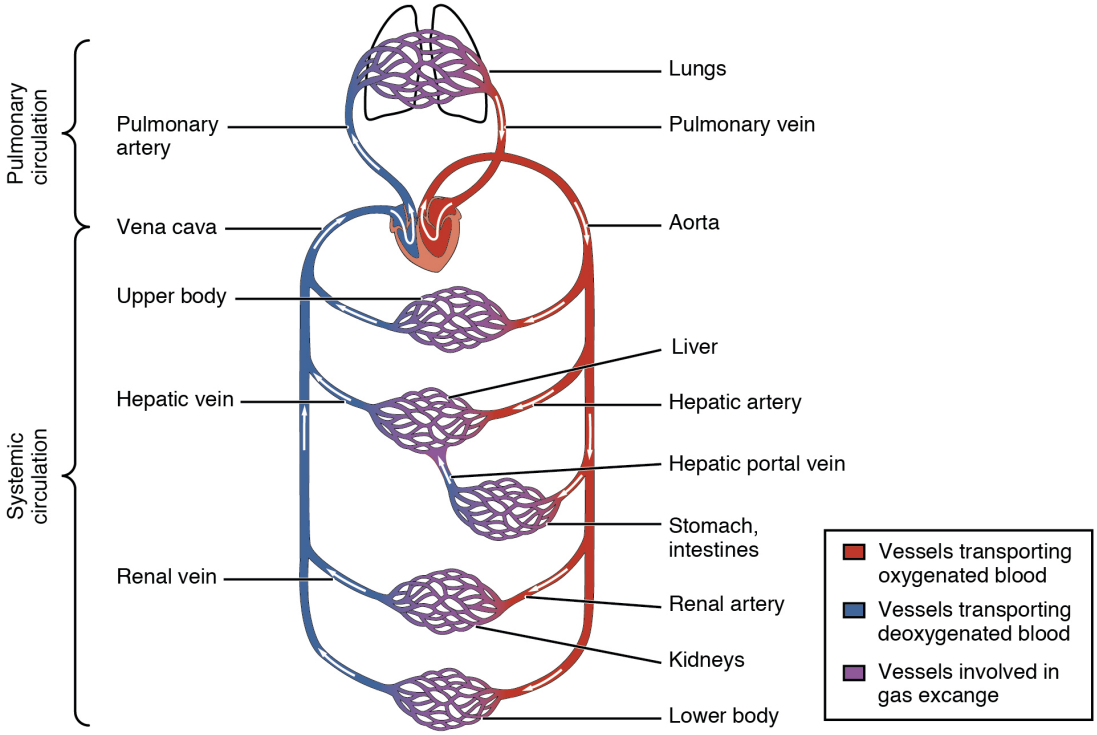

Fig. 6: Circulatory system shown schematically https://upload.wikimedia.org/wikipedia/commons/f/f2/2101_Blood_Flow_Through_the_Heart.jpg (April, 2018)

1.1.1 Cardiovascular diseases

Many diseases affect the cardiovascular system and they are the leading cause of death globally. The most common cardiovascular disease causing death is the coronary heart disease.

Coronary heart disease encompasses a group of diseases concerning the coronary arteries. The common feature of this group is the stenosis of coronary arteries and the following inadequate blood supply of the heart muscle. The reduction of blood flow is mainly caused by atherosclerosis, which is a pathological alteration of the tunica intima of arteries.

Other important cardiovascular diseases caused by atherosclerosis are stroke and peripheral artery disease.

Atherosclerosis is characterized by build-up of lipids and plaques on the tunica intima of vessels. This disease affects elastic arteries and large and medium-sized muscular arteries. Fat, carbohydrates, blood cells, connective tissue and calcium compose plaques.

Atherosclerosis develops over many years, beginning with endothelial dysfunction, which is often caused by hypertension, smoking, hyperlipidemia, immune mechanisms, and hemodynamic factors. The initial lesions appear as fatty streaks, small, yellow, round alterations in the tunica intima. Due to a complex pathological process the lesions grow and plaque develops that narrows the lumen of the arteries, whereby the blood flow gets restricted.

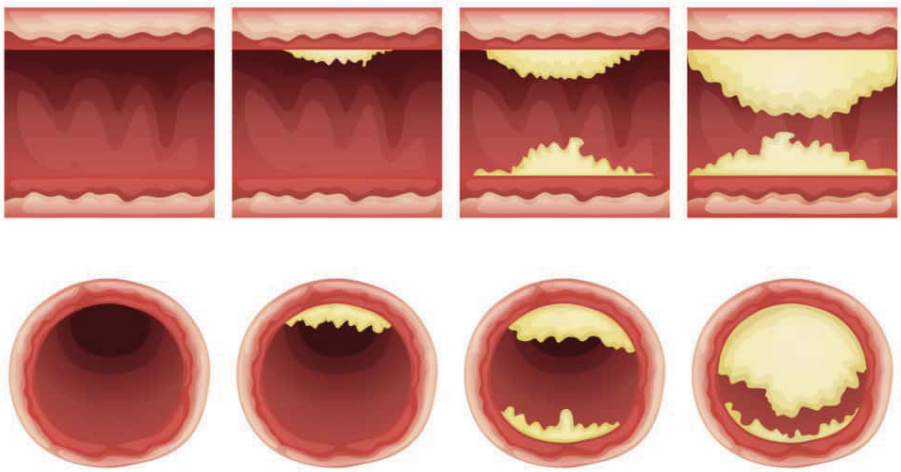

Fig. 7: Progression of atherosclerosis
https://www.kurkuma-superfood.info/wp-content/uploads/2018/04/kyi9_mdt6_121005-1024x544.jpg (July 2018)

If the stenosis of the coronary arteries is more than 75% percent, the blood supply of the heart muscle cannot be ensured anymore. If the supply in the coronary arteries with blood is stopped, often due to a thrombosis based on a plaque, the ischemia causes damage to the heart muscle and may cause death.

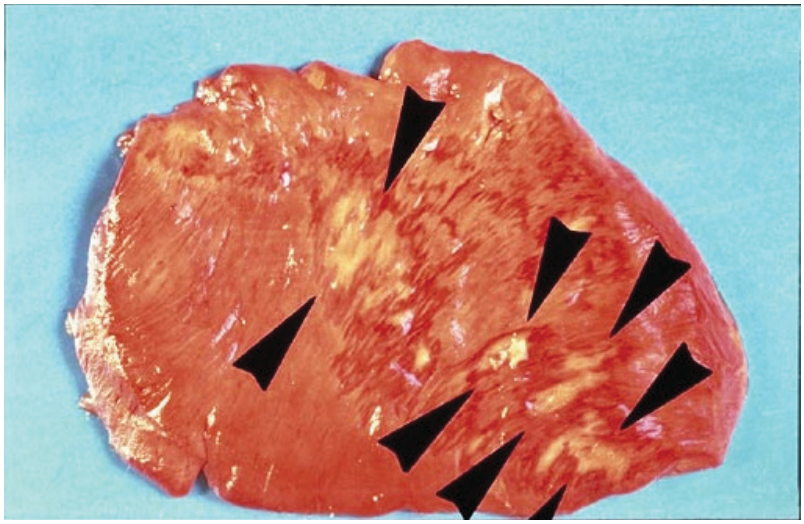

Fig. 8: Pathological preparation of a heart muscle with myocardial infarction http://e-learning.studmed.unibe.ch/webtbs/pat_makropath/images/65_diesem.jpg (July 2018)

Risk factors for atherosclerosis can be classified as modifiable and nonmodifiable. The main risk factors are listed below:

- Hypertension is defined as persisting blood pressure over 140/90 mmHg. Untreated, hypertension damages various organ systems, in particular the heart, the kidney, the brain and the retina. In terms of atherosclerosis, hypertension causes endothelial dysfunction
- Hyperlipidemia can be familial or acquired. Especially elevated LDL (Low Density Lipoprotein), which is a transporter for fat molecules including cholesterol, and decreased levels of HDL (High Density Lipoprotein) contribute to pathogenesis of cardiovascular diseases. Dyslipidemia affects more than 50% of the population aged 40 years and above in western industrialized countries
- Nicotine abuse is known as one of the main risk factors for cardiovascular diseases and atherosclerosis and is highly associated

with increased death rates. Pathophysiological mechanisms might be the impact of tobacco on thrombocytes, fat metabolism, hemodynamic, function of macrophages and on the endothelial function

- Diabetes mellitus leads to damage in endothelial function, causes metabolic dysfunction and accelerates atherogenesis
- Age: Atherosclerosis develops over years, beginning in early ages. With increasing age lesions are more frequent
- Sex: Men are more frequently affected by atherosclerosis at a young age. Estrogens have a protective effect on the vessels of women, after menopauses women “catch-up” in developing atherosclerosis (3)

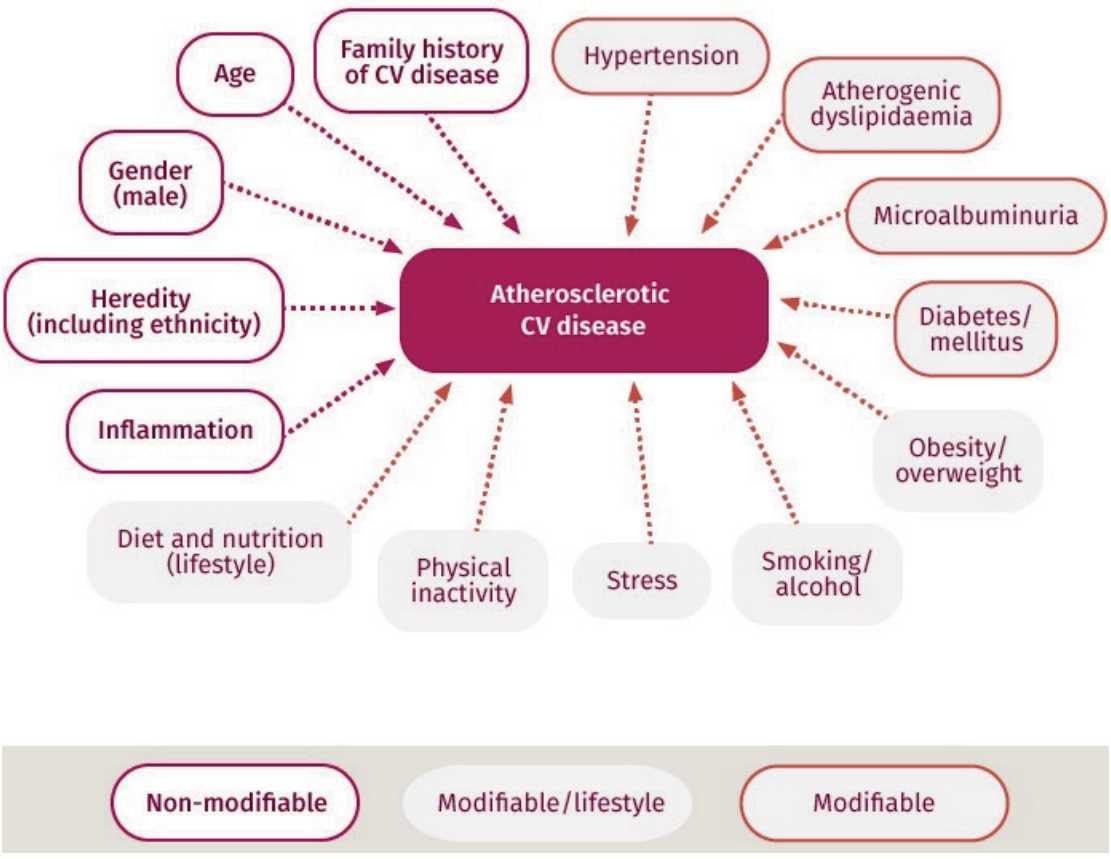

Fig. 9: Modifiable and non-modifiable risk factors of atherosclerosis https://www.thrombosisadviser.com/static/media/images/upload/arterial-thrombosis/major-risk-factors-for-atherosclerotic-cardiovascular-disease.png (July 2018)

1.2 Pregnancy

Pregnancy is the process by which an offspring develops in the womb and takes around 38 weeks.

