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Perinatal and early life factors and their relevance for cardiometabolic health in early adulthood

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SUMMARY

Evidence from Developmental Origins of Health and Disease (DOHaD) studies suggest that environmental factors acting during preconception, fetal, and early postnatal life have profound impact on disease outcomes in adulthood. The DOHaD concept suggests that nutrient deficiencies or excesses in utero during critical periods of fetal development may result in "predictive adaptive responses", which proposes adverse health outcomes when early life and adult environments are "mismatched" (inadequate maternal nutrition in utero followed by overnutrition in adulthood).

The role of early life factors in adulthood disease is an evolving area, with emerging evidence showing potential transmissibility of programmed traits to future generations. This warrants current research in more recently born cohorts to assess the relevance of a range of early life factors on adulthood health, however such studies are scarce.

Thus to fill the research gap and add to scientific knowledge the overall goal of this thesis was to provide new insights on the relevance of perinatal and early life factors to adulthood cardiometabolic health. To this end, three studies addressed early life factors and their relevance to (I) intima-media thickness (IMT) of the common carotid artery in early adulthood (II) markers of cardiometabolic risk in early adulthood. III. A systematic review to assess and judge the evidence for an association between maternal pregnancy weight/BMI (pBMI) or gestational weight gain (GWG) with offspring's blood pressure (BP) in later life. In the first two studies prospective observational data from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD study) were used.

In study I (N=265) an advanced maternal age at child birth was associated with an increased IMT in adulthood among females only. This association was not mediated by adulthood waist circumference (WC), BMI or BP. In study II (N=348), a higher early maternal pregnancy BMI resulted in a higher offspring fatty liver index (FLI), hepatic steatosis index (HSI), pro-inflammatory score and a lower insulin sensitivity (HOMA2-%S). Also a higher GWG resulted in a higher FLI, HSI, pro-inflammatory score but a lower HOMA2-%S among females. Full breastfeeding was linked with a lower FLI. These associations were mediated by offspring adulthood WC or BMI. In study III, no firm conclusion could be drawn on the association between pBMI or GWG with offspring BP, the evidence grade limited-non conclusive was assigned because only few studies could be included in the review due to methodological issues associated with most studies.

In conclusion this thesis shows that early life factors are relevant determinants of risk markers of cardiometabolic diseases in adulthood. This has public health implications for individual health and health care system. Proper dissemination of scientific evidence using public health strategies on risks associated with advanced maternal age, maternal preconception and pregnancy obesity to offspring health to women in the reproductive age may help in decision making on timing of child birth and lifestyle modification. Interventions that promote healthy diet and weight, physical activity in preconception and during pregnancy, promotion of breastfeeding may be beneficial. Preventive strategies against chronic diseases should use an integrated and life course approach in view of the important role of early life events.

ZUSAMMENFASSUNG

Studien zu den Ursprüngen von Gesundheit und Krankheit (Developmental Origins of Health and Disease - DOHaD) legen nahe, dass Umweltfaktoren, die während der Schwangerschaft und in der frühen postnatalen Phase wirken, tiefgreifende Auswirkungen auf den Krankheitsverlauf im Erwachsenenalter haben. Das DOHaD-Konzept geht davon aus, dass Nährstoffmängel oder -überschüsse in der Gebärmutter während kritischer Phasen der fötalen Entwicklung zu adaptiven Stoffwechselveränderungen führen können, die sich negativ auf die Gesundheit auswirken, wenn die Lebensumwelt in der frühen Kindheit und im Erwachsenenalter nicht zu den in utero anvisierten Bedingungen passen (unzureichende mütterliche Ernährung in der Gebärmutter gefolgt von Überernährung im Erwachsenenalter).

Die Rolle frühkindlicher Faktoren bei Krankheiten im Erwachsenenalter ist ein sich entwickelndes Forschungsfeld, wobei sich Hinweise auf eine potenzielle Übertragbarkeit programmierter Merkmale auf künftige Generationen ergeben. Dies rechtfertigt aktuelle Forschungen an jüngeren Geburtskohorten, um die Bedeutung einer Reihe von Faktoren aus der frühen Kindheit für die Gesundheit im Erwachsenenalter zu bewerten, doch gibt es nur wenige derartige Studien.

Um diese Forschungslücke zu schließen und den wissenschaftlichen Kenntnisstand zu erweitern, wurde in dieser Arbeit das übergeordnete Ziel der Relevanz perinataler und frühkindlicher Faktoren für die kardiometabolische Gesundheit im Erwachsenenalter in drei Studien untersucht. Frühkindliche Faktoren und ihre Bedeutung für I. die Intima-Media-Dicke (IMT) der gemeinsamen Halsschlagader im frühen Erwachsenenalter und II. Marker des kardiometabolischen Risikos im frühen Erwachsenenalter. III. Eine systematische Übersichtsarbeit zur Beurteilung der Evidenz für einen Zusammenhang zwischen mütterlichem Schwangerschaftsgewicht/BMI (pBMI) oder Gewichtszunahme während der Schwangerschaft (GWG) und dem Blutdruck (BP) der Nachkommen im späteren Leben. In den ersten beiden Studien wurden prospektive Beobachtungsdaten aus der Dortmunder Ernährungs- und Anthropometrie-Längsschnittstudie (DONALD-Studie) verwendet.

In Studie I (N=265) war ein höheres mütterliches Alter bei der Geburt des Kindes mit einem erhöhten IMT von Frauen im Erwachsenenalter verbunden. Dieser Zusammenhang wurde nicht durch den Taillenumfang (WC), BMI oder Blutdruck im Erwachsenenalter vermittelt. In Studie II (N=348) führte ein höherer mütterlicher BMI in der frühen Schwangerschaft zu einem höheren Fettleberindex (FLI), hepatischen Steatoseindex (HSI), pro-inflammatorischen Score und einer niedrigeren Insulinsensitivität (HOMA2-%S) bei den Nachkommen. Auch eine höhere GWG war mit einem höheren FLI, HSI und pro-inflammatorischen Wert und einem niedrigeren HOMA2-%S-Wert bei Frauen assoziiert. Vollstillen war mit einem niedrigeren späteren FLI verbunden. Diese Zusammenhänge wurden durch den WC oder BMI der Nachkommen im Erwachsenenalter vermittelt. In Studie III konnte keine eindeutige Schlussfolgerung über den Zusammenhang zwischen pBMI oder GWG mit dem Blutdruck der Nachkommen gezogen werden. Die Evidenz wurde als begrenzt bzw. nicht beweiskräftig eingestuft, da aufgrund methodischer Probleme nur wenige Studien in die Überprüfung einbezogen werden konnten.

Zusammenfassend zeigt diese Arbeit, dass frühe Lebensfaktoren relevante Determinanten von Risikomarkern für kardiometabolische Erkrankungen im Erwachsenenalter sind. Dies hat Auswirkungen auf die Gesundheit des Einzelnen und das Gesundheitssystem. Eine angemessene

Verbreitung wissenschaftlicher Erkenntnisse über die Risiken, die mit dem fortgeschrittenen Alter der Mutter, der Adipositas vor und während der Schwangerschaft für die Gesundheit der Nachkommen verbunden sind, kann Frauen im reproduktiven Alter bei der Entscheidungsfindung über den Zeitpunkt der Geburt eines Kindes und der Änderung ihres Lebensstils helfen. Maßnahmen zur Förderung einer gesunden Ernährung und eines gesunden Körpergewichts, körperlicher Betätigung vor und während der Schwangerschaft sowie die Förderung des Stillens können von Nutzen sein. Präventionsstrategien gegen chronische Krankheiten sollten angesichts der wichtigen Rolle, die frühe Lebensereignisse spielen, einen integrierten und lebenslangen Ansatz verfolgen.

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ABBREVIATIONS

95% CI	95% confidence interval
AGA	adequate for gestational age
ALT	alanine aminotransferase
AHA	American Heart Association
AST	aspartate aminotransferase
AA	arachidonic acid
BMI	body mass index
BP	blood pressure
CCA	common carotid artery
CIMT	common carotid artery intima media thickness
CHD	coronary heart disease
(hs)CRP	(high sensitivity) C-reactive protein
CVD	cardiovascular disease
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
DOHaD	Developmental Origins of Health and Disease
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed
DNL	de-novo lipogenesis
FFA	free fatty acids
FLI	fatty liver index
GGT	gamma glutamyltransferase
GLUT	glucose transporter
GWG	gestational weight gain
HbA1c	glycated haemoglobin
HBCS	Helsinki Birth Cohort Study
HDL-(C)	high density lipoprotein (cholesterol)
HOMA2-%S	HOMA of insulin sensitivity
HOMA-IR	homeostasis model assessment of insulin resistance
HSI	hepatic steatosis index
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IMT	intima-media thickness
IOM	institute of Medicine
IGF1	insulin-like growth factor-1
LC-PUFA	long-chain polyunsaturated fatty acid
LDL-(C)	low-density lipoprotein (cholesterol)
LGA	large for gestational age
(MAFLD)	metabolic dysfunction associated fatty liver disease
MRI	magnetic resonance imaging
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
OA	original articles
PPARα	proliferator-activated receptor alpha

PPARγ	peroxisome proliferator-activated receptor gamma
QUICKI	quantitative insulin sensitivity check index
SBP	systolic blood pressure
SGA	small for gestational age
SMCs	smooth muscle cells
TAG	triacylglycerol
T2D (M)	type 2 diabetes (mellitus)
VLDL	very-low-density lipoprotein
WC	waist circumference

1 INTRODUCTION

The prenatal and early years during development have been suggested as critical periods in the etiology of adulthood diseases [1–5]. Cardiometabolic diseases i.e. cardiovascular disease (CVD), type 2 diabetes (T2D) and hypertension have been indicated to have their origins in early life [6]. The Developmental origins of disease and health (DOHaD) hypothesis proposes that adverse exposures during prenatal or early postnatal life can program permanent changes to the physiology, metabolism and epigenome of an offspring which can predispose to disease risk later in life [7–9].

Fetal exposure to a suboptimal in utero environment has been associated with susceptibility to disease [10–12] in later life. Maternal undernutrition during pregnancy due to nutritional imbalances or suboptimal function of the materno–fetal supply channel (uterine blood supply, placental function) could lead to suboptimal supply of nutrients to the fetus. This could result in permanent adaptations or programming of various fetal organs and tissues [2] with priority for brain development given over abdominal visceral tissues, reduced secretion and sensitivity to fetal growth hormones (insulin) and stimulation of the hypothalamo-pituitary-adrenal (stress) axis [4]. Thus, early life represents critical periods of plasticity such that permanent adaptations made to organ structure or metabolism intended to enhance survival in a later inadequate nutritional environment, can become detrimental when later exposed to an obesogenic nutritional environment. This results in a mismatch with metabolic changes and the risk of cardiometabolic diseases [13–16].

Early epidemiological cohort studies by Barker and others have provided evidence of an association between low birthweight and cardiovascular disease [13, 17–21], diabetes [22–27] and hypertension [28, 29] later in life.

Evidence suggests that environmental factors other than undernutrition can program an offspring for later disease. For instance, obesity among women of child bearing age throughout pregnancy could lead to fetal overnutrition [4]. Maternal hyperglycemia, due to high maternal obesity during pregnancy could be a risk factor for fetal programming that can down-regulate glucose tolerance and insulin sensitivity [30–32]. Additionally, women are giving birth at an advanced age in recent times which may have an impact on offspring health outcome in later life [33]. However, prospective evidence of the relevance of these early life factors on offspring cardiometabolic outcomes, specifically common carotid artery intima media thickness (CIMT), fatty liver index (FLI), hepatic steatosis index (HSI), pro-inflammatory score and insulin sensitivity in more recently born cohorts have not yet been assessed.

Although nutrition in early life has received the most focus it is plausible a range of environmental exposures in early life could be relevant for adulthood cardiometabolic disease. Thus this thesis fills the research gap by using data from the DONALD study, to assess the relevance of a range of perinatal and early life factors for cardiometabolic health in early adulthood. A systematic review was also conducted to assess available evidence on the association between pregnancy weight or gestational weight gain (GWG) with offspring's blood pressure (BP). First early life factors and their relevance to the CIMT in early adulthood was assessed (*Aim 1*), secondly the prospective relevance of early life factors for markers of cardiometabolic risk in early adulthood (*Aim 2*) and finally, the association of pregnancy weight or GWG with offspring's BP is examined through a systematic review of the literature (*Aim 3*).

Thesis outline

This thesis comprises of two observational studies based on the DONALD study and a systematic review. The *theoretical background* (chapter 2) gives an overview of current research related to the topic of this thesis. This comprises the relevance of early life factors i.e. birthweight and birthweight by gestation age, maternal and paternal age at child birth, maternal early pregnancy BMI and gestational weight gain, full breastfeeding for cardiometabolic health i.e. cardiovascular disease/CIMT, diabetes/insulin sensitivity, hepatic steatosis, chronic low grade inflammation and BP in adulthood.

In (chapter 3) the *aims and research questions* of this thesis are formulated. In (chapter 4) a brief description of the DONALD study *methodology* and the systematic review undertaken are given. In (chapter 5) the abstracts of the *original articles* (OA) are presented; the original articles are presented as appendices to this thesis. *General discussion* (chapter 6) involves a discussion of the research objectives, methodology and public health relevance in a general view. *Conclusions and perspectives* in (chapter 7) presents a summary of the main results of this thesis and proposes recommendations for future research.

This research finding is based on human data unless otherwise stated. Additionally, as this thesis is cumulative in nature, it does not consist of thorough descriptions of research findings. Details on methodology, statistical analysis, results and discussions are available in the original articles in the appendices (1-3).

2 THEORETICAL BACKGROUND

2.1 Relevance of birthweight and birthweight for gestational age for cardiometabolic health in later life

The relationship between birth weight and later cardiometabolic health has been widely studied [34–36] since the discovery of Barker, that low birthweight or restricted intrauterine growth, especially followed by excessive weight gain in infancy and adolescence [37] is associated with a high risk of CVD in later life [12, 38].

The fetal origins hypothesis states that coronary heart disease (CHD), T2D and hypertension emanates from developmental plasticity following in utero fetal and infancy undernutrition [39, 40] due to inadequate maternal nutrition. Undernutrition during fetal life may lead to disproportionate fetal growth and this can program for disease in later life [14]. Impaired fetal intra uterine growth can be associated with higher allocation of nutrients to adipose tissue in the course of development. Maternal undernutrition may silence placental 11-β hydroxysteroid dehydrogenase type 2, which aids in the inactivation of cortisol and thus can expose the fetus to excess maternal steroid [41]. These may subsequently contribute to increased weight gain and probably higher risk of CVD, T2D and hypertension [1]. The main concept is that regardless of the current nutritional status in Western countries, there are still instances of suboptimal fetal and infant nutrition due to unbalanced available nutrients or due to compromised fetal supply channel [39].

Aside maternal transfer of nutrients, other environmental cues are also transferred to the fetus, thus the fetus makes predictive adaptive responses according to maternal cues, which can lead to alterations in metabolism, hormone synthesis, sensitivity of tissues to hormones, which affects the development of different organs. These could culminate into lasting changes to physiologic and

metabolic homeostatic set points. Thus, the impact of impaired growth in early life and its association with later disease can be viewed as a reflection of long term effects of fetal predictive adaptive responses: that is the inadequate environment anticipated due to a suboptimal environment in utero (or early life) may result in a mismatch when a very abundant nutritional environment is encountered later in life [1, 42].

Epidemiological studies suggest that developmental programming occurs within the full range of birth weights, in addition to those within the normal range of birth size. However, the extremes of the birth weight range are the most definitive indicators of increased risk of adult cardiometabolic dysfunction [27, 43, 44]. Additionally, low birthweight rather than prematurity has also been associated with cardiometabolic health [42].

Small for gestational age (SGA) are infants small in size than normal for gestational age, that is they have birthweight below the 10th percentile for the gestational age [45] whilst low birthweight is a birthweight less than 2500 g, irrespective of gestational age at the time of birth. SGA is not equivalent to low birth weight. An infant can have an appropriate weight for gestational age but yet be considered as having low birth weight [46]. A gestational age of 35 weeks with a birthweight of 2250 g is considered appropriate for gestational age but at the same time low birthweight [47].

Large for gestational age (LGA) is birth weight above the 90th percentile for gestational age and gender [48, 49]. These birthweight ranges are important due to link between fetal and infant growth with adulthood diseases.

SGA infants are indicated to be at risk of later impaired cardiovascular health whilst the association with those born LGA is somewhat controversial [36]. Most SGA children make up for their intrauterine growth restriction with an early catch-up growth whilst the LGA's will probably follow their natural genetic patterns with a catch-down growth [36]. Accelerated catch- up growth (more

rapid growth than expected rate of growth) especially in weight among SGAs has been related to adverse childhood cardiometabolic risk factors i.e. obesity, insulin resistance, and BP [50, 51].

Additionally, some studies have reported high insulin levels and insulin resistance, however, not fasting glucose levels in SGA and LGA children in comparison to those adequate for gestational age (AGA) [52, 53]. Elevated systolic blood pressure (SBP), has also been reported in SGA's across the lifespan [29] and being born LGA has also been shown to increase the risk of high BP in adolescence [54], whilst increased CIMT, an indicator of atherosclerosis has been reported in SGA children [55].

Though birthweight is seen as a proxy (marker) of the in utero milieu [35] it has however, been described as a crude measure of the effect of fetal nutrition on body composition, not an accurate indicator of fetal growth or experience in utero and also it does not indicate attainment of fetal genetic growth potential [27, 56], hence the real effect of fetal growth on subsequent disease is difficult to measure [57], in addition the magnitude of the effect varies for different risk factors. For instance, birthweight is suggested to be associated with considerable changes in indices of insulin resistance, but is it related with minor variations in BP [39]. It has been suggested that after an intra uterine insult the regulatory processes likely maintains homeostasis for a long duration until further injury occurs due to age, obesity etc. that sets into motion a self-perpetuating cycle of escalating functional loss. This is exemplified in the case of hypertension due to low nephron numbers at birth mostly associated with low birthweight [39, 58].

Studies assessing the effect of birthweight on T2D, CVD, and hypertension risk in later life have reported different relationships [34, 59–63]. The relationship between birthweight and T2D is inconsistent in the literature, a meta-analysis reported that there may be an inverse relationship between low birthweight and T2D [64], with a suggestion that high birthweight (>4000g) reduces

the risk of T2D [23, 65, 66], yet others have reported a U-shaped association indicating that low and high birthweight both could increase the risk of T2D [67–70]. The relationship between birthweight and CVD has also been inconsistent with either an inverse [19, 71, 72] or a U-shaped [59, 73] or no association [74], an inverse association has been mostly reported between birth weight and hypertension [63, 75, 76], however, others have suggested that birthweight may be of little relevance to BP in later life [77].

2.1.1 Probable mechanisms of association between birthweight and birthweight by gestational age and offsprings cardiometabolic risk in later life

Though clear mechanisms of association between birthweight and cardiometabolic diseases are yet to be elucidated, low birthweight has been associated with endothelial dysfunction [78, 79], a condition which precedes structural atherosclerotic alterations. Increased CIMT, an indicator of clinical atherosclerosis [80–82] has also been reported in growth restricted and SGA children [36]. Animal studies have shown vascular structural changes i.e. remodeling of aorta and mesenteric arteries, capillary rarefaction (reduced density of arterioles and capillaries within a vascular bed [83], high arterial stiffness, modifications in the composition and structure of the extracellular matrix of the vessels, reduced angiogenesis (reduced new blood vessel growth which impairs delivery of oxygen and nutrients to body tissues) in offspring of protein and caloric restricted dams [80, 84]. The fetal insulin hypothesis also explains the relation between impaired fetal growth and insulin resistance, diabetes and vascular disease. Fetal insulin resistance due to suboptimal in utero milieu can lead to low insulin-mediated fetal growth in utero and insulin resistance in childhood and adulthood [85]. There could be impaired angiogenesis in insulin-resistant fetal tissues with a defect in the production of nitric oxide. These can result in impaired vasodilation, reduced blood

flow, inadequately developed capillary circulation in vulnerable organs and a deficient endothelium-dependent vasodilation [80] which can alter the proper functioning of the heart.

With regards to birthweight and T2D risk in later life the following mechanisms have been proposed: low birthweight infants with rapid postnatal weight gain are said to have a high fat mass in comparison to muscle which has been associated with insulin resistance [37], impaired insulin response to glucose [86] and also a high risk of T2D [39, 87]. It has been indicated that growth restricted offspring may have an impaired ability to secrete insulin due to decreased pancreatic islets. This may trigger an increased demand for insulin, when this demand exceeds the ability of the pancreas, overt diabetes could occur [88]. Adult rat offspring growth restricted in utero have been reported to have a low beta cell mass and pancreatic insulin level [88]. Growth restricted individuals also have an increased demand for insulin due to increased gluconeogenesis. Increased hepatic gluconeogenesis that precedes hyperglycemia has been reported in adult rats who experienced growth restriction in utero. Also increased expression of peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1, a regulator of mRNA expression of glucose-6-phosphatase and other gluconeogenesis enzymes, has been observed in the liver of growth restricted rat offspring, indicating that changes in hepatic glucose production can be attributable to alterations in intracellular signaling [88].

Glucose is transported into the muscle by the glucose transporter GLUT4, an action stimulated by insulin [88]. Offspring exposure to in utero undernutrition and its associated insulin resistance is linked to an impaired regulation of GLUT4 expression in muscle and adipose tissue after insulin stimulation [89]. Taken together, growth restriction is accompanied by alterations in the anatomy of the pancreatic islets as well as changes in intracellular insulin signaling pathways. These changes

limit the ability to produce insulin, with an attendant increased demand for insulin that can lead to glucose intolerance [88].

Finally, in terms of birthweight and mechanisms that relate it to BP in later life, low birthweight has been associated with lesser cells in some vital organs i.e. the reduced number of nephrons in the kidney of low birthweight infants in relation to their body weight [80, 90]. It has also been suggested that hypertension in individuals born small at birth may occur due to decreased number of glomeruli, which leads to high blood perfusion through each glomerulus, subsequently this hyper infiltration could result in glomerulosclerosis and in association with normal age related loss in glomeruli can lead to increase in BP [80, 88, 90].

Taken together, evidence shows that especially low birthweight is associated with adulthood cardiometabolic diseases, probably promotion of adequate maternal nutrition could be beneficial in reducing impaired fetal growth and maybe the incidence of cardiometabolic disease. A summary of the mechanisms is shown in figure 1 below.

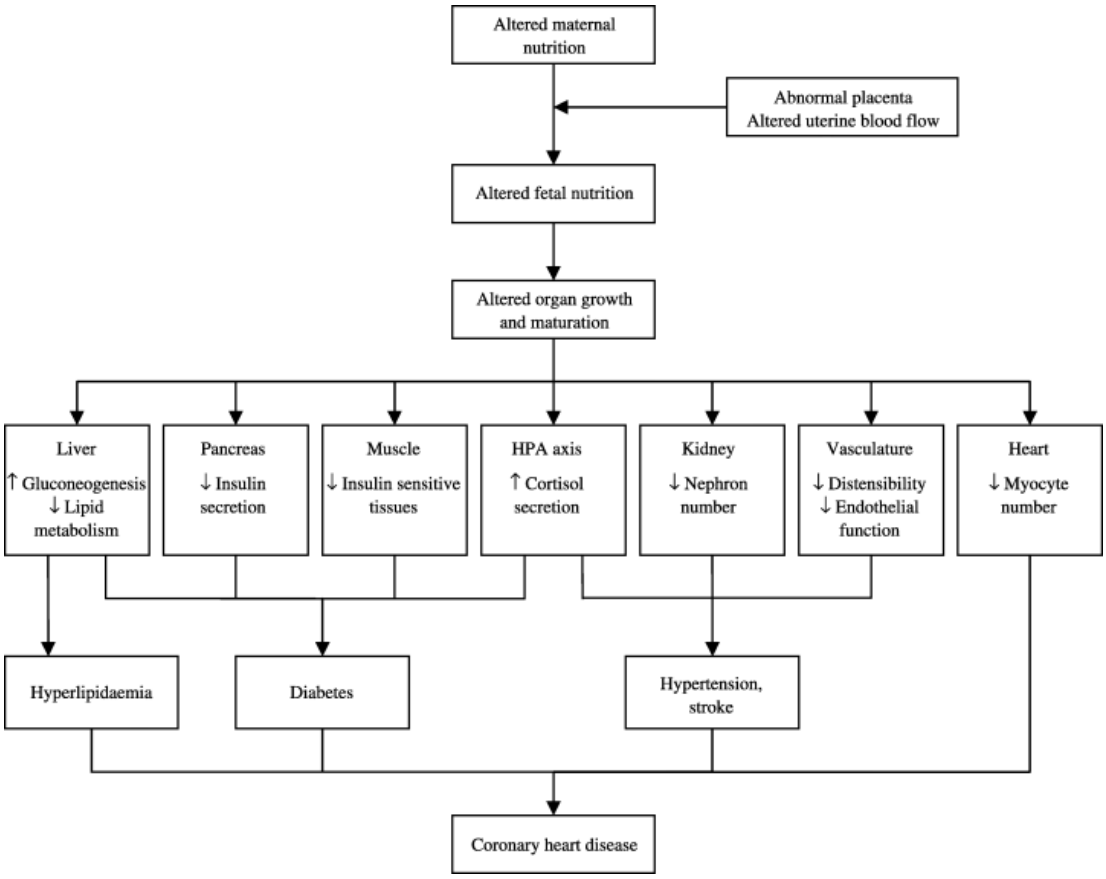


Figure 1 *Effects of altered fetal nutrition on growth and maturation of fetal organ systems and their association with adulthood disease. (Adapted from [91])*

2.2 Relevance of advanced maternal and paternal age for cardiometabolic health in later life

In developed countries there has been an increase in the number of women and men postponing child bearing to an advanced age [92]. The mean age at the birth of a first child is over 30 years in Germany and the UK [92, 93]. Advanced maternal age typically connotes pregnancy in a woman aged ≥35 years at the time of delivery [94, 95]. Although children born to both very young or teenagers and old mothers have been suggested to be at risk of adverse health outcomes i.e. are shorter with higher mortality compared to those born to mothers aged 25 to 34 years [96] emphasis is placed more on advanced maternal age in this write up.