During mating around 200 million of sperms are ejaculated into the vagina, in which they reach the mucus of the cervical canal. Within 4-6 hours sperms ascend through the cavum uteri to the uterine tube, but only a few hundred make it to the ampulla. While this ascension sperms acquire the ability to fertilize an oocyte by a biochemical reaction (capacitation).

While ovulation a human egg is released from one of the two ovaries and is caught by the fallopian tube.

The fertilization of the human egg mostly occurs in the ampulla of the uterine tube. After the fusion with a sperm the zygote is carried towards the uterus, reaching the cavum uteri as morula.

10 days after the fertilization the cells, now known as blastocyst, attach to the uterine wall and the implantation takes place. After this stage of pregnancy the mother can supply the embryo with oxygen and nutrients. (4)

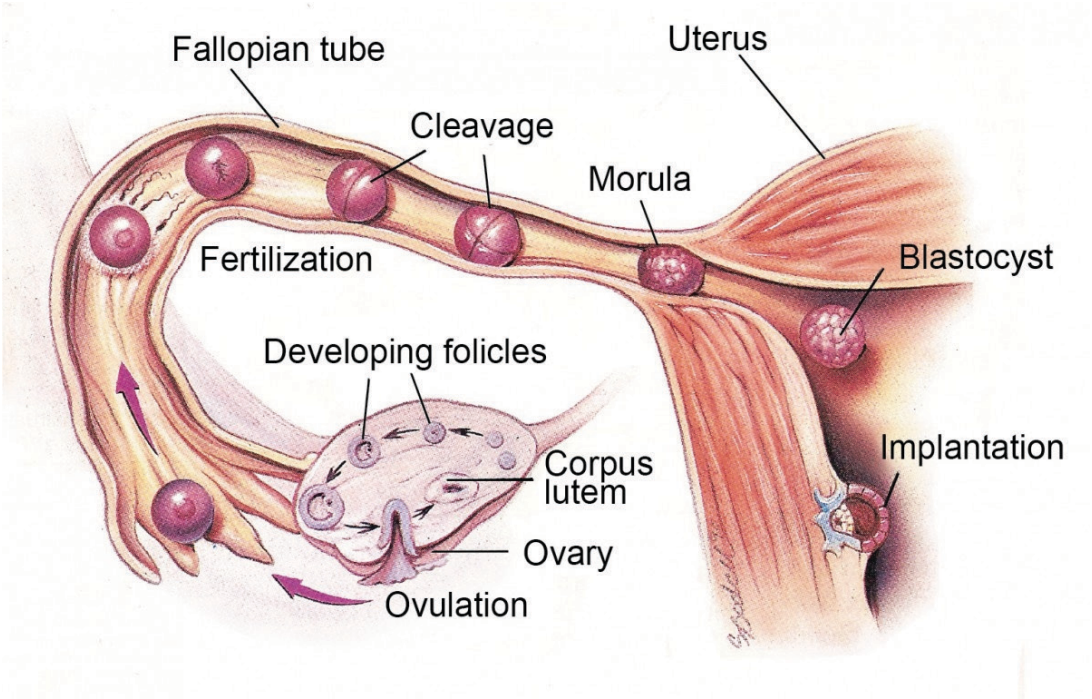

Fig. 10: Migration of the human egg from the ovary to the cavum uteri https://humanbodyanatomy.co/wp-content/uploads/2018/02/fertilization-in-female-female-reproductive-fertilization-anatomy-human.jpg (July 2018)

1.2.1 Placenta

The placenta is an organ that connects the mother with the fetus by the umbilical cord. Having a fetal part, which develops from fetal cells, and a maternal part, which develops from maternal uterine tissue, the main functions of the placenta are the exchange of products of metabolism, of gas and endocrine functions. For the fetus, the placenta is the only access to nutrients, but also toxins, drugs and other substances can pass.

Maternal blood reaches the intervillous space in the placenta through the spiral arteries coming from the decidua of the uterus. In the intervillous space, where fetal villi are present, the exchange of products takes place. Fetal blood, which reaches the placenta through the two umbilical arteries, goes through the capillaries of the villi and returns through the umbilical vein back to the fetus. (5)

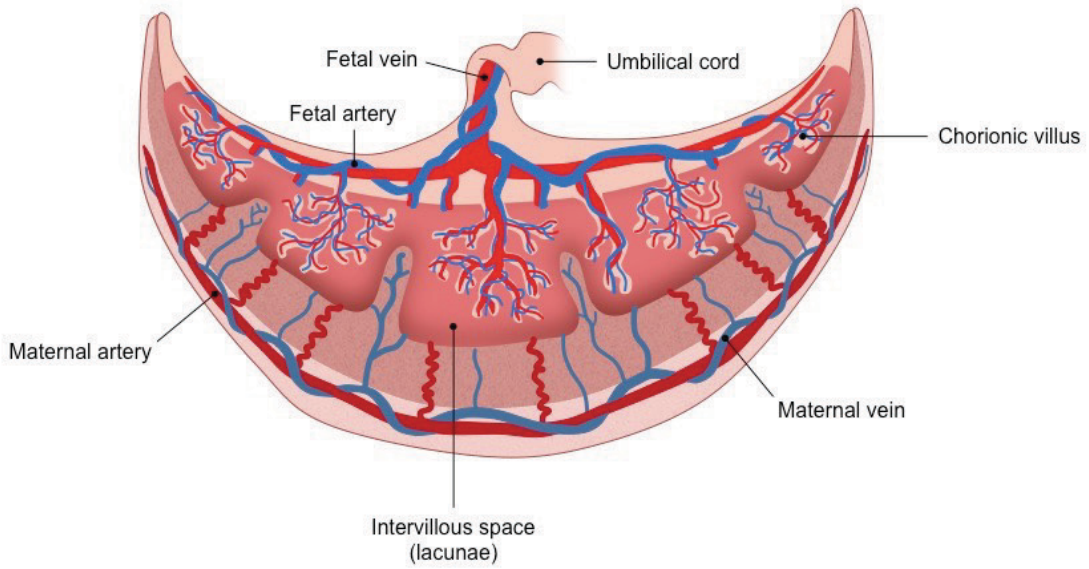

Fig. 11: Chart of the placenta with maternal and fetal blood vessels
http://ib.bioninja.com.au/_Media/placenta_med.jpeg (February 2018)

Because the blood flow in the placenta is not underlying an autoregulation, the perfusion depends on the maternal blood pressure. Low maternal blood pressure leads to a reduced supply of the placenta and can damage the fetus.

The exchange of products between the mother and the fetus are based on 4 mechanisms:

- Diffusion: Each gas follows its partial pressure gradient, O_2 from maternal blood to fetal blood, and CO_2 from the fetus back to the mother. Fat-soluble substances, such as vitamins or drugs, can diffuse as well.
- Facilitated diffusion: specific transmembrane proteins for glucose (GLUT-1-Uniport-Carriers) allow a better energy supply for the fetus without any requirement of energy for the transport itself. Also lactate transport is facilitated to avoid high lactic acid concentrations in the fetal blood.
- Carrier-mediated transport: amino acids, b-vitamins and Ca^{2+}-ions are transported actively between maternal and fetal circulation.
- Endocytosis is an important process for the fetal iron-metabolism and the uptake of immunoglobulins.

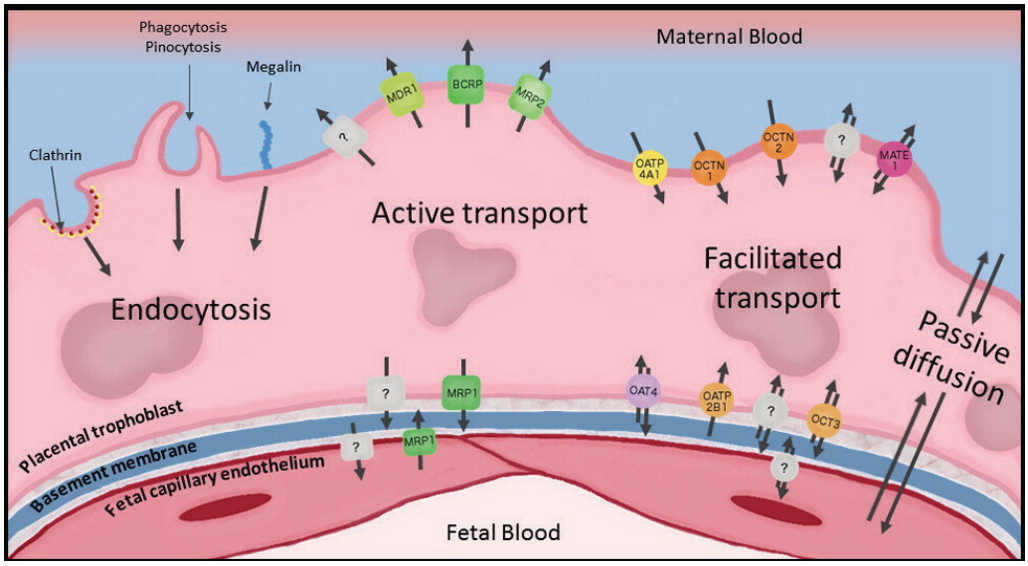

Fig. 12: Placental mechanisms of exchange
https://ars.els-cdn.com/content/image/1-s2.0-S0169409X16302344-fx1.jpg
(July 2018)

The placenta produces important hormones to maintain the pregnancy. Human chorionic gonadotropin (hCG) interacts with the ovaries and stimulates the corpus luteum in the beginning of the pregnancy. After the degeneration of the corpus luteum, the placenta produces progesterone and

estrogen as well. Human placental lactogen take effect on the carbohydrate and fat metabolism, and plays a role on the development of the mammary glands. (4)

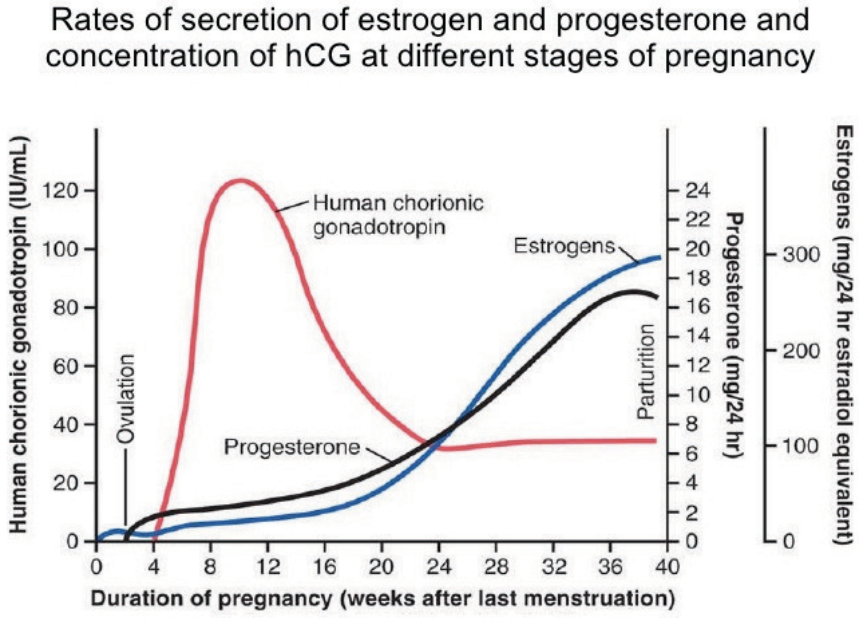

Fig. 13: Estrogen, progesterone and hCG levels in pregnancy https://image.slidesharecdn.com/placentaasanendocrineorgan-111024114409-phpapp01/95/placenta-as-an-endocrine-organ-5-728.jpg?cb=1319456689 (July 2018)

The placenta is fully functional developed after the first trimester of the pregnancy but still grows till the end of the pregnancy. At the end of the pregnancy the placenta weighs approximately 500-600g and its diameter is around 20cm. (5)

The size of the placenta represents its capability for the exchange of nutrients and correlates generally with the baby's size. The crucial factor for metabolic exchange is the placental surface, which can be increased by the surface of the villi, an expand invasion across the uterus, or by a deeper invasion of maternal spiral arteries.

The placental growth is polarized in two axes, a major one, following the largest diameter of the placental surface, and a minor one, bisecting it at right angles. It might be possible that the growth of the major axis orients towards the growth of the rostrocaudal axis of the embryo, while the length of the minor axis depends on the mother's nutrition. (6)

1.3 Embryonic Development

Within nine months the fertilized human egg develops into a full-term newborn, in which it passes the embryonic period and the fetal period. The embryonic period lasts eight weeks (3. - 12. week of pregnancy) and organogenesis happens in this period of pregnancy. In the following fetal period the fetus is growing rapidly until birth. (5)

During this development the embryo and later fetus go through so-called critical periods, in which stimuli cause permanent effects. (7) While stimuli in the embryonic period cause organ malformations (embryopathy), noxae in the fetal period are responsible for disorders in growth and function (fetopathy). (4)

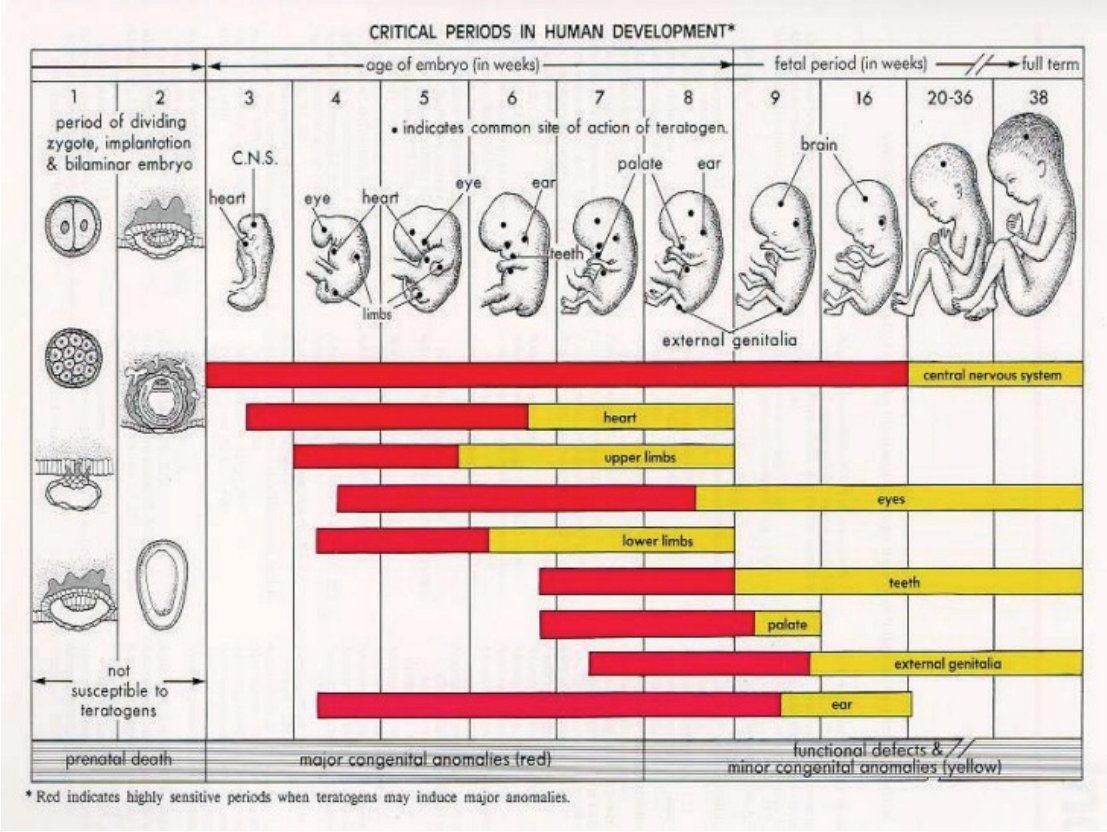

Fig. 14: This chart shows critical periods, when stimuli may induce major anomalies

https://image.slidesharecdn.com/criticalperiodsinhumandevelopment-120102131107-phpapp01/95/critical-periods-in-human-development-1-728.jpg?cb=1325509899 (April, 2018)

The fetal cardiovascular system differs in composition and function. The development of the cardiovascular system starts with the third week after fertilization and the heart begins to beat in the fifth week. The development has its origin in the cardiogenic area with the formation of two tubes. After merging to one single tube, the heart tube, it loops and separates into chambers.

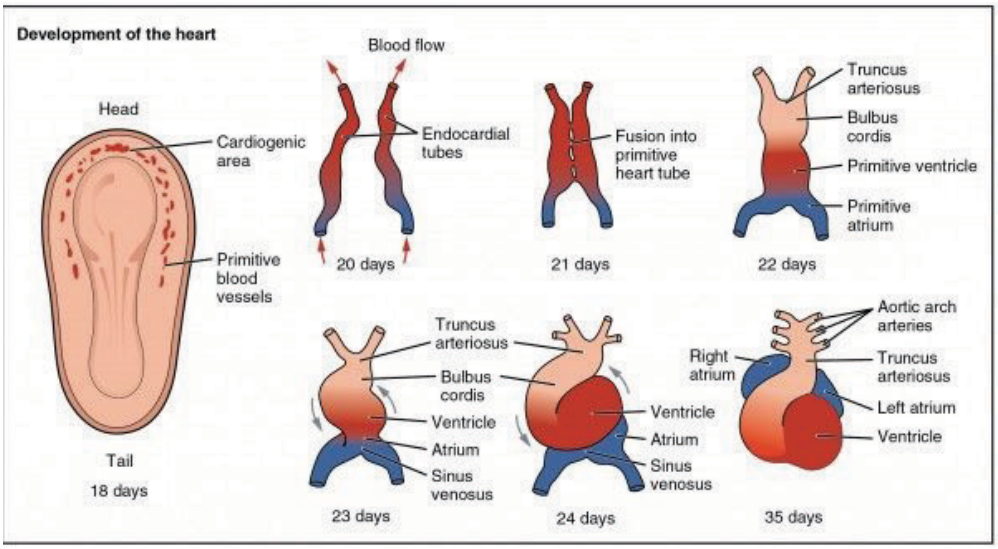

Fig. 15: Development of the heart https://i.pinimg.com/originals/43/c6/9f/43c69f4aab7722150a6e2dac70c2c76f.jpg (July 2018)

Vessels are formed by two mechanisms:

- Vasculogenesis: de novo formation of vessels by merging of angioblasts into endothelial tubes; big vessels underlie this formation mechanism
- Angiogenesis: formation of new vessels based on pre-existing vessels; the majority of blood vessels is formed by angiogenesis (5)

Due to the fact that the mother is giving the fetus all nutrients and oxygen via the placenta and degradation products from the child's metabolism are removed through the umbilical cord, the fetal circulation is adapted to these conditions and uses three shunts:

- Ductus venosus: part of the oxygenated blood, coming from the placenta through the vena umbilicalis, bypasses the liver and flows directly through the ductus venosus into the inferior vena cava.
- Foramen ovale: this shunt connects the right atrium with the left atrium; oxygenated blood, coming from the inferior vena cava, reaches the left heart bypassing the lungs
- Ductus arteriosus: deoxygenated blood from the right ventricle is transported through the ductus arteriosus to the aorta, bypassing the lungs; the ductus arteriosus attaches to the final section of the aortic arch (1)

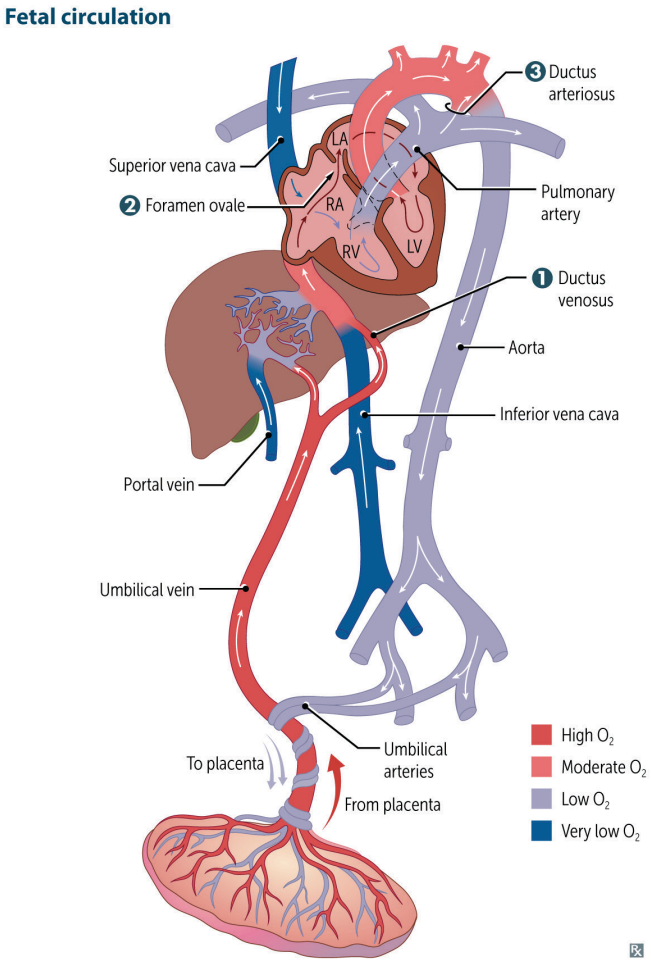

Fig. 16: Fetal circulation with three shunts
http://d1yboe6750e2cu.cloudfront.net/i/3c65f513730f8f685115f62e4b0cf97c0e844d72 (July 2018)

1.4 Fetal programming

1.4.1 Definition

The worldwide average life expectancy at birth was 71.4 years in 2015, ranging from 50.1 years in Sierra Leone to 83.7 years in Japan. (8)

There are many obvious factors influencing life expectancy, but probably most people would not expect, that their birth weight might predict their life span.