A number of reasons has been cited for the postponement of pregnancy to an advanced age in women such as interest in higher educational attainment, availability of reliable contraception and rising economic uncertainty [97]. Notwithstanding these seeming benefits to women, it has been reported that advanced maternal age at child birth has adverse consequences for the woman and the child [92].

Advanced maternal age has been linked to a progressive decline of the intrauterine environment as well as an impaired viability of embryos as a result of an age related reduction in quality of oocyte [98]. Thus women advanced in age have higher risk of pregnancy complications i.e. risks of miscarriage, preterm birth, stillbirth etc. [99, 100].

Whilst some studies have found small or no evidence of an association between advanced maternal age and offspring adulthood health [101, 102], other studies have suggested that advanced maternal age is related to various adverse childhood and adulthood outcomes such as higher risk of hypertension [103], diabetes [104], cancer [105–107], obesity [96] and mortality [108].

Assessment of the impact of advanced maternal age on adult offspring cardiovascular health, T2D, and hypertension is limited in human and epidemiological studies. Thus to better understand the abnormal cardiovascular function or otherwise associated to offspring born to women advanced in age, animal models are used. In a study that examined the long-term effect of cardiovascular programming in both male and female offspring born from animal models of advanced age rat dams, their young adult male offspring had impaired endothelial-dependent vascular relaxation and poor cardiac recovery following an ischemic insult, however, these effects were not observed in female offspring [109].

In a study that assessed the effect of maternal age on the risk of the offspring developing T2D between the ages 15 and 39 years reported a U-shaped association. The study showed that, compared with the baseline maternal age (25.5 years), the risk of T2D in offspring born to very young or old mothers was significantly higher [110]. Additionally, an epidemiological study has reported a higher fasting glucose levels in children of women advanced in age. Mean fasting glucose was higher by 0·06 mmol/L (–0·01 to 0·12) in children of mothers aged 35 years and older compared to 0·05 mmol/L (95% CI –0·01 to 0·10) in children of mothers aged 19 years and younger, there was not much alteration in the association after further adjustment for confounders [111].

Assessment of the link between maternal age and the risk of offspring BP among 5-7 years old children showed that both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in children born to mothers above 30 years old. A 10-year increase in maternal age was associated with a 1.00mm Hg (0.4-1.6) increase in offspring SBP [112]. Similarly, it has been reported that neonatal BP in the first day of life correlates with maternal age, infant SBP increased by 0.8 mmHg for every 5 year increase in maternal age [113]. However, a meta-analysis has

suggested that there is little evidence of an association between maternal age at child birth and offspring BP [103].

Similar to women, emerging studies are showing that men are also delaying parenthood [101], however, whereas it is known that fetal exposure to suboptimal maternal factors in utero is more likely to contribute to adverse metabolic and CVD in later life, the association of adverse paternal factors e.g. advanced age with offspring later health is limited [114]. The "advanced fetal programming hypothesis" suggests that programming incidents linked to paternal genes could affect the fetal phenotype independent of the fetal genome [115]. The hypothesis also indicates that, paternal environmental factors (e.g. body composition, endocrine function, nutritional habits, and age) may affect the offspring phenotype via epigenetic imprinting processes in sperm as the changes in the paternal germline epigenome are passed on to the offspring [114, 116].

Advanced paternal age has been linked with a higher risk of adverse outcomes such as preterm birth, low birthweight [117], patent ductus arteriosus, a subtype of congenital heart defects [118] in the offspring.

The mechanism underlying parental age and offspring outcomes is highly speculative. The adverse relationship between advanced maternal age and adult health could be due to the physiological reproductive aging of the mother. With age the physiobiological functioning of the female body necessary to sustain a healthy conception, fetal growth, low risk birth, and postnatal development degenerates [96] and also due to declining fecundity (the estimate of the number of gametes produced by an individual) [119]. The biological basis for the decline in fecundity are linked to accumulation of DNA damage in germ cells [119], decreasing oocyte quality [120], and placenta weakening [96]. Advanced maternal age could also lead to alterations in hormones and other physiological factors that alter the intrauterine milieu that can affect offspring health [96].

Though the adverse effects of advanced maternal age on the offspring seem to be highlighted more, other studies have suggested that advance maternal age could have positive implications for offspring health. Older parents probably have access to greater resources and of a higher socioeconomic status, which are associated with improved offspring health [92]. Some complications associated with advanced maternal age and its effect for the mother and child are shown in figure 2 below.

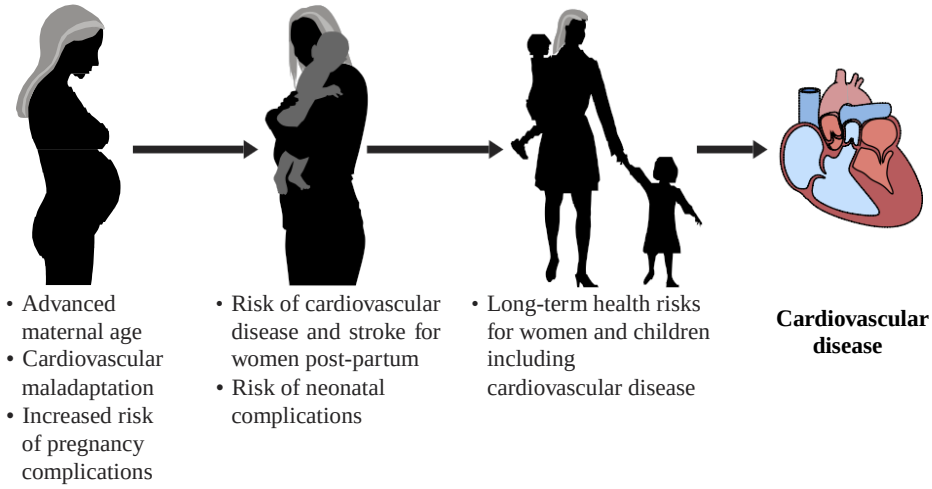


Figure 2 *Implications of pregnancy at an advanced maternal age (Adapted from [94])*

2.3 Relevance of maternal early pregnancy BMI and GWG for cardiometabolic health in later life

Parallel to the surge in global obesity in most populations, the prevalence of obesity (BMI > 30 kg/m^2) among women of child bearing age is also increasing [121, 122]. The prevalence of maternal obesity among pregnant women in European countries is indicated to vary between 7 to 25% and is strongly associated with social and educational disparities [121]. On the basis of the US Institute of Medicine (IOM) guidelines, about 40% of pregnant women are suggested to gain excessive weight in western countries [123, 124].

The intrauterine environment has been shown to play an important role in disease etiology of the offspring in later life, thus GWG is viewed as a simple measure of the state of the intrauterine environment and is considered an important determinant of maternal and child health outcomes [125]. For this reason, the IOM guidelines has proposed optimal ranges of maternal GWG according to a woman's prepregnancy BMI (see table1). These recommendations were proposed based on evidence from observational studies linking high GWG to different adverse child and maternal health outcomes [123, 124].

High maternal weight both before and during pregnancy is suggested to be associated with adverse offspring metabolic and cardiovascular outcomes in later life [126]. In general, maternal prepregnancy obesity, early-pregnancy weight gain (first trimester) [127], seem to be more strongly related to adverse maternal and offspring health outcomes compared to later high GWG [128, 129].

However, the associations of maternal BMI and GWG with offspring health outcomes appears not to be limited to maternal obesity or excessive GWG only, but can be seen across the full-range of maternal BMI and GWG [124]. In a study among 5908 Dutch mother-offspring pairs, early-pregnancy weight gain was associated with adverse childhood cardiometabolic profile (higher

BMI, total fat mass, android/gynoid fat mass ratio, abdominal subcutaneous fat mass and SBP) (OR 1.20 95% CI 1.07-1.35), independent of maternal prepregnancy weight and weight gain in later pregnancy [127]. A similar study among 977 mother–child pairs from Greece, showed that maternal first trimester weight gain was linked to a high risk of childhood obesity and a higher childhood DBP [129]. These studies suggests that early pregnancy weight gain, a period when GWG consists of largely maternal fat deposition and probably a state of adverse metabolic function [129, 130], there could be an interaction between this modified maternal in utero milieu with placental factors that can increase the fuel supplied to the fetus [31], thus maternal weight gain in early pregnancy may represent a critical period for a possible adverse offspring cardiometabolic outcome [124].

Most studies of maternal obesity in pregnancy and its relationship to offspring health outcomes (mostly obesity related outcomes) have been largely focused on childhood and adolescent periods [129, 131–134], with few studies available on the long-term health consequences of maternal prepregnancy BMI or GWG on offspring cardiometabolic health factors in adulthood [135–137]. This could be due to limited cohort studies with the needed data for such long term health studies. Some studies assessing the impact of maternal weight during pregnancy on offspring adulthood health outcomes have shown some association with offspring cardiometabolic health. For instance, using birth records from the Helsinki Birth Cohort Study (HBCS) to study the relationship between maternal BMI during late pregnancy and offspring register-based long-term health outcomes, showed that increased maternal BMI in late pregnancy is an independent predictor of CVD and T2D among the offspring in adulthood [138]. Similarly, a study using birth records from 37,709 participants, to assess whether maternal obesity during pregnancy was associated with mortality and hospital admissions from cardiovascular events (including both coronary events and other vascular events), showed that a higher maternal BMI (overweight or obesity) at the first antenatal

visit was linked to an increased risk of premature death and hospital admissions for cardiovascular events combined in adult offspring. The associations were not altered after adjusting for many confounders which seem to reflect the prenatal and postnatal environment such as maternal social class, maternal parity, gestation at delivery, maternal age at delivery, birth weight, current age and sex of offspring [139].

Furthermore, a study in a mother-offspring pairs of 308 Danish normal-weight women found that higher GWG up to 30 weeks of pregnancy was associated with higher HOMA-IR and insulin levels among 20 year-old male offspring only [135]. Likewise, among 32-year-old offspring, maternal pre-pregnancy BMI was positively associated with offspring insulin level whilst GWG was positively related with adiposity traits but not with insulin or glucose levels [136].

In terms of maternal weight during pregnancy and offspring BP in later life, in an Australian study among 2432 participants, higher maternal GWG was associated with a higher SBP in the offspring at 21 years old [137]. Also a study of 1400 mother-offspring pairs in Jerusalem reported that higher maternal prepregnancy BMI was related to a higher BP in their 32 years old offspring, whilst a higher GWG was positively related with high adiposity traits in adult offspring. However, offspring current BMI explained this association [136].

Of note, most of the relationship reported between maternal prepregnancy BMI and GWG with offspring adverse cardiometabolic outcomes seem to be largely mediated by offspring adiposity or body composition. However, there are suggestions that there could also be direct offspring cardiometabolic programming effects of maternal obesity during pregnancy [124, 136].

Table 1 *Institute of medicine criteria for gestational weight gain*

Recommended gestational weight gain according to institute of medicine criteria	
Prepregnancy body mass index	Recommended total gestational weight gain in kg
Underweight (body mass index <18.5 kg/m^2)	12.5-18
Normal weight (body mass index ≥ 18.5-24.9 kg/m^2)	11.5-16
Overweight (body mass index ≥25.0-29.9 kg/m^2)	7-11.5
Obesity (body mass index ≥30.0 kg/m^2)	5-9

Recommended gestational weight gain guidelines according to women's prepregnancy body mass index. (Adapted from IOM criteria [123])

2.3.1. Possible mechanisms linking pregnancy weight with cardiometabolic risk in later life

In spite of the fact that the mechanisms behind maternal early pregnancy BMI or excessive GWG with offspring cardiometabolic health in later life are yet to be elucidated, firstly, maternal prepregnancy BMI and weight gain in early-pregnancy are suggested to partly reflect maternal fat deposition [123]. Likewise, high weight in pregnancy has been associated with high levels of maternal inflammatory cytokines, non-esterified fatty acids, amino acids, fat deposition as well as increased glucose and insulin resistance [139–141]. These modifications may lead to higher placental transfer of maternal nutrients to the developing fetus usually termed as the "developmental overnutrition hypothesis," can result in programming of offspring adiposity and subsequently adverse cardiometabolic health in later life [122, 132]. Data from animal studies seems to buttress the "developmental overnutrition hypothesis," which suggests that such conditions during pregnancy can lead to permanent changes in appetite control, neuroendocrine functioning, and/or energy metabolism in the offspring that can contribute to risk of adiposity and cardiovascular risk factors in later life [142]. Structural alterations in the heart and vasculature of offspring has been observed in animal models in relation to maternal overnutrition and obesity during pregnancy. The alterations include endothelial dysfunction [143], increased sympathetic tone [144], and accumulation of connective tissue in the heart and myocardial fibrosis [145], such

changes can reduce contractile activity of the heart in the offspring, with a likelihood of susceptibility to long term cardiac dysfunction [139].

Secondly, pregnancy weight gain is associated with relative insulin resistance and other metabolic changes even among normal weight women but is more pronounced among overweight and obese women during pregnancy [146–148]. Though these modifications are intended for efficient transmission of energy to the fetus, it could also bring about altered glucose tolerance in the mother and this could have implications for the child [123]. In animal model studies, maternal obesity during pregnancy have been shown to be associated with increased maternal glucose transport to the developing fetus leading to higher fetal glucose exposure [124], and subsequently to fetal hyperglycemia, hyperinsulinemia, overgrowth of the pancreatic islets, and accelerated fetal β-cell development [149]. This accelerated fetal pancreatic maturation can alter the function of the pancreas [149] and may promote premature β-cell loss [124, 149]. It has been indicated that if such alterations in the pancreas does not revert to a normal state postnatal, it may contribute to altered insulin secretion, and diabetes in offspring in later life [149].

In terms of link between obesity in pregnancy and offspring susceptibility to elevated BP in later life, animal model studies show that perinatal exposure to the metabolic milieu of maternal obesity may irreversibly alter the central regulatory pathways associated with BP regulation. Leptin which is involved in the activation of the efferent sympathetic pathways of the kidney is suggested to be involved in obesity-related hypertension [150]. A study in adult offspring of obese mice during pregnancy reported that the offsprings developed systolic and mean arterial hypertension at 3 months old [143, 150]. Hypertension was also associated with high visceral adiposity and hyperleptinaemia. The study suggests that hypertension in the offspring was obesity-related and also leptin-mediated acting via the central sympathetic pathways [143]. Thus exposure to maternal

overnutrition in utero led to selective leptin resistance with increased sympathetic nervous system activity, which probably predisposed the offspring to hypertension in later life.

Thirdly, high prepregnancy obesity or excessive GWG during pregnancy may lead to high birth weight with a likelihood of obesity in childhood and adulthood. Thus high maternal weight during pregnancy may lead to tracking of body weight and adiposity in life with probable risk of adverse cardiometabolic risk in later life [124, 151].

Lastly, epigenetic alterations due to exposures in early life have been indicated as a probable mechanism involved in cardiovascular and metabolic developmental adaptations. Epigenetic modifications of placental genes due to maternal obesity during pregnancy has been reported in some human studies [152], similarly, maternal weight gain in early pregnancy has also been suggested to be related to epigenetic changes in offspring cord blood [153]. Taken together, there could be a number of mechanisms involved in the intra-uterine pathways associating maternal obesity and excessive weight gain during pregnancy to offspring adverse health outcomes in later life [124]. Figure 3 shows the conceptual model for potential underlying mechanisms for the association of maternal obesity during pregnancy with adverse cardiovascular and metabolic health outcomes in offspring.

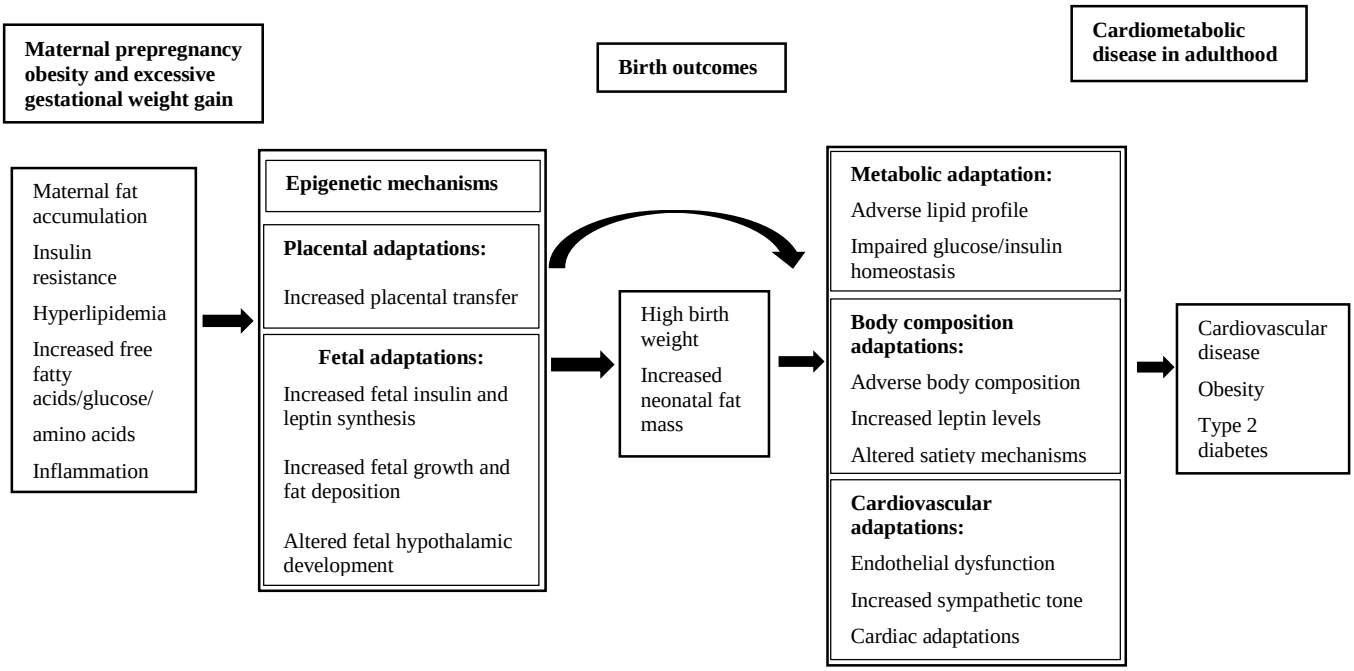


Figure 3 *Maternal obesity during pregnancy and offspring developmental adaptations and potential underlying mechanisms for offspring adverse cardiometabolic risk (Adapted from [124])*

2.4. Relevance of breastfeeding for cardiometabolic health in later life

There is evidence relating nutrition in early postnatal life to health in adulthood [154, 155]. Some early reports from the British Medical Association suggested that early life nutrition plays a role in setting the risk of diseases such as CHD, T2D, etc. in later life [154, 156]. The World Health Organization in recognition of this recommends breastfeeding in infancy to protect against adverse adult health outcomes [157]. Breastfeeding in childhood has been associated with protective effects on CVD risk [158, 159], lower rates of T2D, [160, 161], reductions in BP [162] in adulthood.

Breastmilk contains hormones and growth factors that may be necessary for the development of the cardiovascular system [159]. Breastmilk has low protein and energy content and though high in fat, breastfed infants have slower weight gain in infancy than formula fed infants [163]. Thus the suggested beneficial effects of human milk on cardiovascular health may be due to the slower growth observed in breastfed infants compared to the accelerated growth in formula fed infants [159, 163]. However, evidence from the literature on breastfeeding and long term health outcome is bedeviled with methodological challenges. Besides the fact that most of the evidence is from observational studies mostly comparing breastfed infants with formula fed infants, information on infancy breastfeeding is sometimes based on self-report, it can also be susceptible to the effects of confounding because some maternal and infant factors such as socioeconomic status, maternal pre-pregnancy BMI, birthweight are related with infant breastfeeding and offspring outcomes i.e. diabetes etc. [154, 164, 165].

Evidence from studies assessing the association of breastfeeding and risk of CVD, T2D and hypertension have been inconsistent. In a historic cohort study with a 65-year follow-up, that assessed the association between breastfeeding and atherosclerosis measured by arterial ultrasound in 63- to 82-year-old participants in the Boyd Orr cohort, reported that breastfeeding in infancy

was associated with a reduction in CIMT in adulthood (CIMT; difference 0.03 mm; 95% CI, 0.07 to 0.01) compared to bottle-feeding in a model adjusted for age and sex. Further adjustments for childhood and adulthood socioeconomic factors as well factors likely to be on the casual pathway did not alter this finding [158]. However, other studies have also reported no relationship between breastfeeding and CHD [166, 167], whilst others have suggested prolonged breastfeeding could even be detrimental to arterial distensibility in adulthood, a marker of CHD [168] and a positive relationship between breastfeeding and CHD mortality (hazard ratio: 1.73, 1.17 to 2.55) and incidence (1.54, 1.17 to 2.04) [169].

In terms of the association between breastfeeding and T2D, observational studies have shown that breastfeeding moderately protects against T2D in adulthood [160, 161]. In a prospective study that followed 6,044 individuals from the Copenhagen Perinatal Cohort to assess whether the duration of breastfeeding in infancy was linked with the risk of T2D in adulthood, showed that compared with infants breastfed for a short duration (≤0.5 month), those breastfed for a longer duration (>4 months) had a 51% reduced risk of T2D (HR = 0.49; 95% CI [0.32, 0.75]), suggesting a duration-response effect. However, after adjustment for prenatal and postnatal factors of both infant and mother, the inverse relationship between the duration of breastfeeding and risk of T2D in adulthood was altered and no longer significant at 31% reduced risk (HR = 0.69; 95% CI [0.44, 1.07]), thus likely suggesting that these factors may have confounded the association [165]. Evidence from a systematic review also showed that children and adults breastfed tended to have a lower mean fasting insulin levels compared to formula-fed individuals, with a likelihood of differences in insulin resistance between the breastfed and formula-fed individuals. However, these variations are moderate and not significant, probably due to the effect of confounding [155]. Other studies have also reported no relationship between breastfeeding in infancy and insulin resistance or T2D in later life [169, 170].

With regards to breastfeeding and BP, some meta-analyses have reported beneficial effects of breastfeeding compared to formula feeding on SBP and DBP in later life, Martin et al showed that SBP was lower in breastfed compared with bottle-fed infants with an estimate pooled difference of –1.4 mmHg, 95% confidence interval (CI): –2.2, –0.6), the reduction in DBP was a pooled difference of –0.5 mmHg, 95% CI: –0.9, –0.04) [171], similar observation was made by Owen [172] but there was evidence of heterogeneity between study estimates. There was also the concern of selective publication of small studies with positive findings, which may have overestimated the reported beneficial effect of breastfeeding in infancy on BP in later life. Results from larger studies seem to suggest that breastfeeding in infancy has a modest effect on BP [172]. Other studies have reported no association between breastfeeding and BP [159, 173].

2.4.1 Likely mechanisms linking breastfeeding with cardiometabolic risk in later life

The biological plausibility associating breastfeeding in infancy with a protective effect on adulthood CVD is not clearly defined and is speculative. However, it has been proposed that breastmilk has beneficial effects on the endothelium due to the long-chain polyunsaturated fatty acid (LC-PUFA) in breast milk, that are structural components of the vascular endothelium [174]. Incubation of cultured endothelial cells in breast milk has been shown to increase the production and release of vasodilating prostanoid [175]. The LC-PUFA can also act as an anti-atherosclerotic agent by suppressing pro-inflammatory cytokines [176]. Thus, the components of breastmilk could result in structural and functional alteration in the vascular endothelium that enhance normal flow-mediated dilation responses later in life [177].

Similarly, long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and arachidonic acid (AA) in breastmilk have been suggested as probable links explaining the effect of

breastfeeding on risk of T2D. Breastfeeding results in increased LCPUFAs levels in skeletal muscle membrane, breastfeeding and the resultant increased LCPUFAs levels are also inversely associated with fasting glucose. Additionally, studies show that low levels of DHA and other LCPUFAs in skeletal muscle membrane phospholipid are associated with insulin resistance and obesity in adults [178]. Thus is it possible that early changes in skeletal muscle membrane as a result of LCPUFAs saturation may be protective against insulin resistance, β-cell failure, and T2D [178–180].

Additionally, breastfed infants and formula fed ones differ in insulin secretion and the risk of obesity which could programme for T2D in later life. Infants fed formula have higher levels of insulin which can increase the likelihood of β-cell failure and T2D [179, 181].

In terms of the link between breastfeeding and BP, studies suggest that alteration in insulin-like growth factor-1 (IGF1) level is linked to changes in BP: IGF1 levels in the upper normal range are linked to reduced BP [182]. Nutrition in early life has an effect on IGF1 levels and breastfeeding has been shown to be positively associated with IGF1 [183]. It is possible that IGF1 programming maybe a pathway for the programming of later BP by breastfeeding [179]. IGF1 has been suggested to have vasodilator effects mediated through stimulation of nitric oxide synthesis by endothelial and vascular smooth muscle cells (SMCs) as well as the ability to stimulate vascular SMCs migration and proliferation [182].

2.5 Relevance of outcome variables (risk markers) for cardiometabolic disease

In this thesis a number of cardiometabolic risk markers were considered as outcome variables; their relevance for cardiometabolic diseases are described in the proceeding sections below.

2.5.1 Carotid intima media thickness (CIMT) as a marker of cardiometabolic disease

CVD is the leading cause of morbidity and mortality globally and an essential component of the cost of medical care with immense costs on health systems [184, 185]. In 2017, CVD resulted in over 17 million deaths, 330 million years of life lost and 35.6 million years lived with disability globally [186, 187]. Though over the last decade's mortality rates from CVD declined by 27.3%, the number of deaths rose by 42.4% from 1990 to 2015 [188], it is projected that CVD would continue to be the cause of more than 23 million deaths in 2030 around the globe [189].

Atherosclerosis is a gradual inflammatory condition that begins early in life and progresses over prolonged periods of time. It forms the pathophysiology underlying a number of cardiovascular events [190, 191], with subclinical arterial wall modifications that precede clinical manifestation of CVD [192]. Arterial wall thickness is suggested to indicate atherosclerosis and subclinical CVD risk [191]. It has been shown that an early structural alteration that can be detected in atherosclerosis is an increase in CIMT and such early structural anomalies of the arterial walls can be assessed using B-mode ultrasonography [192]. Thus CIMT a marker of subclinical atherosclerosis (asymptomatic organ damage) [191] can be assessed with B-mode ultrasonography, a simple, noninvasive technique that can detect early stage atherosclerosis [82]. Assessment of CIMT as a proxy of subclinical atherosclerosis early in life can help in early initiation of preventive measures that may delay the development of clinical CVD. Thus the American Heart Association (AHA) encourages noninvasive assessment of CVD risk early in life to enhance early identification of high risk individuals before clinical CVD emerges [191, 193].

Evidence from studies in older subjects shows that an increased CIMT predicts the risk of atherosclerosis in the coronary and peripheral arteries [82, 194, 195] and in young adults high CIMT is related with cardiovascular risk factors such as age, sex, BMI, SBP, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), diabetes mellitus [196, 197], also with an increased risk of developing stroke or myocardial infarction, independent of established cardiovascular risk factors [191].

Though CIMT is considered as a surrogate marker of atherosclerosis, increased CIMT could be due to other events such as intimal or medial hypertrophy or both, an adaptive response to alterations in flow, wall tension, or lumen diameter [198, 199]. Additionally, CIMT increases with increasing age, even in the absence of overt atherosclerosis, due to thickening of both the intimal and medial layers. Also CIMT values vary with sex, males usually have a higher CIMT compared to females. Among a large healthy cohort from Argentina, age –specific reference intervals CIMT values of 0.577 ± 0.003 mm, 0.566 ± 0.004 mm was reported for males and females respectively, with a similar pattern in other populations [200, 201].

Thus, thickening of the intimal-medial characterizes arterial wall aging that is not synonymous with subclinical atherosclerosis, but is linked to it because the cellular and molecular changes that are fundamental to intimal medial thickening have been indicated in the development of atherosclerosis. Taken together, carotid wall thickening is not a confirmation of atherosclerosis, especially when there is no presence of plaque. It depicts subclinical vascular disease, the basic pathophysiology that explains why CIMT is a risk factor and a marker of CVD risk [202, 203].

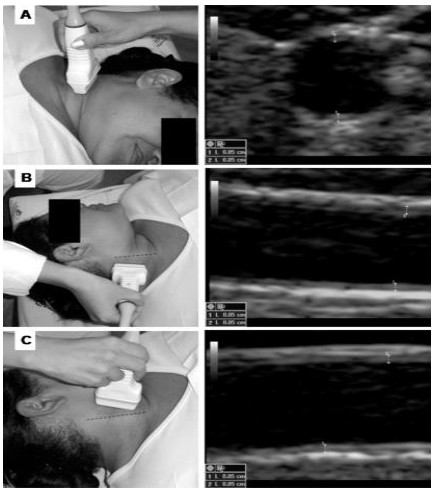
Additionally, even though measurement of CIMT is recommended for cardiovascular risk assessment, there has been concerns about its added value to the Framingham risk factors for cardiovascular-risk prediction. However, it has been shown that addition of CIMT improves the

predictive value of the Framingham risk score [204]. Hence the American Society of Echocardiology concluded in their consensus statement that ultrasonic CIMT measurements can be valuable for refining CVD risk assessment in asymptomatic patients, it could aid in the identification of patients at increased risk of CVD. However, attention should be paid to issues on quality control in image acquisition, measurement, interpretation, and reporting which are important for implementation of the technique in clinical practice [202]. Similarly, an update of the Mannheim IMT Consensus Document (2004-2006-2011) that sought to address issues of standardization of CIMT measurements and to clarify problems related to the classification of early atherosclerotic lesions concluded that CIMT and plaques both indicate increased vascular risk. The presence of plaque shows a higher risk compared to CIMT predictive values. However, CIMT without plaque is still a significant marker of a high risk of vascular events [192].

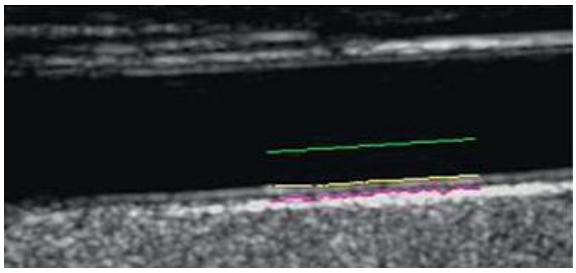
2.5.2 Measurement of CIMT

There are various techniques for measuring CIMT, however, B-mode ultrasonography, a high-resolution technique is one of the best methods used to detect early stages of atherosclerotic disease. It outlines the arterial wall structure with better resolution than similar techniques (e.g. magnetic resonance imaging or radiographic techniques). B-mode ultrasonography is a noninvasive, safe, sensitive, easily performed, reproducible, somewhat cheaper and a widely available method for ultrasonographic scanning of the common carotid artery (CCA). CIMT measurements are obtained with the patient lying in the supine position and with the neck rotated to the opposite side of examination [205]. The IMT is a double-line pattern visualized by echography on both walls of the CCA in a longitudinal image. Two parallel lines, which consist of the leading edges of two anatomical boundaries, the combined thickness of the lumen-intima and media-adventitia

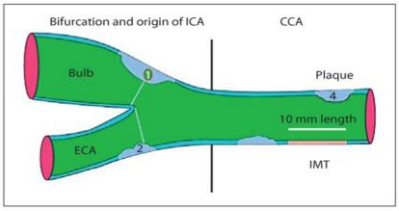
interfaces form the IMT [192, 206]. Figure 4 shows the location of CIMT and its measurement. Taken together, CIMT is a predictor of subclinical atherosclerosis and has been associated with some early life factors.



Angles for intima media thickness measurement: *A. transversal angle, B. posterolateral; C. anterolateral. The dashed lines represent the sternocleidomastoid muscle [205].*



Location of IMT: *distance between the lumen-intima (yellow line) and media-adventitia (pink line) interfaces [192].*



IMT measurement in the diastole [192].

Figure 4 *Location of carotid intima media and its measurement*

2.6 Insulin resistance as a marker of cardiometabolic disease

The number of people with diabetes has increased globally over the past three decades, with 90% having type 2 diabetes mellitus (T2DM), which has been classified as the ninth major cause of death [207]. Though one's genetic disposition and a sedentary lifestyle are major contributors to the current surge in cases, early life factors (intrauterine exposures) have been suggested to play a role in susceptibility to T2DM in later life [207]. T2D is a chronic metabolic disorder associated with persistent hyperglycemia. It is characterized by dysfunction in beta-cell insulin secretion and resistance or low insulin sensitivity to peripheral actions of insulin leading to chronic hyperglycemia [208, 209].

Though the specific mechanisms involved in the development of insulin resistance is not clearly defined, a number of mechanisms have been suggested i.e. (i) accumulation of ectopic lipid (intramyocellular triglyceride and intrahepatic lipid), these fatty acids alter insulin-mediated glucose uptake in muscle by inhibition of pyruvate dehydrogenase leading to reductions in glucose oxidation [210], (ii) impaired GLUT4 function (affects rate of body glucose disposal), (iii) chronic low-grade inflammation due to pro-inflammatory cytokines (tumor necrotic factor- α (TNF-α)) and interleukin-6 (IL-6))- interfere with insulin signaling and insulin action in adipocytes and hepatocyte, (iv) endoplasmic reticulum stress and (v) oxidative stress have all been indicated to alter insulin signaling and contribute to insulin resistance [211, 212]. Following insulin resistance in peripheral tissues (muscle, liver, adipose tissue), β cells in the pancreas increase insulin secretion with an attendant hyperinsulinemia [213]. This further exerts damaging effects i.e. glucotoxicity (damage to pancreatic beta-cell due to excessive exposure to glucose as a result of dysfunctional glucose disposal in insulin resistant peripheral tissues), lipotoxicity (beta-cell destruction due to excessive exposure to lipids-free fatty acids due to impaired lipolysis in insulin resistant adipose

tissue) or combinations of both lipoglucotoxicity and inflammation leading to loss of beta-cell function and eventually leads to overt T2D [208, 214, 215].

Due to the contribution of insulin resistance in the pathophysiology of T2D and other metabolic disorders, quantifying insulin resistance is essential in epidemiological studies. Various methods exist for the assessment of insulin resistance or insulin sensitivity. The *hyperinsulinemic euglycemic clamp* is considered the reference standard for direct measurement of insulin sensitivity. Insulin is infused intravenously at a constant rate after an overnight fast, which leads to a new steady-state insulin level which is above the fasting level (hyperinsulinemia). As a result, glucose disposal in skeletal muscle and adipose tissue is increased, however, hepatic glucose production is suppressed. A glucose analyzer is then used to regularly access blood glucose levels. This method has the advantage of directly measuring whole body glucose disposal at a given level of insulinemia under steady-state conditions, however, it is expensive, time consuming, labor intensive, and needs an experienced person to undertake the procedure, thus not suitable for large scale studies [216]. Other surrogate simple indexes used to assess insulin resistance are the *Homeostasis Model Assessment (HOMA)* which estimates fasting steady state levels of plasma glucose and insulin for any given combination of pancreatic β-cell function (HOMA%B) and insulin sensitivity S_I (HOMA%S) as percentages of a normal reference population. HOMA measurement correlates well with that of the reference standard and is widely used [216–218]. *The quantitative insulin sensitivity check index (QUICKI)* obtained by logarithmic-transformed fasting plasma glucose and insulin concentration gives a reliable, reproducible, and accurate index of insulin sensitivity, it compares well with the gold standard [219]. Finally, the measurement of glycated haemoglobin (HbA1c) which indicates the average blood glucose level over the preceding 2 to 3 months is also recommended for diagnosis of diabetes and it is also a risk factor of cardiovascular outcomes in diabetic and non-diabetic populations [220].

Insulin resistance or impaired insulin sensitivity is a key feature in the etiology of T2D [221, 222] and also an essential component in the pathogenesis of many other diseases, i.e. the metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), atherosclerotic heart disease [210] and hypertension [223]. Thus insulin resistance is an important risk marker for cardiometabolic diseases.

2.7 Hepatic steatosis as a marker of cardiometabolic disease

Hepatic steatosis or fatty liver disease refers to excessive accumulation of fat in hepatocytes [224], and can develop into NAFLD, which is the presence of hepatic steatosis affecting >5% of hepatocytes after elimination of secondary causes of hepatic fat accumulation i.e. excessive significant alcohol consumption, long-term use of steatogenic medication (e.g. corticosteroids, amiodarone, methotrexate), hereditary disorders (e.g. Wilson's disease, alpha-1 antitrypsin deficiency) etc. [225, 226]. NAFLD is the most common chronic liver disease in developed countries with rising incidence and prevalence leading to enormous clinical and economic burden [226, 227].

Liver biopsy with histological assessment is the gold standard for the diagnosis of NAFLD mostly used in severe cases. However, this method is complicated to undertake and sampling variability has been reported for liver histologic assessment which can affect the diagnostic performance of liver biopsy specimens [228–230]. Non-invasive techniques for quantifying liver fat include 1H-magnetic resonance spectroscopy (1H-MRS), ultrasound and computed tomography etc. But these techniques are expensive and require considerable time and are not always available. Thus relatively simple tests using routine laboratory and anthropometric parameters have been developed to assess liver fat. The fatty liver index (FLI) [231] and the hepatic steatosis index (HSI)

[232] are commonly used and seem to produce satisfactory results when validated against ultrasound [228].

The actual mechanisms involved in the pathogenesis of hepatic steatosis are not clearly defined, however, evidence from recent studies show that apart from lifestyle factors, genetics, age, sex ethnicity, epigenetics and nutritional factors may affect one's susceptibility to NAFLD [233, 234]. Several multifactorial events have been indicated to be involved in the development of hepatic steatosis, for instance, under normal conditions the liver does not store triacylglycerol (TAG) but in instances of obesity and abnormal lipid metabolism, (i) ectopic hepatic lipid accumulation occurs contributing to high rates of lipolysis and gluconeogenesis, (ii) enhanced mitochondrial oxidative metabolism leading to oxidative stress and liver damage [235, 236]. Intrahepatic fat and visceral fat are suggested to be independently associated with metabolic dysfunctions [237]. Intrahepatic fat is also linked to multi organ insulin resistance and could be directly associated with the dyslipidemia associated with hepatic steatosis [238]. Dyslipidemia and hyperglycemia are also linked to fatty liver [239]. In a similar vein, hepatic lipid accumulation is also related with lipotoxicity as a result of increased endoplasmic reticulum stress, mitochondrial stress and selective degradation of the mitochondria due to stress [235], similar to mechanisms linked with insulin resistance. Taken together, increased hepatic TAG could trigger metabolic dysfunction leading to insulin resistance, dyslipidemia, CVD, and progression of simple steatosis to nonalcoholic steatohepatitis (NASH)- which is the presence of hepatic steatosis and inflammation with hepatocyte injury with or without fibrosis and other severe forms of liver disease [240].

With regards to importance of hepatic steatosis as a risk marker of cardiometabolic diseases, of note NAFLD is thought to represent the hepatic manifestation of metabolic dysfunction and is strongly related to various metabolic risk factors, i.e. insulin resistance, dyslipidemia, T2D, CVD,

hypertension and obesity [226, 241, 242]. Additionally, in recognition of the need to transform the diagnosis of NAFLD to reflect the metabolic basis in its pathophysiology and to enhance diagnostic clarity in clinical circles, research and clinical trials, the term *metabolic dysfunction associated fatty liver disease* (MAFLD) was lately suggested by some international experts**.** The definition of MAFLD requires evidence of hepatic steatosis in addition to metabolic syndrome, or at least two features of metabolic dysfunction (overweight or obesity, high waist circumference, systolic or diastolic arterial hypertension, hypertriglyceridemia, low plasma HDL-C, T2DM or prediabetes, homeostasis model assessment of insulin resistance (HOMA-IR) score ≥2.5 or high-sensitivity C-reactive protein level >2 mg/L) [233, 243].

NAFLD has been indicated as an independent predictor of CHD and its risk factors, insulin resistance, dyslipidemia and altered flow-mediated vasodilation [244]. Hepatic steatosis has been linked with high CIMT in patients diagnosed with NAFLD [245]. Similarly, high levels of some liver enzymes i.e. gamma-glutamyltransferase (GGT) aspartate aminotransferase (AST) and alanine-aminotransferase (ALT)-surrogate biomarkers of NAFLD, which are possible predictors of CVD have been reported in patients with NAFLD and T2D [245]. Additionally, it has been reported that carotid atherosclerosis occurs earlier in individuals with NAFLD than in those without it [241, 245].

The mechanisms that link NAFLD to CVD are said to be complex and both seem to be indications of end-organ damage of the metabolic syndrome. Thus the specific role of NAFLD in predisposing one to a high CVD risk is difficult to ascertain due to the shared risk factors [226]. Briefly, high lipid uptake and increased de-novo lipogenesis **(**DNL) in NAFLD leads to increased accumulation of triglyceride in the liver with an attendant increased triglyceride-enriched very-low-density lipoprotein (VLDL) production. The high VLDL levels sets in motion an array of plasma

lipoprotein abnormalities and an atherogenic dyslipidemia, characterized by high serum triglycerides and low levels of HDL-C, including an atherogenic lipoprotein phenotype, mainly small dense low-density lipoprotein (LDL), and an accumulation of triglyceride-rich lipoproteins and intermediate-density lipoprotein (IDL) [246–248]. The triglyceride-rich lipoproteins containing apolipoprotein in turn, activate inflammatory cytokines such as IL-1 to IL-6 to CRP (C-reactive protein) with subsequent vascular inflammation and atherosclerotic CVD [226].

NAFLD is also closely associated with both hepatic and peripheral insulin resistance which in turn increases the risk of T2D. The relationship between NAFLD and insulin resistance could be due to liver fat accumulation that could originate from insulin resistance and hyperinsulinaemia [242, 249] or due to direct availability of excessive lipid which leads to insulin resistance [224]. The presence of NAFLD assessed by ultrasonography has been linked to a 2-to 5- fold risk of T2D**,** after adjustment for a number of lifestyle and metabolic covariates [250]. Whilst resolution of fatty liver was linked with a considerable reduced risk in the development of T2D**,** comparable to a level similar to individuals without NAFLD [251].

The role of NAFLD in the onset of hypertension is an ongoing debate, however various cross sectional studies, some of which diagnosed NAFLD ultrasonographically, have shown that the presence and severity of NAFLD are linked with increased BP and both prehypertension and hypertension among various populations, independent of cardiometabolic risk factors However, no causal associations could be established due to the cross-sectional study design of these studies [252–255]. Similarly, some longitudinal studies have also shown that NAFLD diagnosed with surrogate scores, i.e. FLI was prospectively related with hypertension [256, 257]. However, there is the limitation of non-direct measurement of intrahepatic fat content in these studies. Taken together, these studies indicate an association between NAFLD and hypertension. In conclusion

the available evidence shows that NAFLD somewhat contributes to the risk of cardiometabolic diseases.

2.8 Chronic subclinical inflammation as a marker of cardiometabolic disease

Chronic low-grade inflammation is mostly associated with obesity [258] and is triggered by persistent low-grade activation of the innate immune system, mostly in the visceral and ectopic adipose tissues by chemokine activated adipocytes and macrophage infiltration [259]. The infiltration of immune cells leads to the production of cytokines which act as an endocrine mediator [260] enhancing pro-inflammatory, diabetogenic, and atherogenic processes [259].

Obesity associated low-grade inflammation is often referred to as meta-inflammation or metabolic inflammation due to its close link with metabolic diseases [260], and has been suggested to contribute to the pathophysiology of a number of diseases [261] i.e. CVD [262], insulin resistance, BP [263], NAFLD, T2D [264] etc. Inflammatory markers associated with cardiometabolic diseases include the pro-inflammatory IL-6, IL-18, TNF-α, CRP [265], leptin [266], chemerin [267, 268] and the anti-inflammatory adiponectin (both beneficial and adverse effects of adiponectin have been reported on disease risk) [269]. The potential relevance of these markers in cardiometabolic disease is shown in table 2 below.

Though low grade chronic inflammation has been linked to disease risk, there are currently no standard biomarkers for showing the presence of health damaging chronic inflammation. However, research evidence shows that established biomarkers of acute inflammation predict morbidity and mortality, hence it may be used to index age-related systemic chronic inflammation [270, 271]. But, though inflammatory activity tends to be higher with older age, this is not true for all inflammatory markers. Thus, to resolve the limitations of assessing only a few select inflammatory

biomarkers, an approach that assays large numbers of inflammatory markers and combines these markers into more robust indices that symbolize heightened inflammatory activity is used [270, 272].

Chronic inflammation in adulthood maybe linked to exposures in early life which persist across the life course that affect adulthood health outcomes [270]. Exposures to maternal factors (diet, physical activity, smoking during pregnancy) may lead to epigenetic alterations in the offspring that can increase the offspring risk of obesity, low-grade chronic inflammation and associated disease risk in adulthood [270]. Maternal obesity promotes uterine inflammation and cytokine secretion that denotes metabolic inflammation characterized by increased circulating proinflammatory cytokines and adipose tissue macrophage accumulation. The proinflammatory state could extend to the placental area creating a proinflammatory intrauterine milieu [273, 274]. Low birthweight and shorter breastfeeding duration have both been linked to higher CRP concentrations - a marker of inflammation associated with increased risk for cardiovascular and metabolic diseases in young adulthood [275].

Low grade inflammation has been implicated in various pathophysiological stages of cardiometabolic diseases [276]. For instance in atherosclerosis, chemokines and cytokines initiate and promote atherosclerosis through various processes including (i) increased chemokine production and enhanced expression of endothelial cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), (ii) driving additional recruitment of leucocyte into the sub intimal space, (iii) uptake of cholesterol lipoproteins and fatty streak formation, (iv) initiation of smooth muscle cell proliferation and (v) inducing plaque instability and ultimate rupture [275–277].

Activation of chronic pro-inflammatory pathways in target cells of insulin action have been suggested in insulin resistance and T2D [278]. Inflammatory activities in the pancreatic islets,

adipose tissue hypertrophy, and the presence of increased proinflammatory cytokines and macrophage infiltration have been reported in patients with T2D [258, 276]. Local inflammation in target cells of insulin action with the production of cytokines i.e. TNF-α, IL-6 and IL-1b impair the insulin signalling cascade and can lead to insulin resistance [276]. This local inflammatory processes can also activate apoptosis of cells in the pancreas, which reduces the mass of functional pancreatic islets which can develop into T2D [258].

Inflammation is suggested to play a role in the development of hypertension, with reports of higher proinflammatory cytokines observed in hypertensive patients compared to normotensive individuals [279]. Higher plasma CRP [280], IL-6 and TNF-α [281] levels have been reported in hypertensive patients and are also risk factors for overt hypertension.

A potential mechanism by which chronic inflammation may stimulate hypertension could be endothelial dysfunction [282]. The endothelium, a single cell layer that lines the luminal surface of blood vessels regulates vascular tone and structure. Nitric oxide obtained from endothelial nitric oxide synthase (eNOS) is essential in this vascular tone regulation. Nitric oxide from endothelial cells also contributes to smooth muscle relaxation and eventually vasodilation [282]. Hence endothelial dysfunction may induce increased systemic vascular resistance that can culminate into hypertension, which is presented as impaired endothelium-dependent vasodilation as a result of an imbalance between vasoconstrictors and vasodilators [282].

Chronic inflammation can also activate oxidative stress, which has been linked to hypertension [283]. Though reactive oxygen species (ROS) may aid in the preservation of vascular tone and therefore protect against circulatory collapse during severe infection. However, abnormal release of ROS activated by immune cells when no hemodynamic insult is evident can initiate pathologic

rise in BP [283]. Taken together, chronic low-grade inflammation plays a role in cardiometabolic diseases.