In an epidemiological study, Barker showed the correlation between low birth weight and death from coronary heart disease in adult life. 16.000 men and women, born in the early 1900's in Hertfordshire, were traced from birth, and those with low weight at birth had high death rates due to coronary heart disease. (9)

Undernourishment while pregnancy is one of the key factors that lead to low birth weight and persisting alterations to metabolic functions of the fetus. (7)

Markers such as low birth weight, disproportional placentas and a low ponderal index (birth weight/length) are associated with chronic diseases such as cardiovascular diseases and diabetes mellitus in adult life. It is known, that while a critical period of fetal development an insult has lifelong effects on the offspring. Barker first postulated this process, called fetal programming. (10)

1.4.2 Epidemiological Studies

The Hertfordshire records

Due to the concern about the bad health condition of the British people in the early 20th century, Ethel Margaret Burnside, a midwife, arranged, that women in childbirth were attended by trained midwives and were advised in the first year after giving birth. Every newborn baby was weighed after delivery and again at the age of one year. The data, also including illness and development of the baby, were recorded on a card, which was handed in to the county office when the baby reached one year.

The largest set of records was preserved in Hertfordshire, which were investigated by David Barker. (11)

In a follow-up study 16.000 men and women born during 1911 – 1930 were traced from birth. (12) It was shown that death rates from coronary heart

disease fell two-fold between those with low birth weights and those in the high birth weight group. (13)

Table 1: This table shows the correlation between birthweight and death from coronary heart disease (13)

TABLE I.

DEATH RATES FROM CORONARY HEART DISEASE AMONG 15 726 MEN AND WOMEN ACCORDING TO BIRTHWEIGHT

Birthweight lb (kg)	Standardized mortality ratio	Number of deaths
≤5.5 (2.50)	100	57
–6.5 (2.95)	81	137
–7.5 (3.41)	80	298
–8.5 (3.86)	74	289
–9.5 (4.31)	55	103
>9.5 (4.31)	65	57
All	74	941

A Swedish cohort study also investigated the association between mortality from ischemic heart disease and size at birth. All 14.611 babies born during 1915 – 1929 at Uppsala Aacdemic Hospital were followed up to the end of 1995. Containing an almost complete follow up in the cohort, and also considering socioeconomic circumstances, this study proved an inverse association between cardiovascular disease and birth weight. (14)
Also an American Nurses' health study including a cohort of 70.297 women, showed the association between birth weight and adult heart disease. The results demonstrated a similar connection between the relative risk of non-fatal coronary heart disease and birth weight as the Hertfordshire records did. (15)

1.4.3 Fetal development and fetal programming

In the fetal period the fetus is growing mainly due to periods of rapid cell divisions, which goes along with critical periods in development. For cell division an adequate supply of nutrients is needed, otherwise the rate of cell division must be lowered. (16) These critical periods do not occur at one certain point in pregnancy, but are varying on the different tissues and organs.

An example is provided by the gonadal development, which is in a critical period in early pregnancy, whereas the critical period for the kidneys is between 26th and 34th week of pregnancy. (9) As a result of these 'shifted' critical periods undernourishment for a limited phase can cause disproportionate growth. (16)

Interesting findings were made in investigations of the Dutch famine, which was a period of seven months at the end of the Second World War. The effects of the malnutrition on pregnant women were researched. While babies, who were exposed to the famine in early gestation were heavier and longer at birth, children were exposed to this disastrous time in mid gestation lighter, shorter, and had smaller heads than babies with normal nutrient supply. In late gestation, the effect of the famine was apparent in lighter, shorter and thinner babies with smaller heads. The findings, also on the long-term impact, showed that the timing of the insult is crucial on the effect of different tissues. (17)

Table 2: The development of adult disease depends on the timing of the insult in pregnancy (18)

First Trimester	**Second Trimester**	**Third Trimester**
Obesity	Pulmonary Disease	Diabetes
Hypertension	Renal Disease	Depression
Dyslipidemia		Schizophrenia
Coronary Artery Disease		Anti-Social Personality Disorder

Winick and Noble could show in their experiments that growth in rats can be divided into three groups: while in the first 21 days after birth organs grow by cell division, growth between 21 and 42 days growth is based on cell division *and* cell enlargement. After this time cell division in the lung and brain stop, and after 65 days growth is only achieved by cell enlargement. If the overall number of cells is lowered because of under nutrition after the 65th day, cell division cannot be restarted anymore. (19) It is similar to growth in human development, which also changes after birth. In late gestation rates of cell

division falls and after birth growth is mainly achieved by cell enlargement of existing cells. (16)

The development of the cardiovascular system is very complex and a certain sequence of steps is required. The human cardiovascular system is developing and changing all gestation long, but there are two critical periods, in which it is particularly vulnerable. In early pregnancy, in the first six weeks of the embryonic period, the heart is vulnerable to chemical or mechanical stressors “that alter the formation of its structural relationships”. In late pregnancy the final number of cardiomyocytes is fixed and the formation of the coronary tree is finalized at the microvascular level. It is assumed, that the cell division of heart cells is under the influence of the placenta, where hypoxemia, haemodynamic irregularities and the growth factor environment play a key role in determining an abnormal number of cardiomyocytes. In these two periods, deficient supply of nutrients cause permanent alterations on the heart. (6)

1.4.4 Fetal programming and cardiovascular diseases

There is strong evidence from epidemiological investigations in fetal programming, that coronary heart disease is ‘programmed’ in early fetal life.

But not only coronary heart disease, also other chronic diseases are related to birth weight. Many studies prove the correlation between low birth weight and raised blood pressure in childhood and adult life. (20) It is suggested, that birth weight predicts the total glomerular number of a newborn, whereas a reduced number should be associated with essential hypertension. Another factor in the pathophysiology of hypertension is endothelial dysfunction. It has been shown that children and adults with low birth weight developed endothelial dysfunction. (21)

Barker could show that high levels of low-density cholesterol and apolipoprotein B in individuals and low birth weight are correlating as well. In his measurements newborns with low abdominal circumference had increased serum concentrations. Probably the abdominal circumference reflects the size of the liver, which is the important organ in fat metabolism. Growth restriction in late pregnancy, the time when the liver is growing rapidly,

may lead to loss of liver tissue, and further to a persisting change of metabolism. (22)

Analyses also showed increased fibrinogen plasma concentrations of adult men whose abdominal circumference was reduced. Fibrinogen plays a key role in the development of cardiovascular diseases. Table 3 shows the correlation between the mean plasma fibrinogen concentrations of adult men and their abdominal circumference at birth. Adjustments for smoking and waist/hip ratio were made. Significant correlations were not found in women. (23)

Table 3: Mean fibrinogen concentration by abdominal circumference at birth in men and women (23)

	Men			Women		
Abdominal circumference (in.)	Unadjusted	Adjusted for smoking and waist/hip ratio	*n*	Unadjusted	Adjusted for smoking and waist/hip ratio	*n*
⩽11·5	2·60	2·66	25	2·70	2·72	23
11·6–12·25	2·53	2·51	22	2·53	2·53	21
12·26–13	2·49	2·47	35	2·62	2·58	31
>13	2·37	2·36	22	2·68	2·72	21
	$P = 0·03$	$P = 0·006$		$P = 0·9$	$P = 0·9$	

1.4.5 Mechanisms of fetal programming

Fetal Nutrition

Even if the fetal genome determines growth potential in utero, the crucial factors for the offspring's birth weight seem to be the nutritional and hormonal milieu in the uterus. It was shown that half-siblings with the same mother have similar birth weights, but those with the same father are not correlating.

Also in embryo transfer studies, the recipient mother influences the fetus growth more strongly than the donor mother does.

It is assumed that a mother's own fetal growth, her dietary intakes and her body composition effects the fetal demand for nutrients as well as the maternoplacental capacity.

An insufficient maternoplacental supply of nutrients trigger a range of fetal adaptations. Those have benefits for the short-term survival of the fetus, but causes permanent alterations in the organism. (9)

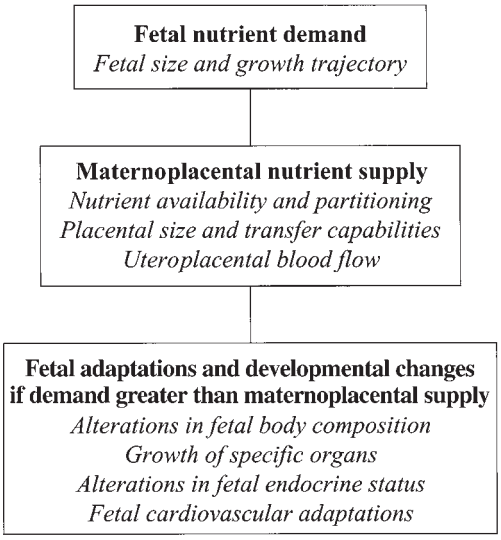

Fig. 17: Framework for understanding the maternal regulation of fetal development and programming (9)

Metabolic Changes

If the mother's supply of nutrients is lower than the fetal demand, the metabolic response of the fetus is catabolism, and it consume its substrate for energy production. It includes oxidation of amino acids and lactic acid and an increased glucose oxidation. If the undernourishment persists, these metabolic adaptions result in delayed growth, reduced substrate use, and lowered metabolic rate, but allow the fetus to survive longer under bad circumstances. (24)

Hyoxaemia

While some organs are affected from metabolic changes, the human organism tries to protect important tissues for its viability and redistributes the blood flow to those organs. In the human body the brain needs protection for survival, but this mechanism goes with loss of tissue of other organs. (10)

Endocrine Changes

Maternal dietary intakes influences fetal hormones very strongly. Fetal insulin and IGF, which are important for growth control, react on fetal nutrition. Responding to undernourishment fetal insulin, IGF and glucose concentrations fall, which is followed by decreased placental transfer of amino acids and glucose and results in reduced fetal growth. Thereby catabolic hormones, like glucocorticoids, which have an important role for cell differentiation, increase. (7)(24)