Table 2 *selected inflammatory mediators with potential relevance to cardiometabolic diseases*

Inflammatory mediators	Probable role in cardiometabolic diseases or risk factors
CRP	• Consistently associated with increased risk of CVD and T2D • Enhances expression of cell adhesion molecules • Leucocyte recruitment into sub intimal space • Uptake of cholesterol lipoproteins and fatty streak initiation • Smooth muscle loss • Destabilization of atherosclerotic plaques
IL-6	• Predicts cardiovascular risk • Induces CRP production • High levels correlate with endothelial dysfunction and subclinical atherosclerosis
IL-18	• Associated with obesity, insulin resistance, hypertension, dyslipidemia and metabolic syndrome • Elevated levels predict development of T2D • Highly expressed in atherosclerotic plaques: plaque macrophages and unstable plaques • Thinning or inhibition of the fibrous cap formation, resulting in vulnerable, rupture-prone plaques • Contributes to plaque destabilization
TNF-α	• Correlate with adiposity and insulin resistance • Associated with CVD
Leptin	• High levels associated with hypertension and insulin resistance • Predictor of high CIMT • High levels linked to features of plaque instability
Chemerin	• Pro-inflammatory and anti-inflammatory roles in immune process • Associated with metabolic syndrome, T2D and obesity • Positively correlates with BMI, fasting insulin, leptin and CRP • Correlates with insulin resistance, adipose tissue and liver inflammation
Adiponectin	• Positive correlation with skeletal muscle and liver insulin sensitivity • Negative correlation with inflammatory markers • High levels predict reduced risk of T2D • Genetic data suggest no cause–effect relationship between low levels and T2D

Abbreviations: CRP- c-reactive protein; IL- interleukin; TNF- tumor necrosis factor. Information presented in the table is based on [266, 268, 269, 284–291].

2.9 Elevated blood pressure as a risk marker of cardiometabolic disease

Though a number of modifiable risk factors namely smoking, dyslipidemia etc. have been linked to CVD, high BP is suggested to be one of the most important risk factors and also that which presents the strongest evidence for causation [292–296]. High BP is defined as SBP ≥140 mmHg or DBP ≥90 mmHg [297]. Hypertension or high BP is the leading preventable cause of CVD mortality and disease burden in most regions of the world [297]. The prevalence of hypertension has increased from 2000 to 2010 mostly in low- and middle-income countries (LMICs). About 31.1% of adults (1.39 billion) worldwide had hypertension in 2010, with the prevalence higher among adults in LMICs (31.5%, 1.04 billion people) compared to high-income countries (28.5%, 349 million people) [298, 299].

High BP is also a predictor of T2D, hypertension and T2D usually have common risk factors and these conditions often coexist [300]. Evidence from longitudinal studies show that high BP is a significant predictor of T2D [301–304]. Evidence also indicates that a suboptimal prenatal and early postnatal environment can lead to a high risk of hypertension later in life [305].

The pathophysiological mechanisms that associate high BP with cardiometabolic diseases (CVD and T2D) are yet to be clearly elucidated, however, some hypotheses have been proposed. High BP has been shown to trigger microvascular dysfunction, which may play a role in the development of T2D. Endothelial dysfunction is linked to insulin resistance, which is also closely related to hypertension [300].

Insulin resistance may induce the development and advance atherosclerosis through the disruption of the blood vessel wall through the promotion of vascular inflammation and endothelial cell dysfunction, derangements of different cell types such as platelets and promotion of coagulation. These could culminate into narrowing of blood vessels and an increase in total peripheral arterial

resistance [306]. Insulin resistance thus can be a common linkage for BP, T2D and CVD [300]. Taken together evidence shows that BP is a risk factor for CVD and T2D.

2.9.1 Conclusive considerations

Evidence from the literature shows that exposures in early life can influence cardiometabolic (CVD, T2D and hypertension) health in adulthood. Thus early life represents a critical period of plasticity such that adverse or suboptimal environmental exposures (nutrient deficiency etc.) during this period of development can permanently change fetal organ structure, function, physiology and metabolism referred to as a predictive adaptive response (PAR). Such modifications intended to enhance later survival in an inadequate nutritional environment can become detrimental when later exposed to a postnatal mismatched obesogenic nutritional environment.

Earlier studies showed the importance of the early environment to disease risk in later life, however studies from Barker on the association between birthweight and CVD, T2D and hypertension really brought the importance of early life factors to disease risk in adulthood to the forefront of research.

Earlier studies also focused on fetal undernutrition evidenced by low birthweight (as a result of maternal malnutrition) and the risk of metabolic diseases in later life, though birthweight is considered an inaccurate indicator of fetal growth or experience in utero and also it does not indicate attainment of fetal genetic growth potential. However, later studies have shown that both low birthweight and high birthweight due to maternal undernutrition and overnutrition (obesity) are associated with offspring adverse cardiometabolic health outcomes, sometimes with conflicting results.

The pathophysiological mechanisms underlying the programming effects of a number of early life factors are not yet clearly understood, however, PAR, epigenetic programming of gene expression

(alterations in gene expression), cellular aging (accelerated by oxidative stress) and genetics have been suggested. Different animal models have been used to study some of these mechanistic processes of fetal programming but these have implications for extrapolating findings into human's settings due to differences in gestational period and how developmental programming may differ in humans and animals.

Current literature evidently shows that suboptimal early life factors during critical periods of development can program physiology in ways that predispose to cardiometabolic disease. Thus modifiable early life events offer an avenue for interventions to optimize the early environment and to promote offspring health in later life, as well as prevent transmission of adverse developmentally programmed traits to the next generation. This will call for a much better insight into the role of early life factors in the surge in cardiometabolic diseases, a more in depth knowledge of the mechanisms behind these associations that could be targets for intervention, use of longitudinal, prospective cohort studies for better research results and the need for probable markers that indicate developmental programming so developmentally programmed individuals can be identified early for intervention purposes.

3 AIMS AND RESEARCH QUESTIONS

A summary of the previous chapters show that perinatal and early life factors are possible determinants of adulthood diseases i.e. CVD, T2D and hypertension. Our interest to assess the prospective relevance of a range of early life factors for cardiometabolic health in young adulthood resulted in three research aims.

AIM 1: Early life factors and their relevance to intima-media thickness (CIMT) of the common carotid artery in early adulthood.

HYPOTHESIS:

CVD is suggested to have its origins in early life and has been associated with early life factors e.g. low birthweight mostly in populations exposed to maternal undernutrition during gestation. High CIMT and endothelial dysfunction have been shown as initial indications of atherosclerotic plaque development. Alteration in the CIMT is also known as a marker of subclinical atherosclerosis, with a high CIMT shown to correlate with cardiovascular risk factors, and to predict CVD.

RESEARCH QUESTION:

Are the following early life factors: indicators of intrauterine growth such as birth weight, birth weight-for-gestational-age (i.e. adequate, small and large for gestational age), pregnancy duration, GWG, parental age and full breastfeeding relevant for adulthood IMT as a surrogate marker of CVD among healthy term-born German participants.

AIM 2: Early life factors and their relevance for markers of cardiometabolic risk in early adulthood.

HYPOTHESIS:

Adverse exposures in early life have been linked with adulthood cardiometabolic diseases. Evidence from human and animal studies lend some credence to the 'developmental programming' of cardiometabolic diseases by early factors including the intrauterine environment, maternal weight during pregnancy etc. Maternal obesity during pregnancy is suggested to partly reflect maternal over nutrition and also partially an increase in adiposity which can alter the intrauterine environment and nutrient transfer to the offspring and thus can have long term health consequences for the offspring.

RESEARCH QUESTION:

Are the following early life factors: early pregnancy BMI, GWG, birth weight, birth weight-for-gestational-age, maternal age and full breastfeeding relevant for markers of cardiometabolic risk i.e. insulin sensitivity, indices of hepatic steatosis and pro-inflammatory biomarkers among healthy young adult Germans.

AIM 3: To assess and judge the evidence for an association between maternal pregnancy weight/body mass index (BMI) or gestational weight gain with offspring's blood pressure in later life.

HYPOTHESIS:

Studies have shown that adulthood BP may be influenced by exposures in early life. Maternal factors such as pregnancy weight and GWG have been purported to be associated with offspring's BP.

RESEARCH QUESTION:

What is the evidence from the literature about the association between pregnancy weight and GWG with offspring's BP?

Table 3 *Research questions addressed in studies I-III*

Aim	Study	Location
1	**I:** Early life factors and CIMT	Appendix 1
2	**II:** Early life factors and markers of cardiometabolic risk	Appendix 2
3	**III:** Maternal pregnancy weight or GWG and BP	Appendix 3

4 GENERAL METHODOLOGY

The research questions of this thesis were answered using data from the DONALD study. A systematic review was also conducted to provide current evidence on the relevance of pregnancy weight or GWG with offsprings BP. This chapter gives a brief overview of the DONALD study design and the procedure for the literature review.

4.1 Population and DONALD study design

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study is a longitudinal, continual, open cohort study undertaken at the Research Institute of Child Nutrition, Dortmund, Germany. The overall objective is to assess the complex interaction between nutritional intake, growth, development, metabolism and health from infancy to adulthood [307]. The main aims of the DONALD study are: analysis of the interactions between nutrition, metabolism, development and growth of healthy children including the identification of nutritional factors with long-term preventive medical potential, the determination of intra- and inter-individual trends in nutrient intake and nutritional behavior among infants, children and adolescents and the determination of metabolic reference data on 24h urinary excretion levels in healthy children and adolescents and the provision of nutritional data for exposure assessment [308, 309].

The inclusion criteria are healthy babies of Caucasian origin with a mother and/or father consenting to participate in a long-term study. Also one parent should be knowledgeable in the German language. Since its commencement in 1985, elaborate records on diet, growth, development, and metabolism has been gathered from over 1,700 children between infancy and adulthood. Approximately, 35–40 infants are newly enrolled each year into the open cohort study, initial examination commences at ages 3, 6, 9, 12, 18, 24 months and then once annually until adulthood. During such visits various assessments and measurements are undertaken depending on

participant's age. These include anthropometry, a 3 day weighed dietary record, socio-demographics and health characteristics, interviews on lifestyle, medical assessments and 24-hours urine collection.

The DONALD study is essentially observational and noninvasive until when participants are 18 years and above. Since 2005, participants are invited for follow up assessment including fasting blood measurement at ages 18, 21 and 25 years and then followed by examination every 5 years. Also parental data such as socio-demographic traits, anthropometry, interviews on lifestyle, medical assessments and health status are repeatedly obtained at certain time points during the study. The information is acquired through personal examination and the use of questionnaire. The study was approved by the Ethics Committee of the University of Bonn. All examinations are performed with written consent of the parent and adult participant [308, 310–313].

The assessments in the DONALD study that are pertinent to this thesis include data on early life factors of participants: (maternal and paternal age at child birth, gestational age, early pregnancy BMI, GWG, birthweight, birthweight by gestation age, full breastfeeding etc.), anthropometric measurements, medical assessment, interviews on lifestyle factors (smoking and physical activity level), CIMT and fasting blood measurement in participants in early adulthood, socioeconomic status, parental health, paternal educational status etc. The children who were initially recruited for the DONALD study differed considerably in age, thus data on the first few years of life was not always available. Additionally, a number of children had not yet reached adulthood at the time of the analysis of the various outcomes used in this thesis. Thus the various study aims in this thesis are answered using different subsample sizes of the total DONALD study participants and also risk markers are considered as outcome variables instead of overt cardiometabolic diseases because

participants are in young adulthood with less manifestation of chronic diseases. Figure 5 shows a summary of the DONALD study design.

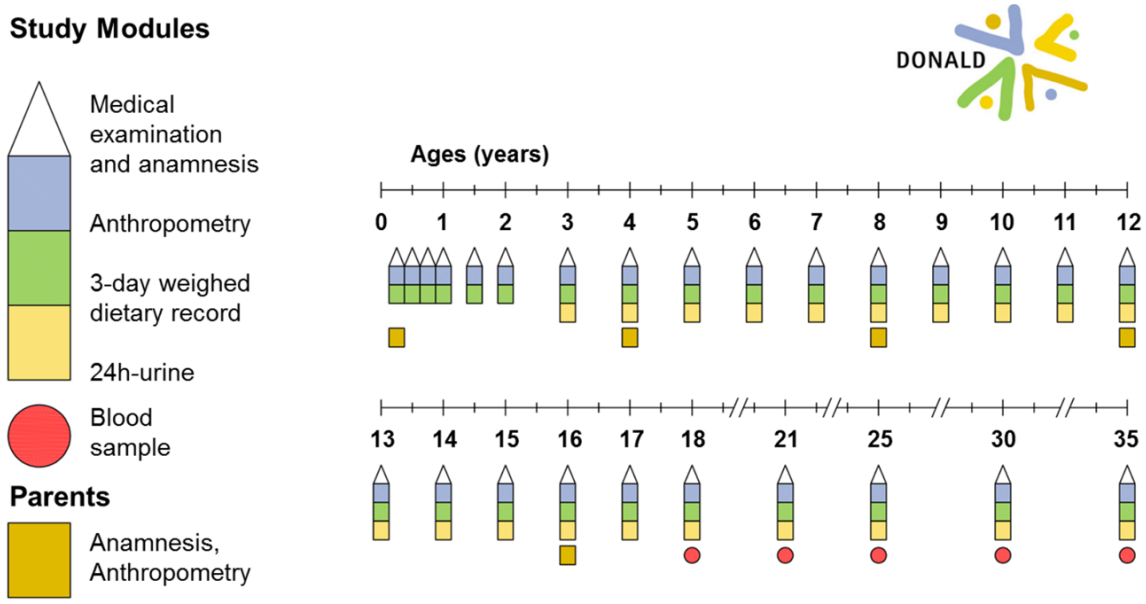


Figure 5 *DONALD study schedule (adapted from [307])*

4.2 Assessment of early life exposures

The DONALD study retrieved data on early life factors measured by gynecologist or midwives during pregnancy and after birth from the "Mutterpass" and breastfeeding data was prospectively collected in the study, thus these variables may be judged to have adequate level of accuracy. Of note various data are collected in the DONALD study, however, only data that are relevant to this thesis are mentioned.

Perinatal and early life factors

Child birth and maternal characteristics were extracted from the "Mutterpass". Gestational duration is calculated based on maternal recalled date according to the mother's last menstrual period.

Maternal weight recorded at the first visit to a gynecologist during pregnancy and at the end of pregnancy weight were abstracted from the "Mutterpass," and from these the GWG was computed. Early pregnancy BMI was also computed from weight and height records.

Birth weight and birth length were recorded at birth. Birth weight-for-gestational-age is defined according to the German sex-specific birth weight and length-for-gestational-age curves [314]. SGA is defined as birth weight and length $<10^{th}$ percentile, and LGA is defined as birth weight and length $>90^{th}$ percentile. All other infants were classified as AGA.

Maternal and paternal age at the time of child birth is assessed at first visit to the study center.

Breastfeeding data was assessed upon the child's admission to the DONALD study. During first visit either at 3 or 6 months the study pediatrician and/or dietitian enquired from the mothers the duration (in weeks) the infant had been fully breastfed (not given solid foods and no liquids daily except breast milk, tea, or water). If the child is still being fully breastfed, the length of breastfeeding is assessed at successive visits at ages 6, 9, 12 and 18 months until commencement of complementary feeding. The duration of feeding formula or solid foods is also assessed during the visits. A coherent check is conducted on all breast feeding information collected such as the recording of breast milk in 3-day dietary records and information acquired by the dietitians to minimize errors. From this information the duration (in weeks) of full breastfeeding is calculated [315, 316].

Anthropometric measurement

Anthropometry of study participants is collected using standard protocol by trained nurses who undergo yearly quality control where intra-and inter observer measurement precision is assessed. Measuring instruments are also regularly calibrated. The participants are dressed in only underwear and are barefooted. Recumbent length of children until 2 years of age is measured to the nearest 0.1 cm using a Harpenden (UK) stadiometer, whilst standing height is measured in children aged older than 2 years to the nearest 0.1 cm with a digital stadiometer (Harpenden Ltd., Crymych, UK). Body weight is measured to the nearest 100 g using an electronic scale (Seca 753E; Seca Weighing and Measuring Systems, Hamburg, Germany). Waist circumference is measured at the midpoint between the lower rib and iliac crest to the nearest 0.1 cm [313]. This same measurement procedure is used to measure anthropometry of parents at regular intervals.

4.3 Outcome Measurements

Intima media thickness

IMT in the DONALD study was measured using the Mindray DP3300, tragbares portable digital system. Participants were measured in a supine position after having rested for 10 min. First, two images were taken each on the right and left common carotid artery on the participants and the images were frozen. Afterwards, measurements were taken at four measurement points on each image. IMT measurements were performed by study physicians and quality control was routinely undertaken to ensure the measurement meet the required criteria [317].

Insulin sensitivity

Insulin sensitivity was assessed in the DONALD study by the use of HOMA of insulin sensitivity (HOMA 2-%S), which is the inverse of HOMA-IR [217, 318]. This method assesses insulin sensitivity from fasting steady state levels of plasma glucose and insulin [217]. HOMA parameters can be calculated using the original HOMA model (HOMA1), a simple mathematical approximation equation developed in 1985 or the 1996 updated computer model (HOMA2) [217]. However, the original HOMA model is widely used compared to the updated version despite the recommendation to use the latter, due to its advantage of being applicable in various insulin assays [217]. It has been recommended that since HOMA is suitable for calculating insulin sensitivity in a steady state, it should not be applied in non-steady state or hypoglycemic state (plasma glucose < 2.5mmol/L) [217]. These recommendations were adhered to in the second study of this thesis (OA2 Appendix 2).

Hepatic steatosis

In the DONALD study hepatic steatosis was evaluated using two noninvasive validated algorithms i.e. hepatic steatosis index (HSI) and fatty liver index (FLI). These indices are calculated based on activity levels of liver enzymes or hepatic transaminases (ALT and AST for HSI, and GGT for FLI) and data from other routine blood and anthropometric measurement (see calculation in OA2 in (Appendix 2).

Chronic low grade inflammation

Sub clinical inflammation was assessed in the DONALD study using various pro-inflammatory markers i.e. hsCRP, IL-6, IL-18, leptin, chemerin, and the anti-inflammatory hormone adiponectin [319]. These pro-inflammatory markers have been associated with increased risk of

cardiometabolic diseases and its risk factors, however, some of these associations are suggested to be non-casual as indicated by Mendelian randomization studies [320, 321]. To depict the general inflammatory state which is an interaction between pro-inflammatory and anti-inflammatory mediators an aggregate score of pro-inflammation was calculated [272] as shown in OA2 (Appendix 2).

4.4 Procedure for systematic review

The systematic review was undertaken and reported according to the (updated) Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and was registered in the international prospective register of systematic reviews PROSPERO (CRD42020197479). The search strategy involved a 2-step systematic literature search in the data bases, MEDLINE, EMBASE (PubMed/EMBASE), Cochrane Library, CINAHL and Web of Science. These five databases were searched until July 24, 2020, using a combination of the following search terms: terms related to "maternal pregnancy weight or BMI" OR "maternal gestation weight gain (GWG)" AND terms related to "offspring's blood pressure (BP)" OR "offspring's cardiovascular variables" AND limitation to human study populations. All search terms were searched both as controlled vocabulary terms (Medical Subject Headings or Emtree) and as free words in the title and abstract. No limitations were set with regards to language, age, year of publication or study type. Figure 6 shows the flow diagram for the systematic review study selection.

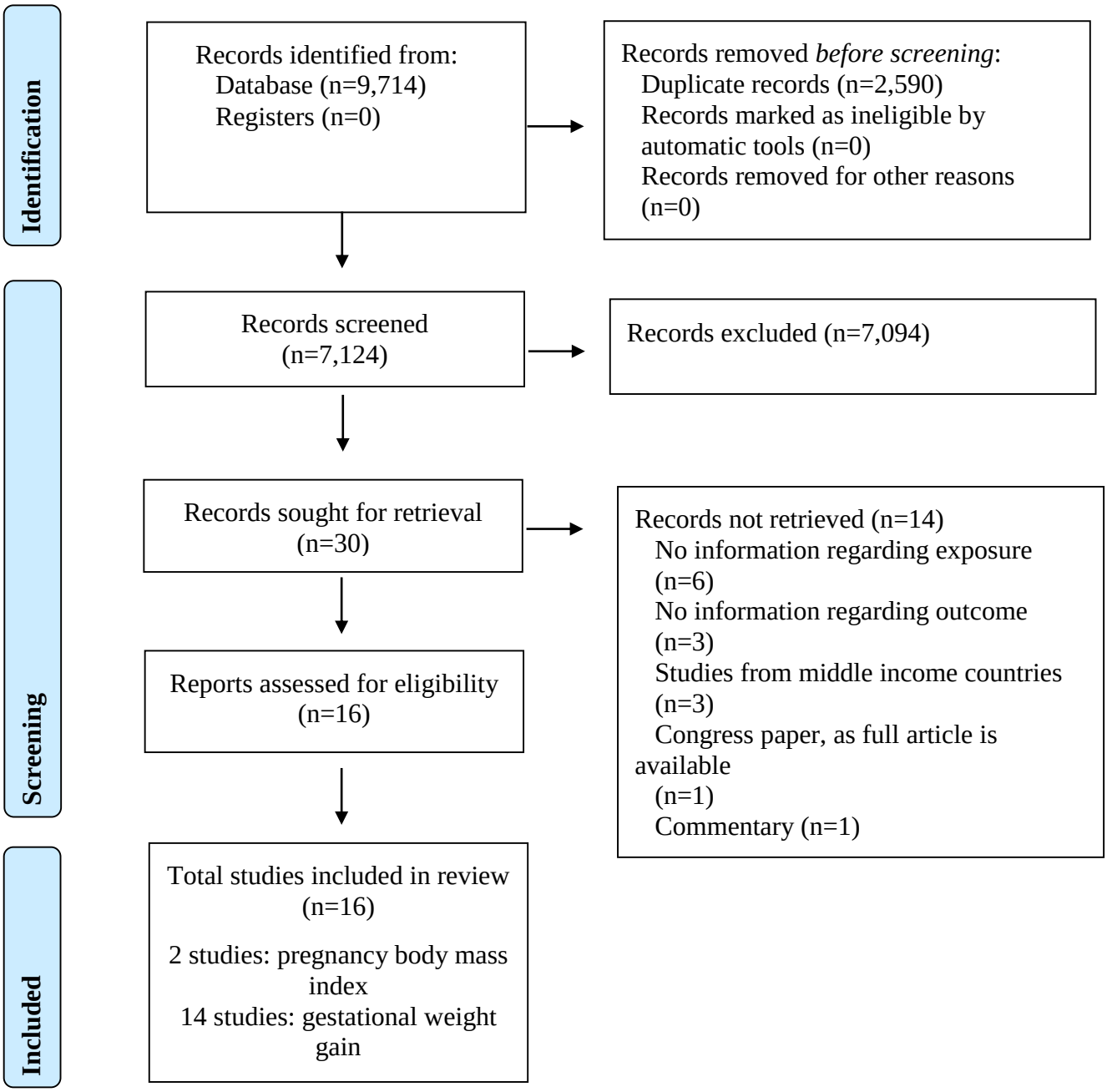


Figure 6 *Flow diagram for systematic review study selection*

5 ORIGINAL ARTICLES

OA1 Early life factors and their relevance to intima-media thickness of the common carotid artery in early adulthood

Nyasordzi J, Penczynski K, Remer T, Buyken A. E.

PLoS ONE 15(5): e0233227. doi.org/10.1371/journal.pone.0233227. [Epub 2020 May 19]

Background

Early life factors may predispose an offspring to cardiovascular disease in later life; relevance of these associations may extend to "healthy" people in Western populations. We examined the prospective associations between early life factors and adult carotid intima-media thickness (IMT), a surrogate marker of atherosclerosis, in a healthy German population.

Methods

We studied term participants (n=265) of the DONALD Study, with bilateral sonographic measurements of IMT (4-8 measurements on both left and right carotid artery) at age 18-40 years and prospectively collected data on early life factors (maternal and paternal age at child birth, birth weight, gestational weight gain and full breastfeeding (>17weeks). Mean IMT values were averaged from mean values of both sides. Associations between early life factors and adult IMT were analyzed using multivariable linear regression models with adjustment for potential confounders.

Results

Adult mean IMT was 0.56mm, SD 0.03, (range: 0.41 mm-0.78 mm). Maternal age at child birth was of relevance for adult IMT, which was sex specific: Advanced maternal age at child birth was associated with an increased adult IMT among female offspring only (β 0.03, SE 0.009 mm/decade, P=0.003), this was not affected by adult waist circumference, BMI or blood pressure. Other early life factors were not relevant for IMT levels in males and females.

Conclusion

This study suggests that advanced maternal age at child birth is of prospective relevance for adult IMT levels in a healthy German population and this association may be of adverse relevance for females only.

Contribution of JN: statistical data analysis, interpretation of results (together with KP and AEB) and drafting of the manuscript

OA2 Early life factors and their relevance for markers of cardiometabolic risk in early adulthood

Nyasordzi J, Conrad J, Goletzke J, Ludwig-Walz H, Herder C, Roden M, Wudy S A., Hua, Remer T, Buyken A. E.

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doi.org/10.1016/j.numecd.2021.03.024 [Epub 2021 April 7]

Background and Aims: Early life exposures could be pertinent risk factors of cardiometabolic diseases in adulthood. We assessed the prospective associations of early life factors with markers of cardiometabolic risk among healthy German adults.

Methods and results: We examined 348 term-born DONALD Study participants with measurement of fasting blood at the age of 18-24 years to assess metabolic indices: fatty liver index (FLI), hepatic steatosis index (HSI), pro-inflammatory score and insulin sensitivity (HOMA2-%S).

Early life factors (maternal weight in early pregnancy, maternal early pregnancy BMI, gestational weight gain (GWG), maternal age, birth weight and full breastfeeding (>17 weeks)) were assessed at enrolment of the offspring into the study. Multivariable linear regression models were used to analyze associations between early life factors and markers of cardiometabolic risk in early adulthood with adjustment for potential confounders.

A higher early pregnancy BMI was related to notably higher levels of offspring FLI, HSI, pro-inflammatory score and a lower HOMA2-%S (all $p<0.0001$). Similarly, a higher gestational weight gain was associated with a higher FLI ($p=0.044$), HSI ($p=0.016$), pro-inflammatory score ($p=0.032$) and a lower HOMA2-%S among females ($p=0.034$). Full breastfeeding was associated with a lower adult FLI ($p=0.037$). A casual mediation analysis showed that these associations were mediated by offspring adult waist circumference (WC).

Conclusion: This study suggests that early pregnancy BMI, gestational weight gain, and full breastfeeding are relevant for offspring markers of cardiometabolic risk which seems to be mediated by body composition in young adulthood.

Contribution of JN: statistical data analysis, interpretation of results (together with AEB) and drafting of the manuscript

OA3 Maternal pregnancy weight or gestational weight gain and offspring's blood pressure: Systematic review. [*Submitted to Nutrition, Metabolism & Cardiovascular Diseases*]

LUDWIG-WALZ H, NYASORDZI J, WEBER K. S., BUYKEN A. E., KROKE A

Objective: An increasing number of studies suggest that maternal weight parameters in pregnancy are associated with offspring's blood pressure (BP). The aim of this systematic review – following the updated Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Statement – was to assess and judge the evidence for an association between maternal pregnancy weight/body mass index (BMI) or gestational weight gain (GWG) with offspring's BP in later life.

Methods: MEDLINE, EMBASE, Cochrane Library, CINAHL and Web of Science were searched without limits. Risk of bias was assessed using the "US National Heart, Lung and Blood Institute"-tool, and an evidence grade was allocated following the "World Cancer Research Fund" criteria.

Results: Of 7,124 publications retrieved, 16 studies (all cohort studies) were included in the systematic review. Overall data from 52,606 participants were enclosed. Association between maternal pregnancy BMI and offspring's BP were analyzed in 2 (both "good-quality" rated) studies, without consistent results. GWG and later offspring's BP was analyzed in 14 studies (2 "good-quality", 9 "fair-quality", 3 "poor-quality" rated). Of these, 3 "fair-quality" studies described significant positive results for systolic BP and significant results, but partly with varying directions of effect estimates, for diastolic BP. MAP was analyzed in 1 "poor-quality" congress paper. Overall, based on the small number of "good-quality"-rated studies, the inconsistency of effect direction and methodological weaknesses, no firm conclusion can be drawn.

Conclusion: Evidence for an association of maternal pregnancy weight determinants with offspring's later BP was overall graded as „limited-no conclusion".

Contribution of JN: screening databases to identify potential relevant studies, assessing full text for eligibility of inclusion into the study, data extraction from included studies (together with HLW) and reviewing manuscript

6 GENERAL DISCUSSION

In this section an overall interpretation of the original research results is undertaken. The overall aims, findings, methodology, study strengths and weaknesses will be briefly discussed. In addition, the practical implications of the findings in terms of its relation to public health nutrition is considered. Detailed aspects of the research findings can be found in the individual studies in appendix OA 1-3. The overall aim of this thesis was to investigate the relevance of perinatal and early life factors for cardiometabolic health in early adulthood and to evaluate the evidence of an association of pregnancy weight or gestational weight gain with offspring's blood pressure through a systematic review of the literature.

6.1 Research Aims

6.1.1 Research Aim 1

The aim of the first study was to assess early life factors and their relevance to intima media thickness of the common carotid artery in early adulthood. The result showed that older maternal age at childbirth was associated with an increased IMT among female offspring in young adulthood, but not in males. This association was not mediated by adulthood waist circumference, BMI, systolic or diastolic blood pressure. This is in line with evidence from studies associating advance maternal age with adverse offspring outcomes.

A similar study reported that advance maternal age was associated with increased IMT among female offspring [322]. Among Finnish offspring aged 15-39 years, a U-shaped association was observed between maternal age and offspring risk of T2D: compared with baseline maternal age (25.5 years), the risk of T2D was higher in offspring of very young or old mothers [110]. Thus both extreme ends of maternal age seem to be associated with adverse offspring effects. Similar reports of adverse offspring cardiometabolic outcomes have been associated with advanced

maternal age in animal studies. Using a rodent model to assess the long-term implications of advanced maternal age on adult offspring cardiovascular health. Male offspring from aged dams showed mild cardiac diastolic dysfunction, female offspring however showed no cardiac dysfunction but had high SBP, which indicates sex-specific differential cardiovascular effects of advanced maternal age on offspring [323] which in is in line with our finding, though our observation of adverse effect was in females. In a study among offspring obtained from embryos of aged female mice (34–39 weeks), crossed with young males and transferred into young embryo recipients, there was impaired body weight gain, BP and glucose metabolism in offspring during postnatal life. Majority of these alterations were sex specific with females offspring being more vulnerable [324]. Hence evidence exists on the adverse effects of advanced maternal age on offspring health outcomes with differential sex specific effects, though other studies have also reported that there seem to be no association of maternal age with offspring long-term morbidities (cardiovascular, endocrine, neurological, hematological, respiratory and gastrointestinal) [325].

Additionally, in animal studies results show that offspring exposed to complicated pregnancies (similar to those observed in advanced maternal age in humans) caused by various maternal manipulations (nutrient restriction, uterine artery ligation) exhibit cardiovascular dysfunction [323]. Apart from these adverse effects on the offspring, advanced maternal age is linked to the risk of maternal complications i.e. preeclampsia, cesarean delivery, gestational diabetes mellitus, preterm birth etc. [326]. Though mechanisms are yet to be established, it has been suggested that advanced maternal age could compromise the intrauterine environment as evidenced by reduced uteroplacental perfusion, impaired vascular function in animal models which led to adverse pregnancy outcomes, these developmental stressors may result in a suboptimal in utero environment that can affect offspring long-term health [327]. However, further studies are needed

to elucidate the underlying mechanisms associating advanced maternal age with offspring adverse health outcomes.

In the US and Canada, births among women advanced in age account for 14% and 18% respectively of total live births [323, 328]. Thus given the current surge in child birth at an advanced maternal age, the likelihood for this trend to continue and its association with adverse maternal and offspring outcomes, it can have major public health and health care cost implications.

Probable interventions maybe that research findings about the adverse effects of advanced maternal age at child birth on maternal and offspring outcome should be widely and routinely disseminated using public health avenues so women in the reproductive age can make informed decisions on the timing of child birth, though this is highly an individual decision. Enacting favorable government policies to improve conditions (economic, educational and health care) in different countries so that women can start a family life relatively early whilst working maybe helpful. Sometimes it is quite challenging to start a family whilst pursuing further studies but probably family or institutional support could ease the burden on women, so that they do not have to wait to obtain their terminal degrees before starting a family life. Additionally, maybe specialized antenatal care services should be given to women advanced in age to improve maternal health and child outcomes.

Taken together, emerging evidence shows advanced maternal age may have adverse consequences for offspring long term health. Hence further experimental research in appropriate animal models is required to better understand the mechanisms linking advanced maternal age at child birth with adverse maternal and offspring outcomes to generate appropriate strategies and solutions to improve maternal outcomes and offspring long term health.

6.1.2 Research Aim 2

The second aim was to assess the prospective relevance of early life factors for markers of cardiometabolic risk in early adulthood. A higher early pregnancy BMI was associated with a higher FLI, a higher HSI, a higher pro-inflammatory score and a lower HOMA2-S%. A higher GWG was also associated with a higher FLI, a higher HSI and a higher pro-inflammatory score with a lower HOMA2-S% among females, but not males. Full breastfeeding was associated with a lower adult FLI. These associations were mediated by adulthood waist circumference and BMI.

This finding is in line with studies assessing the association of maternal prepregnancy BMI and GWG with offspring cardiometabolic risk in young adulthood, though different cardiometabolic parameters were assessed. For instance, the Jerusalem Perinatal Family Study, reported that higher maternal prepregnancy BMI was significantly associated with higher offspring BMI, WC, SBP and DBP, insulin and triglycerides but with a lower HDL-C, whilst a higher GWG was positively associated with offspring adiposity [136]. Similar observations were made by other researchers in addition to higher HOMA-IR in offspring [132, 135]. Evidence also show that maternal obesity during pregnancy is associated with obesity and cardiometabolic disease risk (glucose/insulin homeostasis, hypertension, and vascular dysfunction) [122]**,** indicating that maternal obesity probably induces developmental programming of the offspring [329].

Though human cohort studies are important in unraveling the mechanism of association between maternal obesity in pregnancy and offspring health outcomes, in these studies it is difficult to determine the extent to which adverse effects on offspring are attributable to shared genes, adverse in utero exposure (obesity/diabetes) or early postnatal factors (nutrition) [31, 329]. Thus rodent

models of maternal obesity during pregnancy have been developed by feeding dams high fat, high sugar obesogenic diets to assess the effects of maternal overnutrition on offspring outcomes [31].

Evidence from these models show that feeding a dam a cafeteria or high- diet fat (HFD) results in obesity, insulin and leptin resistance [142, 330–332], hypertension [144, 333, 334], fatty pancreas disease [335], hepatic steatosis and NAFLD in the offspring [336, 337]. The hyperleptinaemia, insulin resistance, as well as the high body weight in the offspring due to maternal adiposity is suggested to persist into adulthood [338]. Increased adiposity, glucose intolerance, and altered brain appetite regulation have also been observed in offspring in response to even mild maternal overnutrition [339, 340].

Of note, though offspring obesity later in life could worsen the effects of maternal obesity on their risk to metabolic disorders i.e. insulin resistance and hypertension etc., these adverse conditions are sometimes observed prior to increased offspring adiposity, probably indicating independent programming of these processes by maternal overnutrition [31].

Animal and human studies suggest that maternal obesity during pregnancy creates an adverse intrauterine milieu for the growing fetus which could programme the offspring to later adverse effects [122, 340, 341]. It has also been shown that the physiological modifications that occur in obese pregnant women differs from that in normal weight women. These alterations are indicated to increase the availability of fuel for fetal growth in obese women [141, 341]. Several of these metabolic changes are suggested to be due to the comparably high adipose tissue in obese pregnant women [341], which contributes to dysregulated release of adipokines (leptin and adiponectin), plasma free fatty acids, and inflammatory markers TNF-α, interleukin (IL)-6) [341, 342].

Apart from the adipose tissue, the placenta also releases leptin, TNF-α, and interleukins, which was evident from assessment of placentas from obese pregnant women which showed increased infiltration of macrophages and increased expression of inflammatory markers [341, 343]. The comparatively high pro-inflammatory cytokine produced by obese women during pregnancy may amplify physiological modifications [341]. Hence these metabolic changes could expose the fetus to high lipid which may affect its growth and development, increased in utero inflammation and blood lipids exposure may also have adverse effects on the development of the liver, adipose tissue, skeletal muscle, pancreas and the brain, with a possible increased risk to metabolic diseases in later life [344].

The pathophysiological mechanisms by which maternal obesity during pregnancy could set in motion programmed alterations (obesity and cardiometabolic diseases) in the offspring are not clearly elucidated, however, (i) changes in development of some key organs i.e. liver, muscle, adipose tissue, and pancreas [122, 143, 335, 337], (ii) altered appetite [143], (iii) and changes in leptin levels [330] have been suggested to play a role. There is evidence of altered leptin production and regulation, changes in hypothalamic regulation of key genes linked to appetite control and energy balance, altered skeletal muscle metabolism in offspring of obese animal models, in addition to effects on placental structure and function of obese dams [340, 345–347].

Additionally, abnormal function of neurons related to appetite regulation (proopiomelanocortin and Agouti-related peptide neurons) has been observed in offspring of hyperglycemic mothers [346].

Epigenetic modification is also suggested as a mechanism by which early life exposures to an adverse intrauterine environment may mediate their effects in later life. Epigenetic modifications which involve DNA methylation, histone marks, non-coding RNAs and transcription factors,

processes that lead to heritable changes in gene function by modifying DNA chemistry independent of changes in the DNA sequence, could be responsible for tissue specific gene expression during development and differentiation [56, 340, 348, 349]. However, the underlying mechanisms of the effects of maternal obesity, on the epigenetic regulation of genes, are still being elucidated [350].

Studies in primates showed that maternal HFD, a proxy of an obesogenic western diet in humans, through epigenetic modifications could alter fetal chromatin structure [351], whilst in rodents, maternal HFD was linked to decreased adiponectin but increased leptin gene expression in adipose tissue of offspring due to changes in both acetylation and methylation of histone H3K9 within the adiponectin promoter and alterations in methylation of histone H4K20 within the leptin promoter, which affect glucose and lipid metabolism [352]. Additionally, maternal HFD is suggested to modify DNA methylation patterns in organs involved in metabolism such as liver etc. [353, 354]. Such epigenetic modifications in fetal liver, muscle and adipose tissue may increase the risk of obesity and metabolic disorders [350, 355].

Moreover, genes responsible for adipogenesis and nuclear receptors (adipogenic and lipogenic transcription factors, peroxisome proliferator-activated receptor gamma (PPARγ) and proliferator-activated receptor alpha (PPARα) can be increased due to fetal overnutrition through epigenetic modifications [350, 356]. These increases in adipogenic, lipogenic and adipokine gene expression in fetal adipose tissue may contribute to obesity and its associated metabolic disorders in later life [357].

These epigenetic modifications are suggested to be transmissible to successive generations. Evidence from epidemiologic studies in humans and animals show that effects of developmental programming are not limited to the generation directly exposed to the environmental stressor

during intrauterine and early postnatal life, but the effects may be transmitted to subsequent generations, [43, 44, 358, 359] even in the absence of continued environmental stressors, thus perpetuating a cycle of obesity and metabolic disorders [360]. Adverse effects on BP, endothelial function, and insulin sensitivity were passed on to F2 offspring of undernourished pregnant rats [361]. For example, direct exposure to an early environmental stressor such as poor maternal diet in a rodent maternal model can induce insulin resistance, which can later be observed in subsequent unexposed (e.g., adequate maternal diet) generations of offspring, though sometimes with a steady diminished magnitude [362, 363]. However, the effects in successive generations may be different from those observed in the F1 generation who were directly exposed to an insult whilst in utero [329].

Some human studies that lend credence to the transgenerational effects of adverse maternal exposure in early life is the Dutch "hunger winter" study, where the observation was that not only adult female offspring of mothers directly exposed to the famine during pregnancy exhibited dysregulated lipid profiles (cholesterol and triglycerides) in comparison to unexposed siblings [364], but that their offspring had higher neonatal adiposity, and as such maybe more prone to suffer from cardiometabolic diseases than the unexposed controls [365]. Similarly, maternal exposure to famine during the first and second trimester in utero resulted in offspring with lower birthweights than mothers not exposed to famine, similar effects were seen in the offspring of the next generation of some of the mothers not exposed to famine [366].

In animal studies maternal exposure to HFD during pregnancy resulted in increase in body size and reduced insulin sensitivity in both F1 and F2 offspring through both maternal and paternal lineages [367, 368] as well as hepatic steatosis [369].

The underlying mechanisms for the intergenerational transmission of developmentally programmed traits are not fully understood. However, the characteristics of these traits suggest a relatively stable and heritable phenotypically plastic response (i.e. epigenetics), rather than one mediated by changes in DNA sequence (i.e., genomic change). Some probable mechanisms suggested to be the basis for these transgenerational transmissions include: (i) those linked to persistence of the abnormal environmental exposures across generations (generation after generation) during early development (e.g. a suboptimal reproductive tract environment), (ii) a single maternal environmental exposure but is able to produce a multigenerational phenotype (altered maternal adaptations to pregnancy), (iii) and transmission of epigenetic information through the germline [44, 359, 360]. Transgenerational transmission of metabolic disorders could even be up to the F3 generation after a range of altered maternal (F0) environments [360] as shown in figure 7.

However, the potential for reversing some components of the metabolic syndrome induced by developmental programming through nutritional or targeted therapeutic interventions during windows of developmental plasticity has been demonstrated in some animal studies. Dietary intervention before pregnancy (reverting to a normal chow diet one month before mating after being on a HFD or being obese) partially prevented maternal weight gain, increases in glucose, insulin, HOMA, fat, and fat cell size, and it completely prevented leptin increases in offspring [370]. Additionally, maternal exercise intervention among obese female rats one month before breeding and continuing the intervention throughout pregnancy, partially prevented a rise in fat and insulin and completely prevented increases in glucose, HOMA, and fat cell size in offspring [371]. Female rats fed a western diet and supplemented with antioxidants reversed oxidative stress and prevented adiposity and glucose intolerance in the offspring [372]. These interventions are

suggested to have a reversal potential of some programmed metabolic disorders [373]. The various interventions to improve outcomes may operate via different mechanisms and combined approaches may produce better effects for the mother and offspring [371]. Though these interventions are promising, there is the difficulty of extrapolating interventions from small animal models to human settings. However, it offers potential for enhancing the understanding of critical determinants and mechanisms involved in human obesity and metabolic disorders [374].

In human studies maternal pre-pregnancy BMI is strongly associated with adverse offspring metabolic outcomes [127]. Hence is it suggested that interventions instituted during the preconception period which prevents obesity before conception may yield better results [130]. Pre-pregnancy lifestyle modifications that improve maternal diet, exercise, weight reduction, which are modifiable factors are interventions of choice [373]. However, these behavioral modifications are very individual based and at times very complex to achieve in human settings [370].

To increase the impact of such interventions they should be evidence based so as to convince women about the adverse effects of maternal obesity to both mother and offspring, and the benefits that accrue to both from obtaining and maintaining an adequate BMI and dietary intake before and during pregnancy [370]. On the other hand, caution should be exercised since poor maternal nutrition induced by sudden and excessive restriction of maternal diet as an intervention also programs the offspring for adverse outcomes due to inadequate nutrient for proper fetal growth [373]. Thus the benefits of interventions (dietary, exercise etc.) especially during pregnancy should be weighed against the overall health outcome for mother and child.

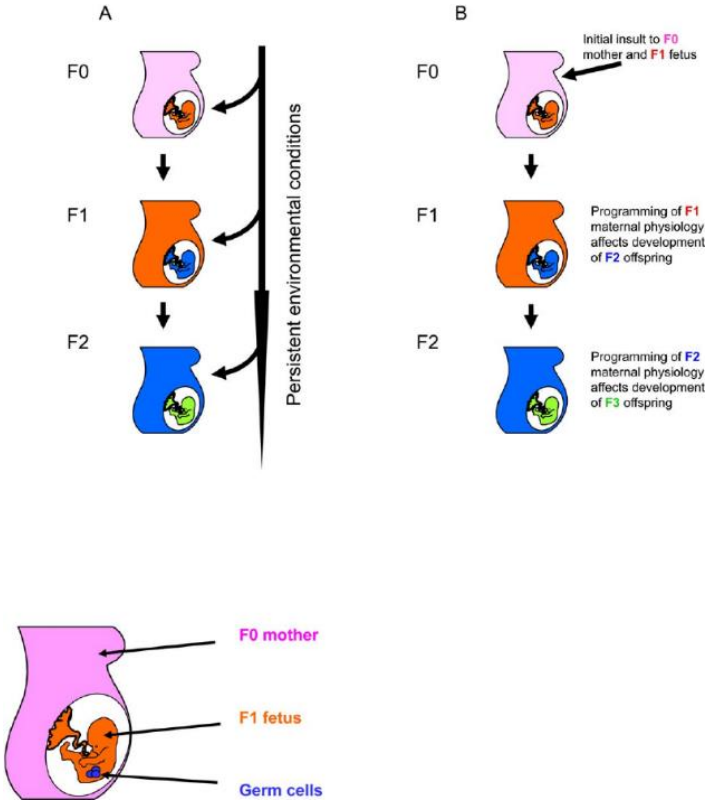


Figure 7 *Mechanisms for intergenerational transmission of programming effects*

(A) Persistence of an adverse external environment can result in the reproduction of the phenotype in multiple generations.

(B) The induction of programmed effects in the F1 offspring following in utero exposure (e.g., programmed alterations in maternal physiology or size) leads to programmed effects on the developing F2 fetus and subsequent generations. An environmental insult during pregnancy to a mother (F0 generation) might not just affect the developing fetus (F1 generation) but also the germ cells which will later form the F2 generation [44, 359].

6.1.3 Research Aim 3

The third aim was to assess and judge the evidence of an association of pregnancy weight/body mass index or gestational weight gain with offspring's blood pressure through a systematic review of the literature. The systematic review showed that only “limited non-conclusive” evidence exist for an association between maternal pregnancy weight /BMI or GWG with offspring's later BP.

To the best of our knowledge, this is the first systematic review providing a summary of the evidence on the association between maternal pregnancy weight/BMI or GWG with offspring’s BP in later life. For maternal pregnancy weight exposure, only 2 studies could be included in this review. Whilst one study reported no significant association of maternal pregnancy weight with offspring’s BP in later life [375], the other study reported a relatively small significant association of maternal pregnancy weight with offspring SBP and DBP [376].

For the GWG exposure, fourteen studies were included in this systematic review. Three studies rated as having “fair quality” indicated a significant positive association of GWG with offspring SBP [136, 377, 378] but these studies had limitations in statistical analysis. Similarly, three “fair quality” rated studies reported a significant association of GWG with offspring DBP [129, 377, 379] however, these studies showed varying directions of effect estimates. In the remaining studies no significant associations with offspring’s BP were reported.

Due to the few studies and methodological issues associated with them no concrete conclusion could be drawn on the association between maternal pregnancy weight or GWG with offspring BP, thus the evidence grade limited-non conclusive was assigned for the association. Thus there is the need for more good-quality studies for a conclusive verdict on this association.

6.2 Methodological Considerations

6.2.1 Study characteristics

The DONALD study is a prospective study design which involves frequent repeated acquisition of various data (see chapter 3) from childhood to adulthood. Data from prospective studies provide more accurate data between exposures and outcomes, and also deals with the issue of temporality an important criteria of causality which indicates that exposure has to precede disease onset for causality to be suggested, thus prospective studies could suggest casual associations compared to data from cross sectional studies [380, 381]. Notwithstanding, as is common with all observational studies, prospective studies are susceptible to attrition bias, miscalculation and measurement error and confounding, limiting its ability for causality determination [380]. Since the DONALD study is a longitudinal study it covers childhood and adulthood periods, hence it runs across probable critical periods of development that could be relevant for the programming of long term disease. The DONALD study is characterized by high frequent measurement during the critical period of early life (childhood) i.e. four visits in the first year of life and two in the second year at the study center. Thus the DONALD study is appropriate for the appraisal of the first two aims of this thesis. The availability of data on early life factors with some retrieved from the "Mutterpass," (a standard document given to all pregnant women in Germany) and data collection upon the child's admission to the study, measurement of CIMT as well as fasting blood parameters in early adulthood permitted the analysis of (early life factors and their relevance to intima-media thickness of the common carotid artery in early adulthood (Aim 1, OA1, Appendix 1)) as well as early life factors and their relevance for markers of cardiometabolic risk in early adulthood (Aim 2, OA2, Appendix 2)).

However, due to the detailed measurements undertaken in the DONALD study (anthropometry, 24-hours urine sampling, blood sampling, 3-days weighed dietary records) annually well over a period of about 20 years, a convenient sample that is not representative of the general population has been recruited which maybe a source of bias. When compared with the general population, parents of participants in the DONALD study are highly educated, are of a higher socioeconomic status and also seem to have a high interest in nutrition and health related issues [308]. Therefore, there is some level of homogeneity in sample participants, thus children with extreme dietary behaviors, anthropometry etc. might not have been included in the study, which can probably reduce the statistical power for detecting associations. Nonetheless, the relative homogeneity could reduce confounding due to unmeasured behavioral and lifestyle factors.

Early life factors (maternal characteristics) such as maternal age at child birth [93], early pregnancy BMI and GWG [121] among the DONALD study participants do not seem to differ from that reported in the sub region. With regards to anthropometry, participants in the DONALD study in early childhood have similar or slightly higher BMI compared to the German reference population [382]. The variation of the DONALD study participants from the general population is of minor significance since exposure outcome relationships in the DONALD study are assessed and the internal validity of the results are not likely to be affected by the above mentioned factors [308].

6.3 Lifestyle and parental characteristics

Assessment in the DONALD study also include measurement of parental anthropometry, personal interviews about lifestyle and family characteristics. These information aid in the assessment of various potential confounders in early life, socioeconomic status, children's behavior and educational status [383]. A limitation of the DONALD study is the unavailability of familial

dietary and other lifestyle (physical activity) habits and especially this data from the mother during and after pregnancy can affect offspring programming.