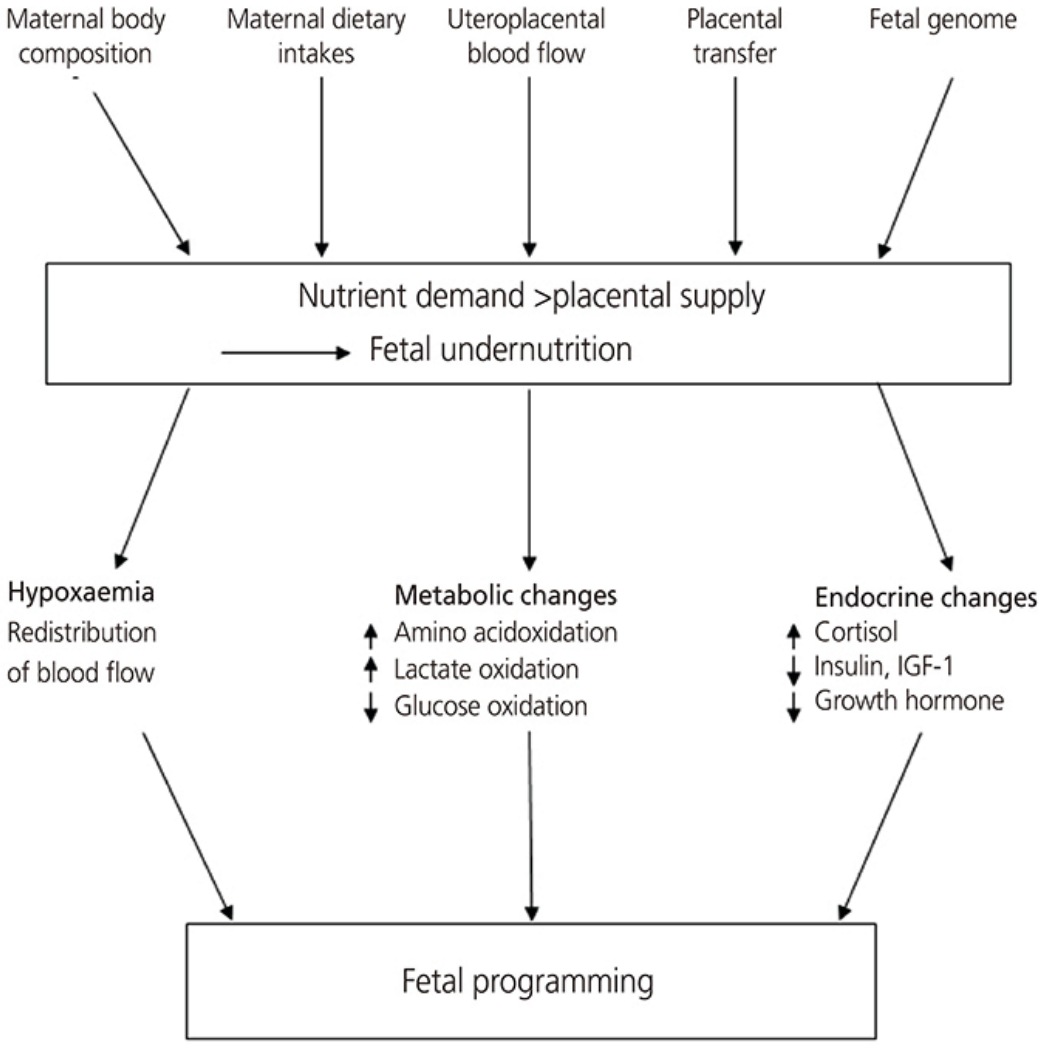

Fig. 18: Fetal adaptations to undernutrition https://synapse.koreamed.org/DOIx.php?id=10.5468/ogs.2017.60.6.506&vmode=PUBREADER#!po=2.27273 (April, 2018)

Genetic mechanisms

It is assumed that fetal programming occurs also on an epigenetic level, underlying two mechanisms: DNA methylation and histone modification. These alterations may be influenced by the maternal dietary intake in early embryonic life and could have consequences in further developmental stages. These processes, known as gene imprinting, modify gene expression and thereby metabolism. (25)

1.4.6 Body proportions

For the predisposition of diseases not only the birth weight is important, but also the body proportions of the newborn. Thinness, stunting and a small trunk are different proportional appearances caused by fetal adaptations to environmental circumstances like undernourishment, (7) even if the body size may be the same. While thin babies, with a normal birth weight, are at risk to develop insulin resistance, short babies in relation to their head circumference and reduced abdominal circumference are at risk that their vascular structure is abnormal, their liver function is limited and their LDL cholesterol level is in disorder. The abdominal – head circumference disproportion might be the result of blood flow adaptations by which the brain gets protected at the cost of abdominal development. (10)

Ponderal Index

The ponderal index measures the thinness and is defined as birthweight/length^3. It was shown, that a low ponderal index at birth correlates with coronary heart disease. Men, who were thin at birth, had death rates from coronary heart disease twice as high as men with a high ponderal index. Table 4 shows the relation between mortality ratios for coronary heart disease, birth weight and ponderal index. (26)

Table 4: Standardized mortality ratios for coronary heart disease in 3302 Finnish men (26)

Birthweight kg [lb]	SMR (No. of deaths)
≤2.5 [5.5]	84 (11)
−3.0 [6.6]	83 (44)
−3.5 [7.7]	99 (124)
−4.0 [8.8]	76 (80)
>4.0 [8.8]	66 (27)
All	85 (286)
P value for trend	0.09
Term babies only Ponderal index at birth (kg/m^3)	SMR (No. of deaths)
≤25.0	116 (59)
−27.0	105 (88)
−29.0	72 (64)
>29.0	56 (33)
All	86 (244)
P value for trend	<0.0001

Disproportion in organ size and catch-up phenomenon

Undernourishment affects organs depending on the stage of gestation, because of the varying growth rate. In late gestation it causes disproportion in organ size and tissues. (7) The kidney, for example, is growing rapidly in that time; slowing of fetal growth leads to reduced growth of the kidney and reduced replication of kidney cells, which cannot be caught up anymore in later life. (10)

A similar problem arises with thin babies, who lack muscle. Muscle cell division happens in utero, but after birth there is little capacity for cell replication. Those babies have an altered body composition at birth, and if they gain weight rapidly in childhood, they develop a disproportional high fat mass. (27) This catch up growth has life long consequences for the individuals and is related to increased death rates from coronary heart disease, hypertension and type 2 diabetes. (24) The highest risk for coronary heart disease have those with low ponderal index at birth and a high body mass index at the age of 11. It was also shown that boys with high ponderal index have a low risk of coronary heart disease, even if they have a high body mass index in the following years. (28)

1.4.7 Role of the placenta

The birth weight depends not only on the mother's dietary, but also on the placenta's transfer capability of nutrients. It seems that the placenta functions as a mediator and regulates the transport of nutrients, in which its potentiality is limited to the nutritional supply and demand. Anyway, the placental size reflects the ability for nutritional transfer. (6)

The research into the Dutch famine found that exposure to the famine in early pregnancy goes along with increased placental weight, (26) whereas smaller placentas result from exposure in late gestation. (17) In sheep farming, the effect of nutrition on the placenta and on the lamb is well known and exploited for breeding. High periconceptional pasture intake followed later by reduced feeding in pregnancy results in better outcomes in growing.

The placental development in size may have life-long consequences for the offspring. In a follow-up study in Sheffield it was shown that placental ratio and later coronary heart disease is U-shaped correlating. While small placentas don't have the capability for adequate supply, disproportionately large placentas may cause fetal catabolism, but both variations may lead to increased death rates from coronary heart disease. (9)

Table 5: Correlation between Birth weight, placental weight and Mean systolic blood pressure of 50-year-old men and women
https://oncohemakey.com/wp-content/uploads/2016/06/table18_3.jpg
(April 2018)

	Placental weight (g)				
Birthweight (kg)	≤454	455–568	569–681	>681	All
≤2.9	149 (24)	152 (46)	151 (18)	167 (6)	152 (94)
3.0–3.4	139 (16)	148 (63)	146 (35)	159 (23)	148 (137)
>3.4	131 (3)	143 (23)	148 (30)	153 (40)	149 (96)
All	144 (43)	148 (132)	148 (83)	156 (69)	149[a] (327)

Figures in parentheses are numbers of subjects.
[a] SD = 20.4.

The correlation between placental size and hypertension in adult life was shown in a study in Preston. Systolic blood pressure in adults is correlating with high placental ratio. The highest blood pressure was shown in the group, whose placental weight was high in relation to their birth weight. Table 5 shows the mean systolic blood pressure of 50-year-old man and women, born at term in Preston.(26)

The shape of the placenta is an important predictor of future diseases in the offspring, and is determined by different conditions, in which pre-eclampsia was investigated by Finnish researcher. In their study anatomic features of the placenta were measured and maternal records investigated. Small placentas were found from women with hypertension without proteinuria, but measures like thickness or length of the two axes were normal. Women with pre-eclampsia delivered altered placentas in length and width and increased thickness. It is controversial whether these alterations are caused by insults during the placental development to protect the fetus, or whether these variations are causative for later diseases. (6)

2. Aims and objectivs

Fetal programming concerns everyone: while the human organism develops in the womb, the lifelong health is determined. Certain insults, like insufficient nutrition, in this critical period of life have impact on the long-term health and can change one's life tremendously.

Barker postulated in his "fetal origin" hypothesis that early life events, like undernutrition, would predispose an individual for several chronic diseases. These insults would lead to fetal adaptations to ensure its short-term survival, but at the expense of long-term health and the development of diabetes, dyslipidaemia, hypertension or cardiovascular diseases.

Barker's hypothesis has been often investigated and many epidemiological and experimental studies have proven the accuracy.

An extended research in this topic is important to understand the mechanisms of the development of chronic diseases. Fundamental understanding of the origin of chronic diseases is essential for various medical disciplines. This knowledge could help to preserve health and inhibit chronic diseases. Further it is possible to link these findings with new treatment approaches that offer the opportunity of a causal treatment.

Even though the danger of teratogens like alcohol and nicotine are known by a substantial part of the public, the long-term effects of adverse circumstances while pregnancy on the health of the offspring are not in the people's awareness. This information is meaningful not only for doctors, but also for politicians, health economists and for all those who work in the service of public health protection. This diploma thesis can help to spread knowledge about fetal programming and especially about its consequences on cardiovascular diseases.

The aim of this diploma thesis is to explore fetal programming and carry out an extensive update of the current literature, with particular emphasis on the risk of cardiovascular diseases.

3. Methodology

For this literature review about “Fetal programming and risk of cardiovascular diseases” a systematic research on the current literature was performed.