6.4 Outcome Measurements

The outcome variables considered in the first two studies of this thesis have some limitations such as (i) measurement of risk markers of cardiometabolic diseases instead of overt cardiometabolic diseases (CVD, T2D) and measurement of risk markers only once in early adulthood. Measurement of risk markers rather than hard disease end points was appropriate, as participants in the DONALD study have not been followed up long enough for the manifestation of overt cardiometabolic diseases. Though the presence of risk factors may not always be indicative of later manifestation of overt cardiometabolic diseases, the risk factors considered in this thesis are recognized as predictors of cardiometabolic diseases due to the persistent associations that have been reported between them [191, 210, 223, 226, 241].

Also evidence exist on the involvement of risk factors in the mechanistic pathophysiology of cardiometabolic diseases (see 2.5). Additionally, risk factors could probably help in unravelling the potential mechanisms by which early factors contribute to later cardiometabolic diseases [384, 385].

(ii) Measurement of risk factors only once during adulthood could induce some level of measurement error since assessment of some blood parameters (lipids, glucose and insulin) do vary under different conditions [386, 387] and also diagnosis of T2D is recommended to be based on repeated test [388].

Intima media thickness

For the measuring range with minimum values of measuring IMT less than 1 mm (0.3 mm), two measuring principles could be considered as sufficiently precise. Measurement using speckle reduction imaging has been shown to provide a better image quality, accurate measurement results and better representation (display) at an IMT of 0.3 mm (value of the thinnest measured IMT), but, due to its high cost this was not used in the DONALD study. However, exact measurements could also be achieved with the fundamental ultrasound, but, this method has the disadvantage of poor representation [389]. The DP 3300 (Mindray), which uses the fundamental ultrasonic mode, was used in the DONALD study. The representation was evaluated by Dr. Wunsch from Children's Hospital Datteln as acceptable [317].

Insulin sensitivity

Insulin sensitivity was assessed in the DONALD study by the use of HOMA of insulin sensitivity (HOMA 2-%S), which is the inverse of HOMA-IR [217, 318]. Though the hyperinsulinemic euglycemic clamp is the gold standard for direct assessment of insulin sensitivity, it is not readily used due to its laborious, high cost and time consuming nature especially in large scale studies. Apart from *QUICKI, HOMA* is a validated simple surrogate index widely used in large scale epidemiological studies and it also correlates well with the glycemic clamp, the reference method hence its usage in the DONALD study [318, 390].

Hepatic steatosis

In the DONALD hepatic steatosis was evaluated using the following indices: hepatic steatosis index (HSI) and fatty liver index (FLI) (see calculation in OA2 in (Appendix 2). These indices offer modest efficacy in the diagnosis of steatosis, however, they are recommended for use in large scale epidemiological studies when the more reliable imaging options (MRI) are not feasible due to their high cost and the difficulty associated with their use in large studies. In the DONALD study imaging techniques were not used due to the reasons enumerated above and additionally, the goal in the second study of this thesis OA2 (Appendix 2) was not to diagnose hepatic steatosis in the relatively young adult's participants who may present mild steatosis, but to use markers of hepatic steatosis as risk factors of cardiometabolic disease.

Evidence suggest that liver enzymes could be risk factors of CVD [391, 392] and metabolic syndrome [393] however, there are disagreements in the association between specific liver enzymes and CVD [391]. Hepatic steatosis indices, HSI and FLI have also been linked to insulin resistance [228], a key component in metabolic dysfunction [226, 241, 242]. Taken together, liver enzyme activity levels and hepatic steatosis indices maybe useful risk factors of cardiometabolic diseases.

Chronic low grade inflammation

An aggregate score was calculated using various pro-inflammatory markers and the anti-inflammatory hormone adiponectin to assess general inflammatory state [272] in the DONALD study. Though portraying such an aggregate state of inflammation is recommended, its application

is difficult as the characteristics and roles of a number of inflammatory markers are not fully known and sometimes conflicting.

There is no agreement on which markers adequately indicate chronic low-grade inflammation [276, 394] or distinction between acute and chronic inflammation or between the various phases of inflammatory responses [395]. Thus a range of cytokines and chemokines (TNF, IL-1, IL-6, IL-8), adipokines (adiponectin) and acute-phase proteins (CRP) are often measured [272, 394, 395]. Notwithstanding, other issues regarding the use of these markers as determinants of low-grade inflammation exist i.e. they are non-specific acute-phase response and pro-inflammatory response markers and there can be disparities in the measurements due to modifying factors (age, diet, body fat, physical fitness and genetics) that affect the concentration of an inflammatory marker at a given time [272].

Taken together, though the inflammatory markers used to generate the pro-inflammatory score in OA2 (Appendix 2) generally show a state of inflammation, the issues raised above indicate the complication of inflammatory processes. Thus even though, a pro-inflammatory score gives an indication of general inflammatory status, it could be a very simple indicator and should be cautiously interpreted. Thus further research is needed on specific biomarkers that are related to cardiometabolic diseases, a more reliable score of a state of general inflammation and effects of modifying factors on these biomarkers for a proper appreciation of the role of biomarkers in cardiometabolic diseases to be made.

6.5 Consideration of potential confounding factors

In the DONALD study information on parental anthropometry, lifestyle and family characteristics etc. were assessed. From these measures various potential confounders in relation to socioeconomic status, children's behavior and educational status were obtained during early life and other periods. However, there is the possibility of measurement error associated with these confounders resulting in residual confounding, such that even after adjustment for these inadequately measured confounders, their confounding effects still persists due to the error in the measurement [396]. As with all observational studies it is impossible to measure all confounding factors and to adjust for them appropriately, thus the issue of unmeasured confounders exists in such studies [396]. Thus residual confounding and unmeasured confounders can affect study findings and conclusions.

A confounder is a variable associated with both the exposure and outcome of interest [397]. A confounder variable should possess these characteristics, (i) be casually related to the outcome of interest, (ii) be non-casually associated with the exposure of interest (iii) it should not lie on the exposure outcome casual pathway [398]. A confounder can falsely obscure i.e. underestimate (negative confounding) or accentuate i.e. overestimate (positive confounding) the relationship between an exposure and the outcome [399]. Thus a confounded research finding can result in an inaccurate conclusion being drawn about the effect of the exposure on the outcome [400], a phenomenon more likely in nonrandomized observational studies [397]. Thus to minimize the effect of confounding in observational studies adequate attention should be given to measurement of these variables at study inception and proper adjustment during analysis.

In study I and II both prospective studies, a hierarchical approach premised on the conceptual framework proposed by Victora et al. [401] was used for the selection of relevant confounders.

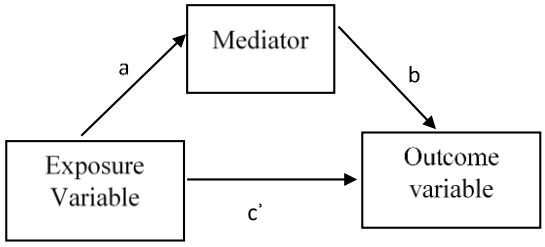
The purpose of this approach is to adequately portray the complex hierarchical interrelationships of multivariate models [401]. The hierarchical conceptual framework was applied by considering covariates individually for potential confounding in a hierarchical manner [401]. Covariates which substantially modified the predictor–outcome associations by (≥10%) or significantly predicted the outcome were included in a hierarchical manner. This resulted in the selection of the following potential confounders in a hierarchical manner (i) early life factors: birth weight, birth weight-for-gestational-age, gestational age, maternal and paternal age at child birth, GWG, first born status, and birth year regressed on age. (ii) socioeconomic factors and parental health: presence of an overweight parent, parental school education, smokers in the household.

In study I sensitivity analyses were conducted in subsamples who had information on either participation in sport or estimated energy expenditure during participation in sports in early adulthood, this was to account for potential confounding due to adulthood physical activity levels. Conditional models were also constructed by adding adulthood waist circumference, BMI, SBP or DBP to the models to investigate whether the observed associations were partly attributable to body composition or BP in adulthood.

In study II the effect of adulthood waist circumference as mediator was tested in a conditional model followed by a casual mediation analysis when the association was nullified in the conditional model. In contrast to a confounder, a mediator is an intermediary variable that lies on the casual pathway between the exposure and the outcome [397], hence it can be useful in explaining the mechanisms underlying the association between the exposure and the outcome. However, caution should be exercised when adjusting for mediators and adjustment for it should be in a separate step as was done in the conditional models of study I and II.

In mediation the association between the exposure variable and the outcome variable is decomposed into two causal paths. One path links the exposure to the outcome directly (known as the direct effect), whilst the other path links the exposure to the outcome through a mediator (known as the indirect effect) as shown in figure 8 below. An indirect or mediated effect suggests that the exposure variable causes the mediator, which, in turn causes the outcome variable [399].

Nullification of the association between maternal early pregnancy BMI and GWG with markers of cardiometabolic risk after inclusion of adulthood waist circumference in the conditional model, and subsequent confirmation by the mediation analysis shows that adulthood waist circumference is a likely mediator between these maternal factors and offspring cardiometabolic risk in adulthood in the sample population studied. For further details on confounders and the level of mediation refer to OA1 and OA2 respectively.



Indirect effect = ab Direct effect = c'

Total effect = ab + c'

Figure 8 *Definition of a mediation effect [399]*

6.6 Public health relevance

From a public health perspective, it is crucial to determine the role of early life factors to the risk of later cardiometabolic diseases (CVD, T2D and BP) in later life. However, less attention seem to be paid to the role of developmental plasticity ("developmental programming") and alterations in phenotypic outcomes, as a result of adverse events occurring in the early life period, in the surge in obesity and cardiometabolic diseases [44, 360]. This has been attributed to the fact that concurrent risk factors are seen to be more influential, the difficulty to identify or attribute risk to distant early life factors, also direct assessment of the probable effect of development on long term disease is arduous, requiring unbiased cohorts that have both perinatal data and health outcomes recorded into middle age. These result in the use of surrogate parameters of disease risk (SBP, fasting insulin/glucose levels etc.) in most studies relating to early life factors [41].

The results of this thesis and other existing evidence show that the causes of cardiometabolic diseases may not be attributable to genetics, lifestyle or events in adulthood only [44] and indicate the important role of early life factors to later disease risk. Cardiometabolic diseases especially T2D, CVD and hypertension constitute a major cause of morbidity and mortality globally and an important component of the cost of medical care with immense costs on health systems [184, 185].

Currently, the number of women giving birth at an advance age is on the rise and it is likely this trend will continue, similarly, obesity among women in the reproductive age group is increasing in parallel to the obesity seen in the general population, thus the contribution of these maternal factors to offspring cardiometabolic risk could have major public health implications for individual and population health. This could worsen the already overstretched health care system, cost, adequate health care provision etc. Offspring developmentally programmed maybe predisposed to

chronic diseases at an early age and may have to depend on the health care system as well as have a lower health related quality of life for a prolonged time period.

Issues such as the relative importance of early life factors influencing intervention strategies during early life (human development) compared to interventions initiated in adult life have been raised. Suggestions have been made that if high risk of CVD, T2D can be attributed to fetal adaptations made in early life due to inadequate maternal nutrition during pregnancy, then improving maternal nutrition during pregnancy to improve fetal growth and health outcomes, using primary prevention interventions should help reverse the trend [44].

However, though the possibility exists for intervention programs during pregnancy and early postnatal periods to help in the prevention of cardiometabolic diseases, evidence from some studies on supplementation of maternal diet in pregnancy with targeted macro and micronutrients to improve fetal growth as well as maternal and child health outcomes have been mixed [402–404]. Other studies have also underscored the greater impact and success of correcting maternal and infant nutrition using nutrition specific and sensitive interventions than interventions that are directed at adulthood especially in the prevention of chronic diseases.

Interventions such as promotion of healthy diet and adequate weight during preconception and pregnancy, physical activity, promotion of breastfeeding have been proven to be effective [405]. Early identification of offspring that have been developmentally programmed [56] by use of probably markers of early gene-environment interactions will enhance acquisition of clinical data [41] so that timely interventions can be prescribed to such individuals before onset of adverse outcomes may be helpful.

Consensus seem to be around the promotion of the health and nutrition of females in the reproductive age group as an avenue for the prevention of chronic disease in future generations

[41]. Applying integrated strategies in the use of these effective interventions as well as recognition of the role of the early environment in cardiometabolic disease etiology and the need to optimize the early environment should be scaled up as routine public health strategies.

7 CONCLUSION AND PERSPECTIVES

In conclusion the findings of this thesis adds to scientific knowledge by showing that early life factors are relevant for cardiometabolic risk in young adulthood. Firstly, advanced maternal age at child birth is associated with an increased IMT, a marker of subclinical atherosclerosis among female offspring in young adulthood but not in males. Due to the possibility of this trend of giving birth at an advanced age to continue, education on its adverse effects to offspring health should be intensified among women during preconception. Secondly, a higher maternal early pregnancy BMI was associated with a higher FLI, HSI, pro-inflammatory score and a lower HOMA2-S%, markers of cardiometabolic diseases. A higher GWG was also associated with a higher FLI, HSI, pro-inflammatory score but with a lower HOMA2-S% among females, but not males. Full breastfeeding was associated with a lower adult FLI.

Maternal obesity among women in the reproductive group parallels the increase seen in the general population and it is likely to continue, hence interventions especially before pregnancy maybe more beneficial. Promotion of adequate weight gain, diet and physical activity need to be intensified. These results agree with existing evidence that indicates that environmental factors acting during preconception, fetal, and early postnatal life are relevant for offspring cardiometabolic health outcomes in adulthood. Lastly, a systematic review of the literature showed that only “limited non-conclusive” evidence exist for an association between maternal pregnancy weight/BMI or GWG with offspring's later BP.

However, further questions remain that need to be addressed in future studies. Most studies on advanced maternal age effects on offspring outcomes have been conducted during neonatal and early childhood periods. The long term effects have not been very much studied, additionally, it is not known whether the adverse effects associated with advanced maternal age are casual or not,

thus experimental research in animal models with long gestation periods and prospective large scale cohort studies are needed to elucidate mechanistic pathways that may serve as targets for intervention to improve maternal and offspring short and long term health.

Though early pregnancy BMI has been associated with offspring obesity and adverse metabolic outcomes, it is not certain if this can be attributed to maternal fat accumulation in early pregnancy, as maternal body composition is not measured during different periods in pregnancy. Further studies in this area to elucidate the mechanisms behind this association maybe helpful to profile solutions. Another area of research could be a longer follow up of DONALD study participants to assess the relevance of these early life factors to overt cardiometabolic disease incidence in late adulthood.

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RESEARCH ARTICLE

Early life factors and their relevance to intima-media thickness of the common carotid artery in early adulthood

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Abstract

Background

Early life factors may predispose an offspring to cardiovascular disease in later life; relevance of these associations may extend to "healthy" people in Western populations. We examined the prospective associations between early life factors and adult carotid intima-media thickness (IMT), a surrogate marker of atherosclerosis, in a healthy German population.

Methods

We studied term participants (n = 265) of the DONALD Study, with bilateral sonographic measurements of IMT (4–8 measurements on both left and right carotid artery) at age 18–40 years and prospectively collected data on early life factors (maternal and paternal age at child birth, birth weight, gestational weight gain and full breastfeeding (>17weeks). Mean IMT values were averaged from mean values of both sides. Associations between early life factors and adult IMT were analyzed using multivariable linear regression models with adjustment for potential confounders.

Results

Adult mean IMT was 0.56mm, SD 0.03, (range: 0.41 mm-0.78 mm). Maternal age at child birth was of relevance for adult IMT, which was sex specific: Advanced maternal age at child birth was associated with an increased adult IMT among female offspring only (β 0.03, SE 0.009 mm/decade, P = 0.003), this was not affected by adult waist circumference, BMI or blood pressure. Other early life factors were not relevant for IMT levels in males and females.

participant information. This has been imposed by the data protection officer Dr. Jörg Hartmann of the University of Bonn as an official ethical restriction. Requests for data access may be sent to the local data protection coordinator Heinz Rinke, email: rinke@uni-bonn.de at the DONALD Study Dortmund of the University of Bonn.

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Conclusion

This study suggests that advanced maternal age at child birth is of prospective relevance for adult IMT levels in a healthy German population and this association may be of adverse relevance for females only.

1. Introduction

Interest in research on early life exposures as possible determinants of disease in later life greatly increased since the discovery of the Barker hypothesis that cardiovascular disease (CVD) has its origins in early life [1]. The atherosclerotic process begins early in life, i.e. many years before cardiovascular complications develop later in life [2]. Fatty streaks which are initial lesions of atherosclerosis have been observed in arteries of children less than a year old which increases with age [3]. Also increases in carotid intima media thickness (IMT) and endothelial dysfunction have been suggested as preliminary indications of atherosclerotic plaque development [4, 5] Similarly, alterations in the IMT have been indicated as a marker of subclinical atherosclerosis [6–8] with a high IMT shown to correlate with CV risk factors [6, 9, 10], and to predict CVD [10, 11].

CVD has been associated with low birthweight mostly in populations exposed to maternal undernutrition during gestation [12, 13]. Though the actual pathophysiological mechanisms underlying the association is not fully clarified, it has been associated with the theory of developmental plasticity, i.e. that the developing offspring's system is plastic and sensitive to the nutritional, hormonal and the metabolic milieu in utero, resulting in various physiological or morphological states due to different conditions during development [14, 15]. A decrease in cell numbers in organs due to changes in the intrauterine environment could also account for CVD in adulthood [15, 16].

It is hence plausible that a range of exposures in early life may be associated with later CVD and thus also with adult IMT. The relevance of these associations may also extend to "healthy" people in Western populations due to the presence of such exposures in this population. Specifically, four groups of early life factors remain to be addressed in these populations: (i) Birthweight, which reflects fetal in utero environment, a potential predictor for which evidence is now also emerging in relation to IMT [17–19]. (ii) gestational weight gain may give rise to an obesogenic intrauterine environment specifically among overweight or obese mothers [20], however, there is no data on its relevance for later IMT; (iii) maternal age, since women are now giving birth at a more diverse age range, with a tendency towards older age in Western countries; (iv) early postnatal nutrition, specifically breastfeeding, which has been related to IMT, however producing controversial results [21–23].

To address these four sets of hypotheses we examined the prospective relevance of (i) indicators of intrauterine growth such as birth weight, birth weight-for-gestational-age (i.e. adequate, small and large for gestational age), (ii) pregnancy duration and gestational weight gain, (iii) parental age and (iv) full breastfeeding for adult IMT as a surrogate of CVD among healthy term-born German participants.

2. Methods

2.1. Study population

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD Study), is a continual, open cohort study undertaken in Dortmund, Germany. Since its commencement in 1985, elaborate records on diet, growth, development, and metabolism has been gathered from over 1,700 children between infancy and adulthood. About 35–40 infants are newly enrolled each year while initial examination commences at the age of 3–6 months, afterwards each child returns for 2–3 more visits in the first year, 2 in the second year and then once yearly until adulthood.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Bonn. The data protection officer is Dr. Jörg Hartmann and requests for data access may be sent to the local data protection coordinator Heinz Rinke, email: rinke@uni-bonn.de at the University of Bonn. All examinations are performed with written consent of parent and adult participant [24–28].

The children who were initially recruited for the DONALD Study differed considerably in age and prospectively collected data on breastfeeding was not always available. Follow-up into adulthood was not planned at the inception of the study. Since 2004 participants are invited to return for further visits at ages 18, 21, 25, 30, 35 etc. However, not all participants followed this invitation. In addition, due to the open cohort design, many DONALD participants had not yet reached young adulthood by the time of this analysis. IMT measurements are offered to adolescents and adult participants since 2008. In this analysis only IMT measurements in adulthood ($\geq$18 years of age) are used. Mean follow-up until IMT measurement is equivalent to the mean age at IMT-measurement. 607 IMT measurements were available with two persons excluded due to the presence of plaques and stenosis in their measurement. 58 others did not have a minimum of four measurements each on the right and left common carotid artery to be included in the analysis whilst 178 persons were not considered because the images did not fulfill the quality control criteria (see below). Among the remaining 369 persons with acceptable IMT measurements, data from 349 persons were considered who were born term (37–42 weeks of gestation) singletons with a birthweight > = 2500g. A further 84 persons were excluded because they did not fulfill the following minimum requirements: Parents had to have provided information on maternal age at birth, only available for 262, paternal age at birth, only available for 256, birth year, birth weight, gestational weight gain only available for 258 and gestational duration. Hence the sample considered for this analysis includes 265 participants with information on IMT collected between 2009 and 2014. See Fig 1, for the sample size of early life factors and for relevant covariates see Table 1.

2.2. Early life exposures

Child birth and maternal characteristics were extracted from the "Mutterpass," a standard document given to all pregnant women in Germany. Gestational duration is calculated according to the mother's last menstrual period.

Maternal weight at first visit at the gynecologist during pregnancy and at the end of pregnancy weight were abstracted from the "Mutterpass," and from these the gestational weight gain was computed.

Birth weight and birth length were recorded at birth. Birth weight-for-gestational-age is defined according to the German sex-specific birth weight and length-for-gestational-age

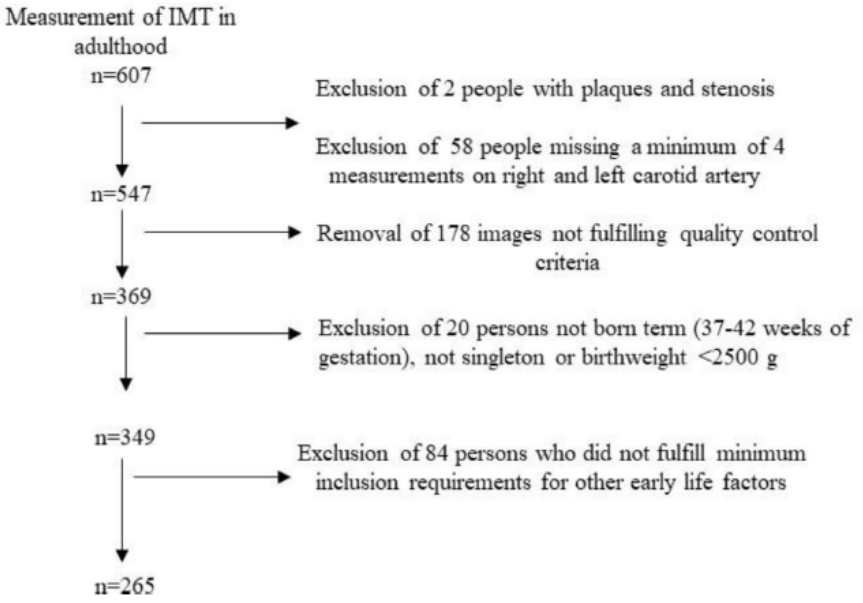

Fig 1. Participant flowchart diagram for IMT and early life factors.

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curves [29]. Small-for-gestational-age (SGA) is defined as birth weight and length <10th percentile, and large-for-gestational-age (LGA) is defined as birth weight and length >90th percentile. All other infants were classified as appropriate-for gestational age (AGA).

Maternal and paternal age at the time of child birth is assessed at first visit.

Breastfeeding data was assessed upon the child's admission to the study. During first visit either at 3 or 6 months the study pediatrician and/or dietitian enquired from the mothers the duration (in weeks) the infant had been fully breastfed (not given solid foods and no liquids daily except breast milk, tea, or water). If the child is still being fully breastfed, the length of breastfeeding is assessed at successive visits at ages 6, 9, 12 and 18 months until commencement of complementary feeding. The duration of feeding formula or solid foods is also assessed during the visits. A coherent check is conducted on all breast feeding information collected such as the recording of breast milk in 3-day dietary records and information acquired by the dietitians before analysis to minimize errors. From this information the duration (in weeks) of full breastfeeding is calculated [30].

2.3. Early adulthood outcome variable: Intima media thickness

Vascular conditions of the left and right common carotid artery (CA) were studied ultrasonographically using high-resolution technology. The Mindray DP3300, tragbares portable digital system was used for this study. The participants were measured in a supine position, head slightly to the right or left after having rested for 10 min. The start point of the measurement was at the beginning of the bifurcation at the left edge of the image with a horizontal vessel course. IMT was measured at 4 points 1 cm before the carotid bifurcation. Images were always taken in the systole. Two images were first taken each on the right and left CA on the

Table 1. Characteristics of participants in early life and young adulthood.