To get familiar with this topic, an Internet search with Google and PubMed was done. In this early stage “fetal programming” was used as a search term and relevant articles were excerpted. Particular attention was given to articles and quotes by Dr. Barker.
Based on the topic of the diploma thesis and the results of this search following fields were identified as relevant:

- Cardiovascular system
- Cardiovascular diseases
- Pregnancy
- Development and function of the Placenta
- Embryology
- Teratology
- Fetal programming

The chapter “Introduction” was set up in order to provide an overview about the physiology and pathology of the cardiovascular system, the development of human organism and fetal programming. Information about the cardiovascular system, pregnancy and embryonic development were obtained from secondary literature, which were found in the library catalogue of the Medical University of Graz. These books were in my personal possession or were provided from the library of the Medical University of Graz and from the Institute of Gynecology and Obstetrics. For the subchapter “Fetal programming” secondary literature as well as papers were used for gathering information. The papers found in the Internet search were used for this section and additional papers were gathered from the List of Reference.

Searches for the literature research were performed in using PubMed in July 2018. The search term used first was “fetal programming” which resulted in 157291 hits. To narrow the search “cardiovascular disease” was added and resulted in 11065 hits. Next “fetal programming” was set as a search term only in titles, whereas “cardiovascular disease” was used for all fields, which resulted in 101 articles. To get the latest literature, the results were limited to the last 5 years, whereby 25 articles remained.

Theses papers were evaluated with a main focus on relevance in content with the topic of this diploma thesis. Following criteria were regarded:

- The article was written in English
- The article was relevant for this diploma thesis
- Novelty of the article
- Review articles, animal studies and clinical trials were included

12 papers gave a general overview about fetal programming or did not meet the criteria; 13 papers remained as relevant and were arranged according their thematic aspects into 5 sub-groups:

- Hypertension and fetal programming
- Oxidative stress and its effects on fetal programming
- Impact of glucocorticoids on cardiovascular diseases in fetal programming
- Epigenetic mechanisms in fetal programming of cardiovascular diseases
- Diet influences on fetal programming

Additionally, from review articles relevant references were used for the discussion of this diplomat thesis.
Figures for this diploma thesis were found in Google.
Zotero was used for organizing the references in this work.

4. Update of the literature

For the literature review 13 papers were identified as relevant and they were classified into 5 sub-categories (sections 1-5). Section 1 deals with how fetal programming affects the development of hypertension in adult life. In section 2 the effects of oxidative stress in connection with fetal programming on the cardiovascular system is explored. Section 3 examines the impact of glucocorticoids on cardiovascular diseases in fetal programming. Next, section 4 discusses epigenetic modifications. Finally, section 5 deals with the effects of maternal diet on cardiovascular diseases of the offspring.

4.1 Hypertension and fetal programming

Author	**Year**	**Major finding**	**Article type**
Lopez-Lopez	2015	Link between Fetal Programming, Inflammation, Muscular Strength and Blood Pressure;	Review article
Brøns	2015	Muscles of low birth weight young men are not restricted due to mitochondrial dysfunction	Clinical trial
Jensen	2008	Muscles of low birth weight young men have altered muscle signaling proteins	Clinical trial
Ruiz	2008	Muscle strength as a risk factor for chronic diseases	Clinical trial
Lopez-Jaramillo	2014	Low handgrip strength is a predictor of cardiovascular mortality	Clinical trial
Steene-Johannessen	2012	High muscle strength goes along with low levels of CRP	Clinical trial
Singh	2015	Glomerular and tubular adaptations initiate hypertension;	Review article
Hoy	2006	A reduced number of nephron is associated with hypertension	Clinical trial
Walker	2012	Mice with a high nephron number are	Animal

		less susceptible to hypertension	study
Scherrer	2015	Assisted Reproductive technologies have effects on the development of cardiovascular diseases;	Review article
von Arx	2015	Vascular dysfunction in children conceived by ART	Clinical trial
Ceelen	2008	Blood pressure levels are higher in ART conceived children	Clinical trial
Chiossi	2016	Adverse in utero environment, placental perfusion, nitric oxide synthase and genetic traits are responsible for the development of hypertension;	Animal study
Tain	2016	Melatonin and N-acetylcystein can prevent L-NAME induced hypertension;	Animal study

It is already known from early research that hypertension and birth weight correlate and the link with fetal programming is obvious. Detailed mechanisms and pathways are subjects of recent studies and reviews.

Good physical shape and physical activity are generally considered as important factors for the prevention of cardiovascular diseases.

It is well known that the body composition is determined by in utero environment. In utero, malnutrition causes a reduced muscle mass in the offspring. **Brøns et al.** reported that muscles of low birth weight young men are not restricted due to mitochondrial dysfunction, (29) but it seems that size, fiber type, neuromuscular function and morphology of the muscles are involved. (30) An example is given by an altered function of the muscle: impaired muscle signaling proteins cause metabolic changes in glucose and fat metabolism and may lead to obesity and metabolic diseases. One of these signal proteins is identified as Akt-1, which plays a role in muscle protein synthesis as well as in insulin signaling. (31)

Even more, **Ruiz and colleagues** suggested recently that muscle strength was a risk factor for chronic diseases, in particular for cardiovascular diseases (32) and **Lopez-Jaramillo et al.** found out that low handgrip strength is a predictor of cardiovascular mortality and all-cause. (33)

Even though exact mechanisms, which explain the relationship between muscle strength and cardiovascular diseases, are not clear yet, **Steene-Johannessen et al.** added that higher muscle strength goes along with lower levels of CRP, a marker for chronic inflammation. (34) Moreover fat cells secrete proinflammatory cytokines, which are responsible for the production of CRP.

Lopez-Lopez and colleagues could show with their research team that inflammatory processes are involved in the development of hypertension, endothelial dysfunction, built-up of plaque and thrombotic events. Further they reported the correlation between high levels of CRP and increased blood pressure and hypothesized that low-grade inflammation is an independent risk factor for essential hypertension. (30)

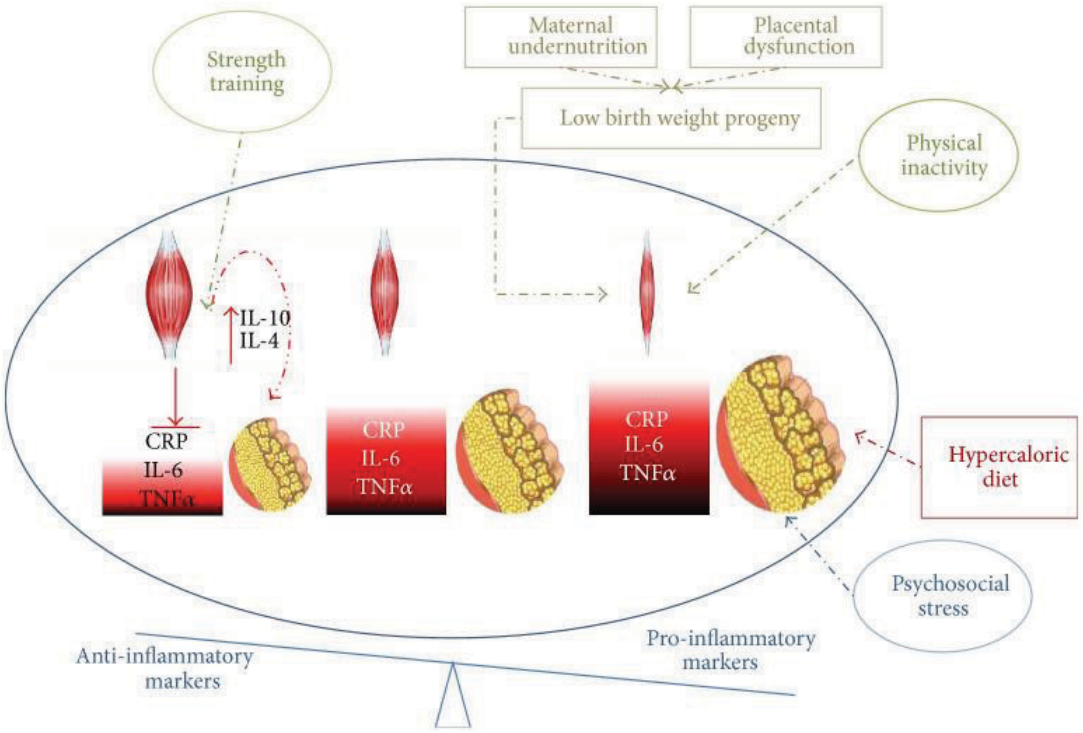

Fig. 19: Impacts on the balance between anti-inflammatory markers and pro-inflammatory markers

https://www.ncbi.nlm.nih.gov/corecgi/tileshop/tileshop.fcgi?p=PMC3&id=594907&s=54&r=1&c=1 (August 2018)

The kidney plays an important role in the regulation of blood pressure and the development of hypertension is connected with renal dysfunction.

Singh and colleagues discuss in their work that a reduced number of nephrons due to an adverse environment in fetal development is assumed as a reason for hypertension in adult life. (35) There is evidence that the renal mass and the number of nephrons correlate with blood pressure: while **Hoy et al.** investigated, that indigenous populations, having a reduced number of nephrons, suffer from hypertension, (36) **Walker et al.** demonstrated that modified mice with a high nephron number are less susceptible to hypertension. (37) However, various studies have shown that a reduced number of nephrons alone is not a sufficient reason for adult hypertension.

However, a kidney with few nephrons must adapt to compensatory growth of the glomerulus and tubule to maintain the fluid homeostasis. In addition, ion channels and transporters alter in number and function due to the increased load. These alterations can be seen in the gene expression of ion channels as well. Those adaptations increase the risk of renal dysfunction and cardiovascular disease.

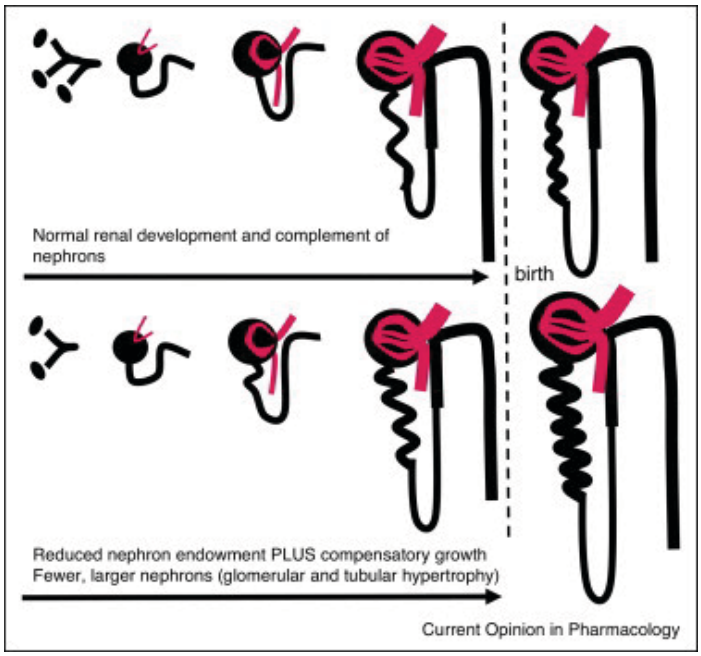

Fig. 20: compensatory glomerular and tubular hypertrophy growth schematically shown
https://ars.els-cdn.com/content/image/1-s2.0-S1471489214001775-gr1.jpg
(July 2018)