Variables		Males		Females
	N		N	
Measurements in young adulthood				
Average IMT (mm)[1]	120	0.57 (0.06)	145	0.55 (0.05)
Age at IMT measurement (yrs)	120	23.3 (5.7)	145	23.9 (5.1)
Waist circumference (cm)	120	83.6 (8.3)	145	75.3 (8.3)
BMI at IMT measurement (kg/m^2)	104	24.2 (3.3)	142	23 (4.2)
Systolic blood pressure (mm Hg)	103	121.3 (10.7)	140	110.5 (9.8)
Diastolic blood pressure (mm Hg)	103	75.9 (9.4)	140	72.8 (8.1)
Participation in sport (yes/no) [2]	91	88 (96.7%)	123	118 (96.0%)
Energy expenditure (kcal) [3]	55	641 (263)	68	363 (260)
Early life factors				
Maternal age at child birth (yrs)	120	30.6 (4.2)	142	30.3 (4.2)
Paternal age at child birth (yrs)	119	33.3 (5.1)	137	33.2 (5.0)
Pregnancy duration (wks)	120	40 (40, 41)	145	40 (39, 41)
Gestational weight gain (kg)	117	12.9 (4.0)	141	12.9 (3.6)
High gestational weight gain n (%)[4]	117	20 (17.1%)	141	20 (14.2%)
Birthweight (g)	120	3583 (443)	145	3411 (437)
Birth weight < 3000 (g)		5 (4.2%)		28 (19.3%)
Birth weight ≥ 3000 to ≤ 4000 (g)		93 (77.5%)		104 (71.7%)
Birth weight > 4000 (g)		22 (18.3%)		13 (9%)
Birth weight by gestation age[5]	120		145	
SGA		12 (10%)		16 (11.0%)
AGA		92 (76.7%)		111 (76.6%)
LGA		16 (13.3%)		18 (12.4%)
Full breastfeeding	104		130	
Never (0–2 weeks)		29 (27.8%)		37 (28.4%)
Short duration (3–17 weeks)		40 (38.5%)		49 (37.7%)
Long duration (>17 weeks)		35 (33.7%)		44 (33.9%)
Additional potential confounders				
Birth year	120	1989 (1985, 1992)	145	1988 (1985, 1991)
Firstborn status (yes/no)	104	58 (55.8%)	129	74 (57.4%)
Maternal overweight (yes/no)[6]	116	38 (32.8%)	141	41 (29.1%)
High paternal educational status (yes/no)[7]	119	70 (58.8%)	136	73 (53.7%)
Smokers in the household (yes/no)	106	37 (34.9%)	134	55 (41.0%)

Values are presented as means (SD) medians (IQR) or frequencies (percentage).

AGA: appropriate for gestational age, LGA: large for gestational age, SGA: small for gestational age.

AGA, LGA and SGA defined according to German sex-specific birth weight and length-for-gestational-age curves.

[1]Average IMT: mean of intima media thickness (IMT).

[2]Participation in organized or unorganized sport: (yes/no).

[3]Estimated energy expenditure during participation in organized or unorganized sport.

[4]High gestational weight gain: yes (>16kg), no (≤16kg).

[5] Birth weight by gestation age

[6]Maternal overweight: yes (≥ 25kg), no (<25kg).

[7]High educational status: yes (≥12yrs of school attendance), no (<12yrs of school attendance).

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participants and the images were frozen. Subsequently, measurements were taken at four measurement points on each image.

Quality control was carried out on all images and only images that met the criteria were used for analysis. The criteria for IMT measurement quality control are based on 1) clear representation of the Intima-Media-Complex of the "far wall "shown as echo-rich/echo-poor uninterrupted line, 2) echo-free imaging of the vessel lumen and clear separation of the intima from the lumen, 3) localization of the image section at the beginning of the bifurcation and 4) horizontal course of the vessel within the image. Individual measurement points were discarded, if they were set incorrectly (i.e. the point for measurement was set below or above the visible IMT lines). Mean IMT values were firstly averaged for the right and left sides (i.e. 4–8 measurements), and then an overall mean was calculated from the two averages.

All measurements were performed by the study physicians. Each year, a quality control of IMT measurement by the physicians is carried out. Coefficient of variation (CV) which considers the precision of the measurements within and between the physicians is computed and from 2009 to 2015, the values are $CV_{intra} = 6.95$ and $CV_{inter} = 3.70$. An acceptable precision is given at a value less than 10.

2.4. Potential covariates

Anthropometry of study participants were taken at each visit using standard protocol by trained nurses. The participants are dressed in only underwear and are barefooted. Recumbent length of children until 2 years of age is measured to the nearest 0.1 cm using a Harpenden (UK) stadiometer, whilst standing height is measured in children aged older than 2 years to the nearest 0.1 cm with a digital stadiometer (Harpenden Ltd., Crymych, UK). Body weight is measured to the nearest 100 g using an electronic scale (Seca 753E; Seca Weighing and Measuring Systems, Hamburg, Germany). Waist circumference is measured at the midpoint between the lower rib and iliac crest to the nearest 0.1 cm. The trained nurses who perform the measurements undergo quality control, conducted with healthy young adult volunteers [28]. This same measurement procedure is used to measure anthropometry of parents at regular intervals.

The number of smokers in the household was enquired and from this smoking exposure was assessed. The years of schooling was also enquired and from this a proxy of parental socio-economic status was created. A high educational status is defined as ($\geq$ 12 years of schooling).

2.5. Statistical analysis

All statistical analysis was conducted using SAS 9.4. Prospective association between early life factors and IMT during young adulthood were analyzed using multivariable linear regression models. IMT was adjusted for age and sex using the residual method.

To evaluate whether sex modifies the association between early life factors and IMT, an interaction analysis was carried out and if a significant sex difference existed, analysis was carried out separately for men and women. Interaction analysis indicated sex interactions for maternal age at child birth and breastfeeding ($P_{interaction} = 0.03$ to 0.09).

Initial regression models (A) included IMT as the dependent continuous variable and individual inclusion of an early life predictor as the independent variable, adjusted for age at IMT measurement, sex and the physician measuring IMT.

Next, multivariable adjusted models (B) were constructed considering covariates individually for potential confounding in the models in a hierarchical manner [31]. Covariates which substantially modified the predictor–outcome associations by ($\geq$10%) or significantly predicted the outcome were included in the final multivariable adjusted models.

These early life factors were considered as mutual potential covariates in this model (1) early life factors: birth weight (g) considered as both a continuous and categorical variable (i.e.,

<3000g, ≥3000g to ≤4000g and >4000g), birth weight-for-gestational-age as a three level categorical variables (AGA, SGA, LGA), gestational age (weeks), maternal and paternal age at child birth (years) were considered as continuous variables, gestational weight gain (kg) as a continuous and categorical variable (i.e.≤16kg and >16kg), breastfeeding for >2 weeks (Yes/No and >16 weeks (Yes/No), first born status (Yes/No) and birth year regressed on age at IMT measurement as a continuous variable. (2) Socioeconomic factors: paternal school education ≥12 years (Yes/No), presence of an overweight parent BMI≥25 kg/m^2, (Yes/No), smokers in the household (Yes/No). Sensitivity analyses were conducted in subsamples who had provided either information on participation in sport (Yes/No) (n = 123) or on estimated energy expenditure during participation in sports (n = 68) in early adulthood, so as to account for potential confounding arising from adult physical activity levels.

Finally, four sets of conditional models were constructed adding adult waist circumference, adult BMI or adult systolic or diastolic blood pressure to the models, so as to investigate whether observed associations were partly attributable to these variables in adulthood.

Results from regression analysis are presented as adjusted least-square means (95% confidence interval (CI) by tertiles of the respective predictor while P-value is obtained from models using the predictors as continuous and categorical variables. Significance was determined at a p-value of 0.05.

3. Results

Early life and young adulthood characteristics of the participants in this analysis are presented in Table 1 according to sex. The minimum and maximum average IMT ranged from 0.41mm to 0.78mm. The mean age at IMT measurement was 23 years in males and 24 years in females. Participants lost to follow up differed slightly from those included in this analysis: mothers of males and females were younger i.e. 29.4 and 29.8 years, children were born earlier i.e. in 1987 and fewer offspring were fully breastfed for a long duration, i.e. 31% and 27% among male and female offspring (for details see S1 Table).

Paternal age at birth was not related to adult IMT (S2 Table). In multivariable analysis, increased maternal age at child birth was associated with an increased IMT among female offspring during young adulthood (P = 0.003, Fig 2), but not in males (P = 0.2, Fig 3) (S2 Table). These associations were not affected by adjusting for paternal age at birth. In addition, inclusion of adult waist circumference, BMI, systolic or diastolic blood pressure in separate conditional models did not affect the relationships (S3 Table).

Sensitivity analyses in the subsample of females, for whom data on participation in sport (n = 123) or data on estimated energy expenditure during participation in sport (n = 68) was available, additional consideration of these variables did not affect the association of maternal age at birth with IMT (S4 Table).

In multivariable analysis on the relevance of full breastfeeding for adult IMT, there was no association in females (P = 0.1, model A and B, Table 2). In males, there was a trend for an association between full breastfeeding and IMT in young adulthood (P = 0.09, model B), which remained when considering adult waist circumference, BMI, systolic or diastolic blood pressure in separate conditional models (data not shown).

When analyses were repeated in subsamples of males with data on participation in sport (n = 91) or estimated energy expenditure during participation in sport (n = 55) in adulthood, full breastfeeding was no longer associated with adult IMT levels, irrespective of considering adult physical activity (data not shown).

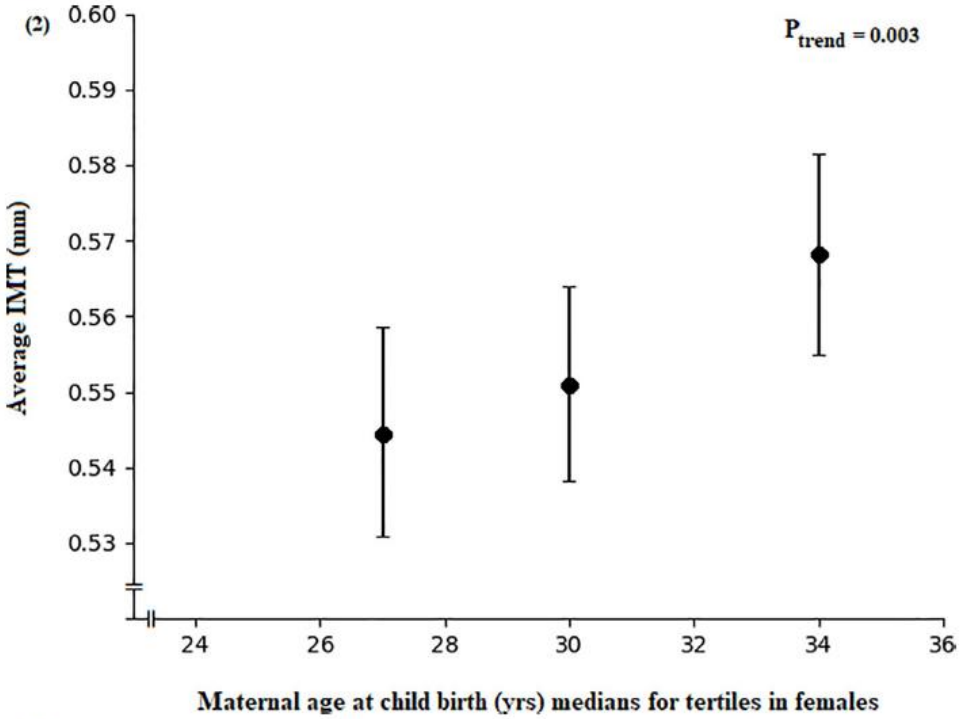

Fig 2. Association between maternal age at child birth and intima media thickness. Maternal age at child birth by tertiles of average IMT adjusted for age by the residual method in young adulthood among female participants. Data are means and 95% CI adjusted for adult age at IMT measurement, the physician taking the IMT measurement and birth year (residuals of birth year were calculated on age at IMT measurement), (n = 142).

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There was no association of IMT with other early life factors i.e. pregnancy duration, gestational weight gain, birthweight, birthweight according to gestational age (S5 Table and S6 Table).

4. Discussion

This study indicates that older maternal age at child birth is associated with an increased IMT of the offspring in young adulthood. Whereas increased maternal age at child birth was associated with an increased IMT in female offspring's, this was not evident in males.

Other early life factors were not associated with IMT in this study. Birthweight and birthweight according to gestational age were not associated with IMT, probably because term infants with mostly adequate birthweight were included in our study.

It is likely advanced maternal age at child birth may programme an offspring for the onset of cardiovascular disease later in life: maternal age at birth was found to be associated with higher infant systolic blood pressure [32] and impaired adult glucose metabolism [33]. In our study, IMT was not associated with paternal educational status (data not shown) and the relevance of maternal age at birth for adult IMT was not influenced by paternal age at birth, hence it is likely the association with maternal age is due to the intra uterine environment rather than socioeconomic factors.

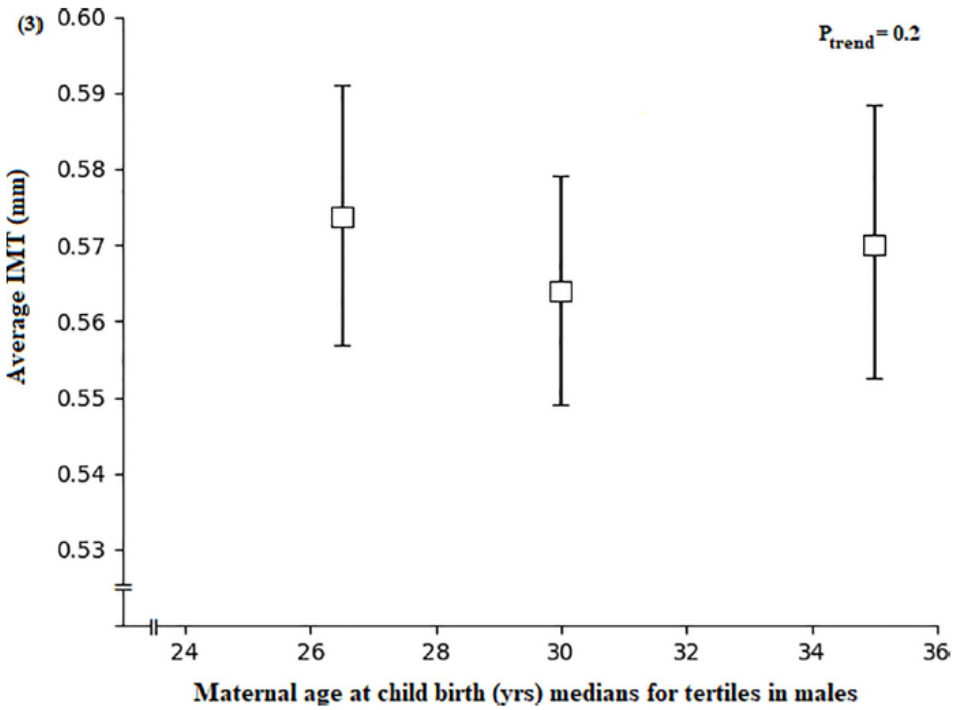

Fig 3. Association between maternal age at child birth and intima media thickness. Maternal age at child birth by tertiles of average IMT adjusted for age by the residual method in young adulthood among male participants. Data are means and 95% CI adjusted for adult age at IMT measurement, the physician taking the IMT measurement and birth year (residuals of birth year were calculated on age at IMT measurement), (n = 120).

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In line with our study, a study among Chinese participants reported that older maternal age at delivery was adversely associated with IMT among females only [34], with an effect size comparable to ours (ß: of 0.02mm per decade compared to ß: 0.03mm per decade) in our study. Mothers in that cohort were approximately 4 years younger than mothers in our cohort, which may explain the slightly more pronounced effect size in our cohort.

The overall mechanisms between maternal age at child birth and IMT of offspring are not known, yet state of health in mothers advanced in age and placental nutrition could affect fetal development due to variations in uterine artery flow or hormone synthesis in comparison to younger mothers. Likewise, mothers advanced in age are prone to higher levels of CVD risk factors such as higher blood pressure, dyslipidemia or increased oxidative stress [34, 35]. A study using a rat model of advanced maternal age, reported that pregnancy complications were partly due to development of maternal hypertension and altered vascular function in the aged female rats [36]. Hence, both the study in humans and animals suggest that advanced maternal age at child birth may programme an offspring for cardiovascular disease later in life.

In terms of the sex-specific nature of our results it should firstly be noted that men and women have similar CVD risk factors, however, there are considerable variations in the first manifestation and in clinical signs [37]. There is evidence suggesting that IMT among females are more vulnerable to metabolic disorder: Insulin resistance is related to IMT and atherosclerosis solely among females [34], and blood glucose and triglycerides levels associate strongly

Table 2. Association of full breastfeeding categories and IMT in young adulthood among females and males.

		Average IMT (mm)		
	N	Full breastfeeding categories		P trend
		0–17 wks	>17 wks	
Females	**130**	86 (76.7)[1]	44 (23.3)[1]	
Model A		0.55 (0.54, 0.56)[2]	0.56 (0.55, 0.58)[2]	0.1
Model B[3]		0.55 (0.54, 0.56)	0.56 (0.55, 0.58)	0.1
Males	**104**	69 (61.1)	35 (38.9)	
Model A		0.57 (0.56, 0.58)	0.56 (0.54, 0.58)	0.6
Model B[4]		0.57 (0.56, 0.59)	0.55 (0.54, 0.57)	0.0917

Average IMT: average of means of right and left side intima media thickness (IMT).

Linear trends (P trend) were obtained in linear regression models with IMT as a continuous variable.

[1]Values are frequencies (percentages) of breastfeeding durations.

[2]Values are adjusted least squares means (95% Confidence Interval (CIs)) of IMT.

Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement.

[3]Model B among females additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement) and maternal age at child birth.

[4]Model B among males additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).

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with IMT among females only [38]. Such sex differences could be due to hormonal differences as well as genetics [39]. Specifically, sex chromosomes may be involved in sex variations in disease development [40]. The X chromosomes in females carries clues related to inflammation [41] and the unstable nature of the X chromosome in the outer blood cells (due to loss of the second X chromosome) is associated with autoimmune diseases as well as cardiovascular diseases in females [42, 43].

Secondly, mechanisms linking early life factors to adult IMT levels in a sex-specific way are not clear, yet female placentas have been found to be more responsive to maternal emotional distress: in female fetuses, high emotional distress of the mothers was associated with lower mRNA levels of fetal genes which prevent glucocorticoid transfer to the fetuses as well as higher mRNA levels of placental glucocorticoid receptors. In turn, in male placentas, high emotional distress was associated with high mRNA levels of genes which raise glucocorticoid inactivation providing a protective effect [44]. Animal studies have shown that high glucocorticoid exposure can result in structural alterations that can hamper heart function [45–47]. Additionally, increased maternal emotional distress was associated with high insulin like growth factor IGF2 and IGF2R mRNA levels in female but not in male placenta [44].

The strength of this study is its long follow up of participants and the fact that exposure variables were collected prospectively; careful consideration was given to the prospective assessment of potential confounders.

The study is limited by its observational nature, thus any conclusion drawn should be done with circumspection and the relatively small sample size. Attrition bias is a possibility, yet those lost to follow-up differed only slightly in their early life characteristics from those included in the analysis. Participants in the DONALD study are from a relatively high educational and socioeconomic status, so the results cannot be uncritically extrapolated to other socioeconomic (sub) populations. Data on physical activity in young adulthood were only available for subsamples, yet their consideration did not change our main findings. Nonetheless, the non-availability of behavioral variables for all participants as well as variables in other moments throughout childhood and adolescence is a limitation of our study.

Conclusion

In conclusion, our study suggests a sex specific association between older maternal age at child birth and increased IMT in early adulthood among females.

Supporting information

S1 Table. Characteristics of participant's loss to follow up in adulthood. Values are presented as means (SD), medians (IQR) or frequencies (percentage). Full breastfeeding defined as breast milk including water given to the child.
(DOCX)

S2 Table. Association of maternal or paternal age at child birth and IMT in young adulthood. Average IMT: average of means of right and left side intima media thickness (IMT). T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with IMT as a continuous variable. [1]Values are medians (25th, 75th percentiles) of early life factors. [2]Values are adjusted least squares means (95% CIs) of IMT. Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement. [3]Model B additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).
(DOCX)

S3 Table. Conditional models for the association of maternal age at child birth with adult IMT in females. Average IMT: average of means of right and left side intima media thickness (IMT). T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with IMT as a continuous variable. [1]Values are medians (25th, 75th percentiles) of maternal age at child birth. [2]Values are adjusted least squares means (95% CIs) of IMT. Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement. [3]Model B additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).
(DOCX)

S4 Table. Association of maternal age at child birth and IMT in young adulthood among females with data on physical activity. Average IMT: average of means of right and left side intima media thickness (IMT). T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with IMT as a continuous variable. [1]Values are medians (25th, 75th percentiles) of maternal age at child birth. [2]Values are adjusted least squares means (95% CIs) of IMT. Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement. [3]Model B additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).
(DOCX)

S5 Table. Association of pregnancy duration or gestational weight gain with IMT in young adulthood. Average IMT: average of means of right and left side intima media thickness (IMT). T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with IMT as a continuous variable. [1]p values less than 0.025 are considered significant according to Bonferroni adjustment. [2]Values are medians (25th, 75th percentiles) of early life factors. [3]Values are adjusted least squares means (95% CIs) of IMT. Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement. [4]Model B additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).
(DOCX)

S6 Table. Association of birthweight for gestational age or birthweight and adult IMT. AGA: appropriate for gestational age, LGA: large for gestational age, SGA: small for gestational age. AGA, LGA and SGA defined according to German sex-specific birth weight and length-for-gestational-age curves. Linear trends (P difference) were obtained in linear regression models with IMT as a continuous variable and birthweight according to gestational age as a 3 level categorical variable (0 = SGA; 1 = AGA; 2 = LGA with AGA set as the reference category in the models). [1]p difference and trend less than 0.025 are considered significant according to Bonferroni adjustment. [2]Values are frequencies (percentages) of birthweight according to gestational age. [3]Values are adjusted least squares means (95% CIs) of IMT. [4]Values are medians (25th, 75th percentiles) of birthweight. Model A adjusted for adult age at IMT measurement and the physician taking the IMT measurement. Model B additionally adjusted for birth year (residuals of birth year were calculated on age at IMT measurement).
(DOCX)

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Formal analysis: Juliana Nyasordzi.

Project administration: Thomas Remer, Anette E. Buyken.

Supervision: Anette E. Buyken.

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Early life factors and their relevance for markers of cardiometabolic risk in early adulthood

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Gestational weight gain

Abstract *Background and aims:* Early life exposures could be pertinent risk factors of cardiometabolic diseases in adulthood. We assessed the prospective associations of early life factors with markers of cardiometabolic risk among healthy German adults.
Methods and results: We examined 348 term-born DONALD Study participants with measurement of fasting blood at the age of 18–24 years to assess metabolic indices: fatty liver index (FLI), hepatic steatosis index (HSI), pro-inflammatory score and insulin sensitivity (HOMA2-%S).

Early life factors (maternal weight in early pregnancy, maternal early pregnancy BMI, gestational weight gain (GWG), maternal age, birth weight and full breastfeeding (>17 weeks)) were assessed at enrolment of the offspring into the study. Multivariable linear regression models were used to analyze associations between early life factors and markers of cardiometabolic risk in early adulthood with adjustment for potential confounders.

A higher early pregnancy BMI was related to notably higher levels of offspring FLI, HSI, pro-inflammatory score and a lower HOMA2-%S (all $p < 0.0001$). Similarly, a higher gestational weight gain was associated with a higher FLI ($p = 0.044$), HSI ($p = 0.016$), pro-inflammatory score ($p = 0.032$) and a lower HOMA2-%S among females ($p = 0.034$). Full breastfeeding was associated with a lower adult FLI ($p = 0.037$). A casual mediation analysis showed that these associations were mediated by offspring adult waist circumference (WC).

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Conclusion: This study suggests that early pregnancy BMI, gestational weight gain, and full breastfeeding are relevant for offspring markers of cardiometabolic risk which seems to be mediated by body composition in young adulthood.

Introduction

Evidence from studies on the Developmental Origins of Health and Disease (DOHaD) hypothesis suggests that adverse exposures in early life are linked to adult non-communicable diseases [1–3]. Though genetics and the current obesogenic nutritional environment contribute to the epidemic of obesity and cardiometabolic diseases, evidence from human and animal studies also lends some credence to a 'developmental programming' of obesity and cardiometabolic diseases by early factors including the intrauterine environment [4–10].

Maternal obesity during pregnancy with high birth weight as a frequent consequence has become more prevalent in recent decades in Western countries [11,12]. The increased prevalence of maternal obesity at conception and during pregnancy has warranted research interest into the roles of these factors on the offspring's cardiometabolic risk in later life. However, prospective evidence on the relevance of early life factors from more recently born cohorts potentially exposed to maternal over nutrition is scarce.

To the best of our knowledge no study has yet assessed the association between such a range of early life factors and some specific markers of cardiometabolic risk considered in this study. The long time lapse between early life exposures and onset of cardiometabolic diseases demands that risk factors are considered as intermediate outcomes, hence the consideration of these risk markers in this study.