Conversely, the physiological increase of glomerular filtration rate of a newborn to levels of an adult person are reached by the following mechanisms: (35)

- Increase of glomerular capillary hydrostatic pressure
- Increase in permeability of the glomerular capillaries
- Increase in glomerular surface area
- Maturation of tubule

Interestingly, the number of nephrons in connection with hypertension is crucial at the time of birth: adult uni-nephrectomies halve the amount of nephrons, but rarely cause hypertension. To compensate the loss of a kidney, the remaining organ grows by cell enlargement. This hypertrophy differs from prenatal organ growth essentially: in neonates growth of kidneys is obtained by cell proliferation. (35)

Remarkable findings were made that show the connection between assisted reproductive technologies (ART) and development of hypertension. It was assumed that ART embryos are exposed to adverse environment in early embryonic life that affects the cardiovascular system. (38) Investigations by **von Arx et al.** showed that children conceived by ART had signs of vascular dysfunction, (39) which, as **Ceelen et al.** found out, might result in increased arterial blood pressure. Further on, ART children showed an increased blood pressure compared with control children. (40) **Scherrer et al.** report in their review that in ART mice epigenetic mechanisms are responsible for altered expression of the endothelial nitric oxide synthase (eNOS) in the Aorta, which is suggested to be one of the reasons for premature vascular ageing. (38)

eNOS is an enzyme that catalyzes the synthesis from the protein L-arginine to nitric oxide (NO), a muscle relaxing molecule that plays an important role in the regulation of the vascular tone. In the fetal blood supply NO is crucial to maintain the uteroplacental blood flow. **Chiossi et al.** studied in a mouse model the interactions between lack of eNOS, altered vascular programming and development of hypertension. The design of their study combines the impact of uteroplacental perfusion on the offspring with the inheritance of

traits. Therefore, mice lacking a functional eNOS and wild-type mice were crossbred:

- WT: wildtype mice (eNOS $^{+/+}$)
- KO: mice lacking eNOS (eNOS $^{-/-}$)
- KOM: maternally derived heterozygous (eNOS $^{+/-}$)
- KOP: paternally derived heterozygous (eNOS $^{+/-}$)

Due to the fact that the group KOM is maternally derived heterozygous, the mice were exposed to an adverse in utero milieu, while KOP mice had a normal in utero environment. The results showed that the lowest blood pressure was found in WT mice, followed by KOP and KOM, while the highest blood pressure was measured in KO mice. Interestingly, KOM, those who were in an adverse in utero environment, had higher blood pressure than KOP mice. Furthermore, they could show that the impact of the uterine environment is stronger than the genetic background. (41)

The NO-Synthase can also be blocked by inhibition with N^G-Nitro-Larginine-methyl ester (L-NAME), a mechanism that during pregnancy leads to induced 'programmed' hypertension in the offspring as well. In addition to the decreased bioavailability of NO, an increase of reactive oxygen species (ROS) is assumed to be another key factor in the programming of hypertension.

Tain et al. showed in a recent study that melatonin as well as N-acetylcystein could prevent L-NAME induced hypertension in adult offspring. In their study pregnant rats got L-NAME subcutaneously, while one group of the rats got melatonin, the other group got N-acetylcysteine in the drinking water during the pregnancy and lactation. A third group of rats was only given L-NAME, without any addition of melatonin or N-acetylcysteine. Melatonin is known for its antioxidant properties and as a radical scavenger. N-acetylcysteine can reduce oxidative stress as well and is a precursor of L-cysteine, the substrate for the reaction to hydrogen sulfide (H_2S), which works as a vasodilator similar like NO.

The results show that after 12 weeks of age the groups with melatonin or N-acetylcysteine had lower systolic blood pressure and mean arterial pressure. (42)

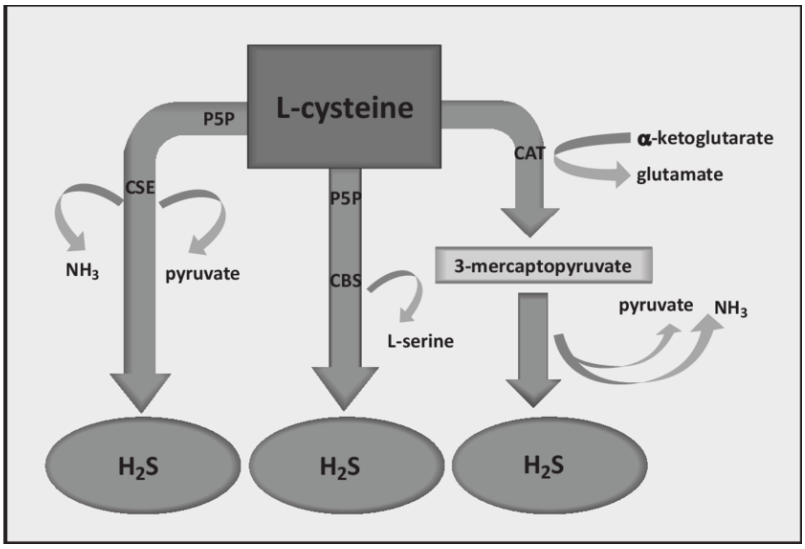

Fig. 21: Mechanisms of H2S production from L-cysteine https://www.researchgate.net/profile/John_Wallace11/publication/277144384/figure/fig1/AS:601679892316190@1520463073691/Fig-1-Enzymatic-pathways-of-hydrogen-sulfide-H2S-synthesis-H2S-is-synthesized-from.png (August 2018)

Similar approaches were discussed by **Singh et al.** for the treatment while pregnancy: L-arginine, the substrate for NOS, and Tempol, a superoxide scavenger were tested and showed a delay in the onset of cardiovascular and renal diseases. (35)

4.2 Oxidative stress and its effects on fetal programming

Author	**Year**	**Major finding**	**Article type**
Perrone	2016	Connection between oxidative stress and fetal programming	Review article
Rodriguez-Rodriguez	2018	Connection between oxidative stress, fetal programming and cardiovascular diseases	Review article
Cambonie	2007	Lazaroid administration prevented blood	Animal

		pressure elevation in rats	study
Herrera	2010	Vitamine C and E improves the cardiovascular function in pups after dexamethasone treatment	Animal study

As already mentioned oxidative stress is deeply involved in the development of fetal programmed chronic diseases. Oxidative stress occurs if the production of ROS or nitrogen oxygen species (RNS) is higher in the human body than the ability to detoxify the reactive species. Different mechanisms like the mitochondrial respiratory chain, the formation of NO, inflammatory cell activation or activated neutrophils, eosinophils and macrophages, just to name a few, are sources of those damaging reactive species. (43) To 'defend' against those reactive species, antioxidants like melatonin, enzymatic systems and vitamins are present.

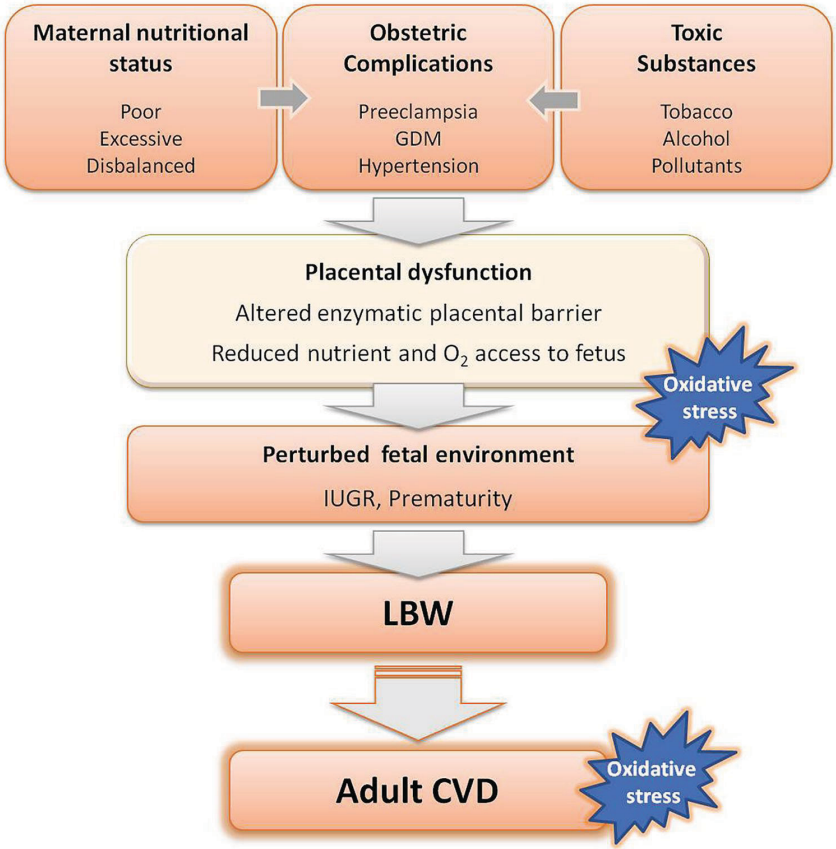

Fig. 22: Oxidative stress can occur at different stages in the development of adult cardiovascular diseases
https://www.frontiersin.org/files/Articles/355419/fphys-09-00602-HTML/image_m/fphys-09-00602-g001.jpg (August 2018)

Even if ROS are involved in many physiological mechanisms while pregnancy, oxidative stress is associated with suboptimal fetal development and low birth weight. Here malnutrition and other stress factors cause catabolism, affecting the placenta and increasing oxidative stress, while at the same time a poor nutritional status reduces the ability to produce antioxidants. Moreover, children with low birth weight have shown increased levels of lipid peroxidation connected with an altered metabolic profile. In animals it has been shown that oxidative stress impairs the cardiovascular system and causes endothelial dysfunction as well as cardiac hypertrophy.
Even if several treatments for oxidative stress were studied in animal models with promising results (e.g. Lazaroid investigated by **Cambonie et al.** could prevent hypertension and vascular dysfunction, (44) and Viatamin C and E studied by **Herrera and colleagues** could improve the cardiovascular function in pups after dexamethasone treatment), (45) clinical trials have not yielded clear results so far. (46)

4.3 Impact of glucocorticoids on cardiovascular diseases in fetal programming

Author	**Year**	**Major finding**	**Article type**
Benediktsson	1997	Maternal cortisol is converted to inactive cortisone in the placenta	Clinical trial
Moisiadis	2014	Elevated glucocorticoid levels lead to alterations in the offspring	Review article
Moisiadis	2014	Elevated glucocorticoid levels while pregnancy might have life long consequences	Review article
Nyirenda	2009	Prenatal dexamethasone administration resulted in elevated 11β –HSD1 mRNA expression in the offspring	Animal trial
Holmes	2006	11β –HSD2 deficiency leads to low birth weight in mice	Animal trial

As **Benediktsson et al.** could show, the fetus is normally protected from maternal glucocorticoids by an enzymatic barrier in the placenta that converts active cortisol to inactive cortisone. (47) Malnutrition impairs this barrier in the development or function followed by an increased glucocorticoid load for the fetus. (46) **Moisiadis and colleagues** report that not only malnutrition, also maternal or fetal stress, like depression or hypoxia, and treatments with glucocorticoids in pregnancy or perinatal lead to higher glucocorticoid levels. (48) Also the hypothalamic-pituitary-adrenal axis is very plastic in early life, and raised levels of glucocorticoids influence the cardiovascular development and might have life long consequences. (49)

Nyirenda et al. made similar findings in nonhuman primates, where dexamethasone administration in pregnant marmoset resulted in elevated expression of 11β-Hydroxysteroid-Dehydrogenase-1 mRNA in the offspring. These alterations were found in metabolically active tissues like the liver or the pancreas and go along with elevated glucocorticoid concentrations, which might lead to obesity and other metabolic alterations. (50)

Also **Holmes et al.** studied the impact of prenatal glucocorticoid exposure in mice. They could show in their animal model that offspring of 11β-Hydroxysteroid-Dehydrogenase-2 knockout mice have decreased birth weight. (51) Hyperglycemia, hypertension, dyslipidemia and vasoconstriction are known effects on the offspring of high glucocorticoid exposure. (48)

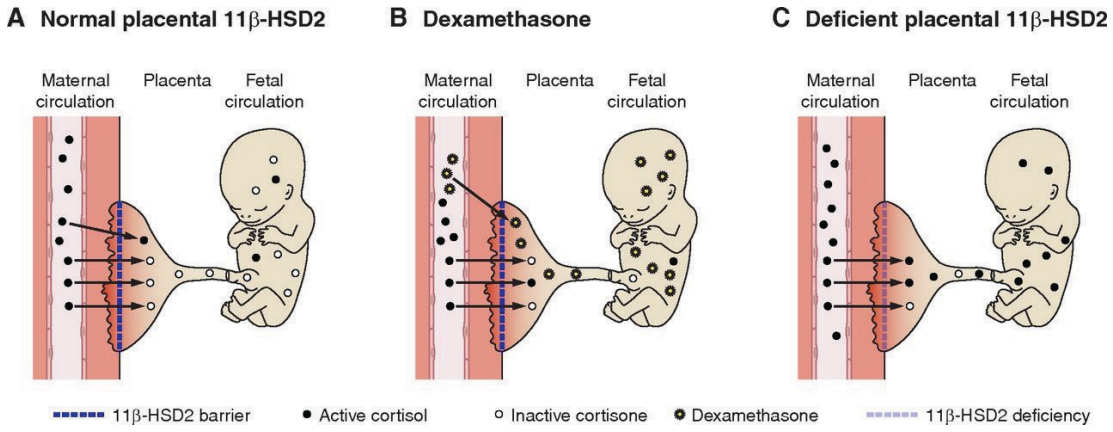

Fig. 23: Enzymatic barrier for glucocorticoids in the placenta https://www.physiology.org/na101/home/literatum/publisher/physio/journals/content/physrev/2013/physrev.2013.93.issue-3/physrev.00020.2012/production/images/large/z9j0031326580012.jpeg (October 2018)

4.4 Epigenetic mechanisms in fetal programming of cardiovascular diseases

Author	Year	Major finding	Article type
Lane	2014	Uteroplacental insufficiency is related to epigenetic alterations;	Review article
Fu	2009	Intrauterine growth restriction in rats leads to epigenetic alterations	Animal study
Scherrer	2015	Assisted Reproductive technologies influence epigenetic mechanisms;	Review article
Rimoldi	2014	Oral Vitamin C and E administration improves vascular function in ART children	Clinical trial
Kubota	2018	Inheritance of acquired epigenetic modifications;	Review article
Popp	2010	Demythelation of epigenetic traits can be incomplete	Animal study

It is known that fetal programming has also impact on epigenetic patterns that regulates the gene expression. Those epigenetic alterations do not change the DNA sequence itself, but modifies the chromatin structure or produces different RNA species to regulate the gene expression. This mechanism is used by the organism in order to adapt to the environment.

Lane et al. provide an interesting example with insulinlike growth factor 1 (IGF-1), a polypeptide that is important in growth and is suggested as a participant for the development of fetal-programmed diseases like insulin resistance and coronary artery disease. Uteroplacental insufficiency lowers the IGF-1 levels in early life and goes along with disorder of IGF-1 in adult life. (18) Furthermore, **Fu et al.** demonstrated epigenetic alterations of the IGF-1 gene in rats due to induced intrauterine growth restriction: this resulted not only in decreased hepatic and serum IGF-1, but also in persistent epigenetic alterations along the entire hepatic IGF-1 gene. (52)

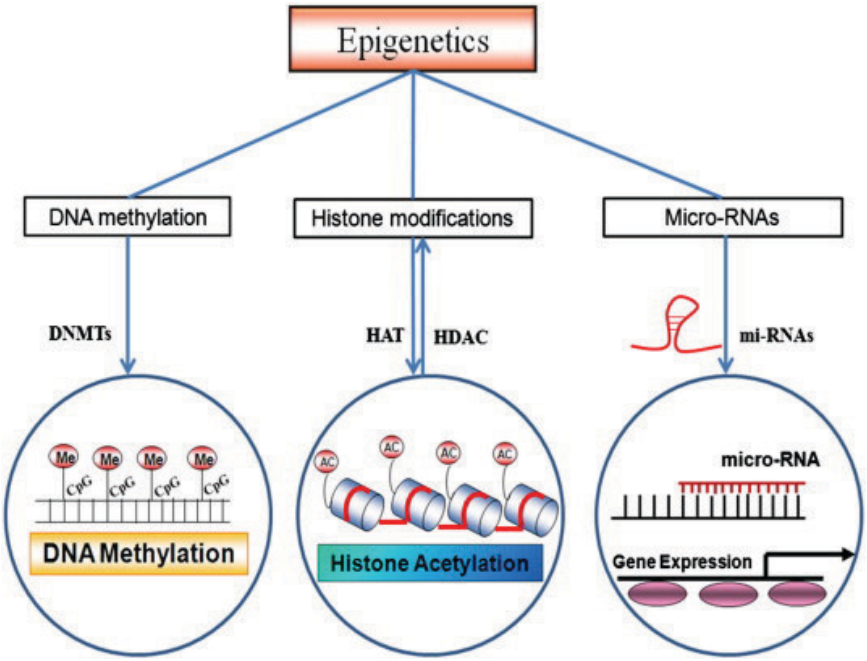

Fig. 24: Epigenetic mechanisms
https://ars.els-cdn.com/content/image/1-s2.0-S0167527314003611-gr1.jpg
(August 2018)

As already mentioned before, ART can alter the function of the cardiovascular system. **Scherrer et al.** report that in the aorta the promotor of the gene coding for eNOS is epigenetically altered. Consequently, the eNOS expression was lowered, followed by decreased NO plasma levels. Fortunately it was possible to reverse epigenetic and phenotypic alterations by the application of different drugs. (38) **Rimoldi et al.** could demonstrate that Vitamin C and E, known antioxidants, improve the NO bioavailability as well as the vascular responsiveness in ART children. They concluded that vascular dysfunction in ART children is reversible. (53)
Recent findings have proven that these acquired epigenetic modifications can be inherited through generations. Epigenetic traits are usually erased by demethylating factors and set up again by the following generation. (54) **Popp et al.** could show that in mice this mechanism does not work completely in the whole genome and epigenetic traits might be transmitted through generations. (55)

4.5 Diet influences on fetal programming

Author	Year	Major finding	Article type
Reichetzeder	2014	Vitamin D deficiency leads to slower growth after birth, impaired glucose tolerance and increased mortality in mice; Vitamin D deficiency in human is associated with low birth weight, low gestational age and low APGAR score;	Animal study / Clinical trial
Roberts	2018	Diet and obesity influence the development and the health of the offspring;	Review article
Bohlen	2006	Leptin inhibits growth of arterial vascular smooth muscle cells	In-vitro study
Saad	2016	High fructose maternal diet leads to metabolic dysfunction, hypertension and adult obesity in the offspring;	Animal study

Undernourishment was one of the factors that got associated with fetal programming first. But pregnancy requires an optimal provision of different nutrients, also including micronutrients like vitamins. Recent studies have investigated influences of diet on fetal programming.

Reichetzeder et al. studied the effects of Vitamin D Deficiency in an animal model as well as in a prospective clinical study. Vitamin D is known for participating in the mediation and regulation of calcium and phosphate homeostasis and is supposed to have an important role in the human development. In pregnancy Vitamin D has important functions in the adaption of the endometrium for the implantation and probably also in the production of pregnancy hormones.

In their study the F1 generation of mice with lack of 25 hydroxy vitamin D_3 (25OHD) before and during pregnancy due to an insufficient diet was analyzed and compared to F1 mice with sufficient 25OHD diet.

Even if there was no significant weight difference of the litters, it was shown that 25OHD deficiency results in slower growth after birth in mice. Interestingly the offspring of 25OHD deficient mothers had impaired glucose tolerance and also the mortality rate was higher in the follow-up.

Conversely the clinical trial showed that 25OHD deficiency is linked with low birth weight and low gestational age in humans. Further on babies of mothers with 25OHD deficiency had a lower APGAR score. (56)

Optimal provision of nutrients not only means sufficient diet, but also adequate diet. Consumption of high caloric food and its consequences like obesity is an increasing and severe health problem. The impact on the fetus is subject of current literature.

High-Fat diet is one determinant that leads to decreased uterine blood flow. It is suggested that obesity impairs the development of the placenta, which leads to altered fetal hemodynamics and altered fetal development. Anyway, adipose tissue is metabolically active and secretes among others leptin, (57) which **Bohlen et al.** could show in a in-vitro study, is responsible for inhibition of arterial vascular smooth muscle cells growth. (58) **Roberts et al.** added, that children from obese mothers have more likely higher blood pressure, congenital heart defects and myocardial hypertrophy as well as a greater atherogenic lipid profile. (57)

In a recent study by **Saad and his colleagues** the effect of high fructose diet in pregnancy on the offspring was investigated. After getting only fructose solution (10% wt/vol, a percentage of fructose, that is similar to fructose-sweetened soft drinks) while pregnancy as a drinking fluid, different parameters were measured in the pups. The findings show that high fructose maternal diet leads to metabolic dysfunction, hypertension and adult obesity in the offspring. From epidemiological studies it can be assumed that the effects are very similar in humans. (59)

5. Conclusions and future direction

Even if the world hunger is decreasing, millions of people are still suffering from malnutrition, consequences of war and adverse circumstances. In addition, modern lifestyle is responsible for elevated stress levels and unhealthy diet. All these factors impact future generations and they could potentially contribute towards the development of chronic diseases.

The concept of fetal programming is known now for many decades and more and more mechanisms are now being put forward. Apart from initial epidemiological studies, animal experiments help to understand pathophysiological pathways in this field. Having said that, there is still a need to research this field further.

The future challenge will not only be to elucidate the missing elements of understanding the whole concept of fetal programming, but also to develop strategies how to preserve future generations from the burden of cardiovascular diseases. This will include the development of therapeutic measures, but also an awareness of protecting pregnant women from unfavourable circumstances.

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