Obesity, hepatic steatosis, low-grade inflammation and insulin resistance are considered risk factors for and describe three different, yet interlinked pathways towards the development of cardiometabolic diseases [13]. Hepatic steatosis characterized by ectopic accumulation of triglycerides in hepatocytes is an inflammatory condition that can lead to a range of other metabolic comorbidities i.e. non-alcoholic fatty liver disease (NAFLD), insulin resistance, type 2 diabetes (T2D) and cardiovascular disease (CVD) [14]. Hepatic steatosis has been associated with high carotid intima-media thickness [15], impaired endothelial function and lower adiponectin levels [16] all of which promote CVD. NAFLD also predicts the incidence of T2D independent of common risk factors, probably through its promotion of hepatic insulin resistance [14]. Though insulin resistance has mostly been associated with T2D, it has also been indicated to affect different processes related to atherogenesis, progression of atherosclerotic lesions and plaque susceptibility [17]. Taken together, fatty liver disease and its frequent association with insulin resistance is highly related with markers of the metabolic syndrome and is viewed as the hepatic manifestation of the metabolic syndrome [15], that can promote cardiometabolic disorders.

We hypothesize that different exposures in early life may be relevant for markers of cardiometabolic risk. We addressed four different groups of early life factors: (i) birthweight and birth weight-for-gestational-age, i.e. indicators of in utero environment; (ii) early pregnancy BMI and GWG, i.e. factors reflecting possible fetal exposure to an over-nourished environment, for which no data exist on their relevance for the assessed markers of cardiometabolic risk; (iii) maternal age at birth, and (iv) early postnatal nutrition, i.e. breastfeeding.

We examined the prospective relevance of these exposures for markers of cardiometabolic risk i.e. insulin sensitivity, indices of hepatic steatosis and pro-inflammatory biomarkers, among healthy young adults in Germany.

Methods

Study population

This analysis is based on data from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD Study), an ongoing, open cohort study carried out in Dortmund, Germany. The DONALD Study has been described in detail elsewhere [18]. Comprehensive data on diet, growth, development, and metabolism have been obtained from over 1700 participants between infancy and adulthood since the study's inception in 1985. Around 35–40 infants are newly recruited each year and initial examination commence at the age of 3–6 months, afterwards each child returns for 2–3 more visits in the first year, twice in the second year and then once annually until adulthood.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Approval for the study was given by the Ethics Committee of the University of Bonn. Dr. Jörg Hartmann is the data protection officer and requests for data access may be sent to the local data protection coordinator Heinz Rinke, email: rinke@uni-bonn.de at the University of Bonn. Written parental and adult participant's consent is acquired before all examinations are performed.

There was considerable difference in the age of the children recruited at the start of the DONALD Study and breastfeeding information was not always available.

Although follow-up into adulthood was not anticipated at the conception of the study, from 2005 onwards, adolescents and adult participants were invited for follow-up studies including blood withdrawal every 5 years. Due to the open cohort nature of the study, most of the participants had not yet attained young adulthood at the time of this analysis.

This analysis includes 348 participants for (FLI, HSI and pro-inflammatory score outcomes) and 346 for (HOMA2-%S) who had data on early life exposures and fasting blood sample in young adulthood (18–24 years) for the assessment of markers of cardiometabolic risk.

Participants also had to meet the following minimum requirements: be born as term (37–42 weeks of gestation) singletons with a birthweight ≥2500 g, have data on early life exposures, anthropometry, as well as relevant covariates. The sample size of outcome variables and early life factors are depicted in Fig. 1, and those for the relevant covariates are shown in Table 1.

Early life exposures

Data on child birth and maternal characteristics were abstracted from the "Mutterpass," a standardized document given to all pregnant women in Germany. Gestational duration was calculated based on the recalled date of mother's last menstrual period.

Maternal weight recorded at the first visit to a gynecologist during pregnancy and at the end of pregnancy were abstracted from the "Mutterpass" and used to compute GWG. Early pregnancy BMI was computed from weight recorded by the gynecologist and height measurements taken from the mother at the first visit to the DONALD study center.

Birth weight-for-gestational-age is defined according to the German sex-specific birth weight and length-for-gestational-age curves [19]. Small-for-gestational-age (SGA) implies birth weight and length ˂10th percentile, large-for-gestational-age (LGA) is defined as birth weight and length ˃90th percentile. All other infants were categorized as appropriate-for gestational age (AGA).

Maternal age at the time of child birth was assessed at first visit at the study center.

Information on breastfeeding was assessed upon the child's inclusion in the study. At the first visit at 3 or 6 months, a pediatrician and/or dietitian enquired from the mothers about the duration (in weeks) they had fully breastfed their infant (no foods or liquids other than breast milk, tea, or water). In case of continued full breastfeeding, the duration of breastfeeding was evaluated during ensuing visits at ages 6, 9, 12 and 18 months until introduction of complementary food. Time of initiation of formula or solid foods was also assessed. All breastfeeding data were checked for consistency by comparing data collected by the pediatricians, the recording of breast milk in 3-day dietary records, and information acquired by study dietitians to exclude any potential source of error. This information was used to calculate the duration (in weeks) of full breastfeeding [20,21].

Markers of cardiometabolic risk in early adulthood

Blood analysis

Venous blood samples were taken after an overnight fast, centrifuged within 15 min at 4 °C and frozen at −80 °C at the Research Institute of Child Nutrition, Dortmund, Germany. Fasting plasma glucose was assessed on a Roche/Hitachi Cobas c 311 analyzer (Basel, Switzerland).

The following blood measurements were performed at the German Diabetes Center with assay characteristics as described [22–25]: plasma activities of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), gamma-glutamyltransferase (GGT) and plasma concentrations of triglycerides (TG) and high-sensitivity C-reactive protein (hsCRP) were quantified on a Roche/Hitachi Cobas c311 analyzer (Roche diagnostics, Mannheim, Germany). Commercially available assays were used to measure concentrations of high-sensitivity interleukin-(IL)-6 (Human IL-6 Quantikine HS), adiponectin (Human Total Adiponectin/Acrp30 Quantikine ELISA, all from R&D Systems, Wiesbaden, Germany) and chemerin (Human Chemerin ELISA kit, BioVendor, Brno, Czech

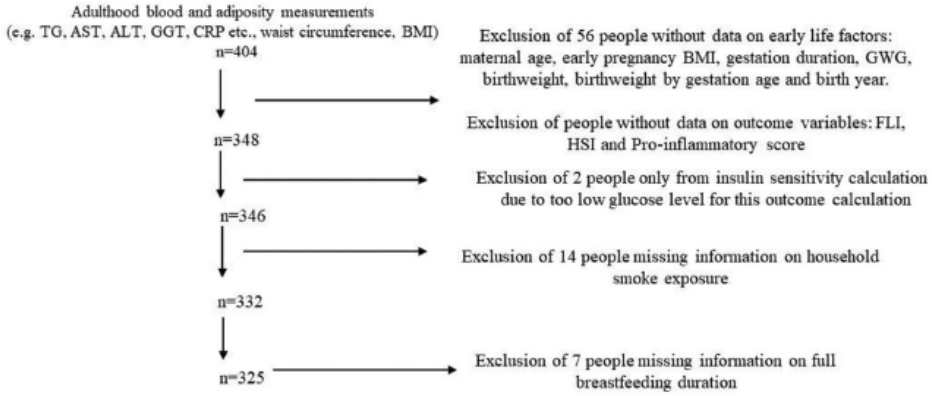

Figure 1 Participant flowchart diagram for analytical population.

Table 1 Characteristics of participants in early life and young adulthood.

General characteristics (N = 348)	
Sex: male, n (%)	169 (48.6)[a]
Adult age (yrs)	21 (18, 24)[b]
Birth year	1987 (1984, 1991)
Adult measurements	
Glucose (mmol/L)	95 (90, 101)
Insulin (pmol/L)	11 (9, 14)
Insulin sensitivity (%) (HOMA2-%S)	69 (54, 87)
ALT (U/L)	16 (13, 22)
AST (U/L)	21 (19, 25)
Hepatic steatosis index (HSI)	30 (28, 33)
GGT (U/L)	15 (11, 19)
TG (mmol/L)	91 (71, 121)
Fatty liver index (FLI)	7.9 (4.5, 17.5)
Adiponectin (μg/mL)	7619 (5046, 10,579)
Chemerin (ng/mL)	154 (133, 179)
hsCRP (mg/L)	0.08 (0.04, 0.19)
IL-6 (pg/mL)	0.67 (0.47, 1.03)
IL-18 (pg/mL)	252 (208, 311)
Leptin (ng/mL)	6624 (2423, 13,076)
Pro-inflammatory score	−0.1 (−0.41, 0.35)
Waist circumference (cm)	76 (71, 82)
Offspring BMI (kg/m^2)	22 (21, 25)
Early life factors	
Maternal BMI (kg/m^2)	23 (21, 25)
Maternal age at child birth (yrs)	30 (28, 33)
Pregnancy duration (wks)	40 (39, 41)
Gestational weight gain (kg)	12 (10, 15)
Birthweight (g)	3450 (3150, 3800)
AGA (n (%)	270 (77.6)
SGA	39 (11.2)
LGA	39 (11.2)
Full breastfeeding (wks) (n = 325)	
Never (0–2weeks)	95 (29.2)
Short duration (3–17 weeks)	87 (26.8)
Long duration (>17 weeks)	143 (44)
Family characteristics and additional covariates	
Maternal overweight (n (%)	96 (27.6)
Maternal high educational status (n (%)	171 (49.2)
Smokers in the household (n (%) (n = 332)	115 (34.6)

ALT: alanine-aminotransferase, *AST:* aspartate-aminotransferase, GGT: γ-glutamyltransferase, *HOMA2-%S:* updated homeostasis model assessment of insulin sensitivity, *hsCRP:* high-sensitivity C-reactive protein, *IL-6:* interleukin 6, *IL-18:* interleukin 18, *LGA:* large for gestational age, *SGA:* small for gestational age. AGA: appropriate for gestational age. AGA, SGA and LGA defined according to German sex-specific birth weight and length-for-gestation-age curves. Maternal overweight in early pregnancy: BMI ≥25 kg/m^2 (yes/no). Full breastfeeding defined as breast milk including water and tea given to the child. High maternal educational status: ≥ 12 years (yes/no). Smokers in the household: yes (≥1 smoker in the household), no (0 smoker in the household), *TG:* triglycerides.

[a] Values are frequencies (percentages) for categorical variables.

[b] Values are presented as medians (25th, 75th percentile) for continuous variables.

Republic) in plasma as well as leptin (Leptin Quantikine ELISA kits, R&D Systems, IL-18 (Human IL-18 ELISA kit MBL, Nagoya, Japan) in serum. Plasma insulin concentration was analyzed at the Laboratory for Translational Hormone Analytics of the University of Giessen with an immunoradiometric assay (IRMA, DRG Diagnostics, Marburg, Germany) [26].

Insulin sensitivity was calculated using the updated HOMA2 sensitivity (in %) based on fasting insulin and blood glucose level with the HOMA2 calculator https://www.dtu.ox.ac.uk/homacalculator/download.php [27].

Indices of hepatic steatosis were calculated as follows:

$$HSI = 8 \times ALT / AST + BMI\ (+2, \text{if female};\ +2, \text{if diabetes mellitus})$$ [28],

Fatty liver index (FLI): $FLI = e^x / (1 + e^x) \times 100$

$$x = 0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times \text{waist circumference} - 15.745$$ [29].

To assess associations with low-grade inflammation, a pro-inflammatory score, presumed to be more predictive of inflammation than single markers [30], was calculated by averaging circulating concentrations of five biomarkers of low-grade inflammation (hsCRP, IL-6, IL-18, chemerin, leptin, and adiponectin an anti-inflammatory biomarker), that were each standardized (z) by sex (mean = 0, SD = 1) beforehand. To align the anti-inflammatory adiponectin in the pro-inflammatory score it was multiplied by −1.

Pro-inflammatory score = [(z-hsCRP + z-IL-6 + z-IL18 + z-chemerin + z-adiponectin × (−1) + z-leptin)]/6.

Potential covariates

Trained nurses performed anthropometric measurements using standard procedures. Recumbent length is measured in children until 2 years of age to the nearest 0.1 cm using a Harpenden (UK) stadiometer whilst standing height is measured in children aged older than 2 years to the nearest 0.1 cm using a digital stadiometer (Harpenden Ltd., Crymych, UK). Body weight is measured to the nearest 100 g with an electronic scale (Seca 753E; Seca Weighing and Measuring Systems, Hamburg, Germany). Adulthood waist circumference was measured at the midpoint between the lower rib and iliac crest to the nearest 0.1 cm. This same measurement procedure is used to measure anthropometry of parents at regular intervals.

Smoking exposure in a participant's household and the number of years of maternal education used as a proxy of socioeconomic status were assessed by questionnaires. A high educational status is defined as ≥ 12 years of schooling.

Statistical analysis

All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Prospective associations between early life factors and offspring markers of cardiometabolic risk in early adulthood were analyzed using multivariable linear regression models.

To obtain normal distribution in the outcome variables they were transformed as follows: $\log_e$ transformation for HOMA2-S%, HSI and pro-inflammatory score and double $\log_e$ transformation for FLI.

An interaction analysis showed an interaction of the association between GWG and HOMA2-%S with sex ($P_{interaction}$ = 0.0361) only, thus sex-stratified analysis was performed for this association.

Initial regression models (A) included markers of cardiometabolic risk as the dependent continuous variables and individual inclusion of an early life factor as the independent variable, adjusted for sex and age at blood sampling in adulthood. Next, multivariable adjusted models (B) were constructed considering covariates individually for potential confounding in the models in a stepwise approach. Individual early life factors were considered as mutual confounders and in a hierarchical manner: Pregnancy and child birth related confounders were firstly considered followed by socioeconomic factors. Covariates which substantially modified the predictor–outcome associations by ≥ 10% or were significantly associated with the outcome were included in the final multivariable adjusted models.

The following variables were considered as mutual potential covariates in model B:

- Early life factors: birth weight (g), birth weight-for-gestational-age as a three-level categorical variable (AGA, SGA, LGA), gestational age (weeks), maternal age at child birth (years), early pregnancy weight (kg) and GWG (kg), full breastfeeding for 0–2 weeks, 2–17 weeks, >17 weeks as a three-level categorical variable and birth year regressed on age at blood sampling in adulthood as a continuous variable;
- Socioeconomic factors: maternal school education ≥ 12 years (yes/no), smokers in the household (yes/no), parental overweight or obesity (BMI ≥ 25 kg/m^2 yes/no).

To enhance comparability of results, models were adjusted identically for related outcomes i.e. parameters of hepatic steatosis (FLI and HSI) whilst the other outcomes (pro-inflammatory score and HOMA2-S %) were not adjusted identically.

A conditional model was constructed with the addition of adult offspring waist circumference or adult age BMI to examine whether observed associations were partly explained by body composition in adulthood.

Retransformed results from regression analysis are presented as adjusted least-square means (95% confidence interval (CI)) by tertiles or categories of the respective exposure while p-values are obtained from models using the exposure as continuous and categorical variables. Statistical significance was determined at a p-value of <0.05.

Results

Characteristics of the study participants in early life and young adulthood are presented in Table 1. Median age at blood sampling in young adulthood was 21 years (range 18–24 years).

In multivariable analysis (model B), a higher maternal early pregnancy BMI was associated with a higher FLI (P < 0.0001, Fig. 2a), a higher HSI (P < 0.0001, Fig. 2b), a higher pro-inflammatory score (P = 0.0017, Fig. 2c) and a lower HOMA2-S% (P = 0.0007, Fig. 2d). These associations were no longer significant upon additional inclusion of offspring adult waist circumference (WC) in the model (P-value > 0.1, Table S1). Inclusion of adult age BMI showed similar results (data not shown) as adult WC. A casual mediation analysis with adjustment for relevant covariates to test the potential mediatory role of offspring WC on outcomes showed that the 'total effect' of early pregnancy BMI on FLI was (β 0.0421 (0.0246–0.0595, p<0.0001)). The 'natural indirect effect' of early pregnancy BMI on FLI via WC was (β 0.0370 (0.0227–0.0514, p<0.0001)). Whilst the 'natural direct effect' of early pregnancy BMI on FLI was (β 0.0050 (−0.0057–0.0158, p = 0.356)). The proportion mediated by WC was 88%.

Similarly, a higher GWG was associated with a higher FLI (P = 0.044), a higher HSI (P = 0.016) and a higher pro-inflammatory score (P = 0.032), and with a lower HOMA2-S% among females (P = 0.034), but not males (P = 0.624) in model B. These associations were no more significant after inclusion of adult WC in the model (P-value > 0.1, Table 2) or adult age BMI (data not shown). In a mediation analysis the 'total effect' of GWG on FLI was (β 0.0109 (0.0011–0.0206, p = 0.029)). The 'natural indirect effect' of GWG on FLI via WC was (β 0.0107 (0.0027–0.0187, p = 0.009)). Whilst the 'natural direct effect' of GWG on FLI was (β 0.0002 (−0.0056–0.0059, p = 0.958)). The proportion mediated by WC was 99%.

Full breastfeeding (>17 weeks) was associated with a lower adult FLI (P = 0.037, model B, Table 3); the association rendered non-significant with additional consideration of adult WC (P = 0.438). The mediation analysis showed the 'total effect' of full breastfeeding on FLI was (β −0.0531 (−0.1010 to −0.00528, p = 0.030)). The 'natural indirect effect' of full breastfeeding on FLI via WC was (β −0.0410 (−0.08034 to −0.00169, p = 0.041)). The 'natural direct effect' of full breastfeeding on FLI was (β −0.0121 (−0.0397–0.0155, p = 0.390)). The proportion mediated by WC was 77%. The results from the mediation analysis suggest these associations were mediated by offspring WC. A similar mediatory role comparable to that of adult waist circumference was observed when adult BMI was used in the model, however, adult height did not show a potential mediatory effect. Similar results of mediation were observed for early pregnancy BMI, GWG and the other outcomes (data not shown).

Full breastfeeding was not associated with HSI (P = 0.462, model B, Table 3), pro-inflammatory score (P = 0.149, model B, Table 3) or HOMA2-S% (P = 0.155, model B, Table 3).

There was no association between maternal age at child birth, birthweight and birthweight by gestational age and any of the markers of cardiometabolic risk (Tables 4 and 5).

Discussion

This study shows that a higher early pregnancy BMI and GWG among mostly normal-weight mothers are associated

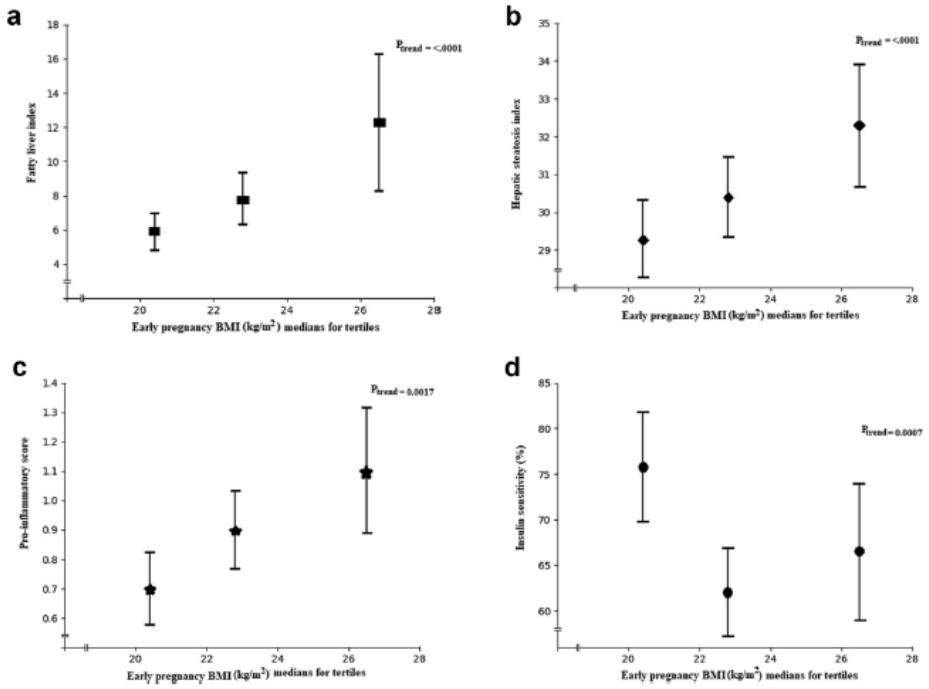

Figure 2 **a. Association between early pregnancy BMI and fatty liver index**; Fatty liver index (FLI) in young adulthood by tertiles of early pregnancy BMI. Data are least−square means and 95% CI adjusted for adult age at blood sampling, sex, gestation duration, birthweight, birth year and maternal overweight or obesity: (BMI ≥ 25 kg/m² yes/no), (n = 348). **b. Association between early pregnancy BMI and hepatic steatosis index**; Hepatic steatosis index (HSI) in young adulthood by tertiles of early pregnancy BMI. Data are least−square means and 95% CI adjusted for adult age at blood sampling, sex, gestation duration, birthweight, birth year and maternal overweight or obesity: (BMI ≥ 25 kg/m² yes/no), (n = 348). **c. Association between early pregnancy BMI and pro-inflammatory score**; Pro-inflammatory score in young adulthood by tertiles of early pregnancy BMI. Data are least−square means and 95% CI adjusted for adult age at blood sampling, sex, maternal educational status: high educational status: ≥ 12 years (yes/no) and maternal overweight or obesity: (BMI ≥ 25 kg/m² yes/no), (n = 348). **d. Association between early pregnancy BMI and insulin sensitivity**; Insulin sensitivity (HOMA2-%S) in young adulthood by tertiles of early of pregnancy BMI. Data are least−square means and 95% CI adjusted for adult age at blood sampling, sex, birth year and maternal overweight or obesity: (BMI ≥ 25 kg/m² yes/no), (n = 346).

with a higher adult FLI, HSI and pro-inflammatory score in their offspring. Whilst a higher early pregnancy BMI was associated with lower insulin sensitivity in both male and female offspring, a higher GWG was associated with a lower insulin sensitivity only among female offspring. According to our results early pregnancy BMI was more strongly associated with markers of cardiometabolic risk compared to GWG. Full breastfeeding was only associated with a lower FLI. All of the above associations were no longer significant after additional consideration of adult WC or adult age BMI, which indicates a possible mediation by increased offspring adiposity. Other early life factors were not associated with markers of cardiometabolic risk.

Our study advances current knowledge, since associations between maternal factors throughout conception, e.g. maternal prepregnancy obesity, GWG and cardiometabolic outcomes of the offspring have been researched more intensively in childhood [31,32] and adolescence [33−35], yet the longer-term follow-up until adulthood was addressed scarcely only.

Our results indicating that maternal body weight before and during pregnancy is relevant for their offspring's adult insulin sensitivity is in line with previous findings from the Raine study [36]. The Australian Raine study reported that a higher maternal prepregnancy BMI was associated with a higher HOMA-IR whilst a higher GWG in early but not in late pregnancy was associated with a higher abdominal fat but not with other adverse cardiometabolic risks among 17 year-old offspring. These results showed that maternal prepregnancy BMI was strongly associated with offspring cardiometabolic outcomes: BMI, waist circumference, systolic blood pressure, insulin, glucose and HOMA-IR than with early pregnancy weight gain [36]. The authors reported that after consideration of adolescents' current BMI, the associations were no longer significant and concluded that the associations were mediated by adolescents' current BMI [36]. Furthermore, a study in Denmark among normal-weight women found that higher GWG up to 30 weeks of pregnancy was associated with higher HOMA-IR and insulin levels among 20 year-old male offspring only [37] whilst our observation of low HOMA2-S% was in females. In a study among 32 year-old offspring, maternal pre-pregnancy BMI was positively associated with offspring insulin level whilst GWG was positively related

Table 2 Association of gestational weight gain and markers of cardiometabolic risk in young adulthood.

	(N = 348)			P_{trend}
	T1 (n = 113)	T2 (n = 124)	T3 (n = 111)	
Gestational weight gain (kg)	9 (7, 10)[a]	12 (12, 13.5)	16 (15, 18)	
Fatty liver index (FLI)				
Model A	7.4 (6.4, 8.7)[b]	8.3 (7.1, 9.7)	8.8 (7.5, 10.5)	0.005
Model B[c]	7.7 (6.6, 9.1)	8.4 (7.2, 9.7)	8.4 (7.1, 9.9)	0.044
Conditional model[d]	7.9 (7.2, 8.7)	8.5 (7.8, 9.3)	8.1 (7.3, 8.9)	0.950
Hepatic steatosis index (HSI)				
Model A	30.3 (29.5, 31.2)	30.5 (29.7, 31.4)	31.1 (30.3, 32.0)	0.003
Model B[c]	30.5 (29.6, 31.4)	30.6 (29.8, 31.4)	30.9 (30.0, 31.8)	0.016
Conditional model[d]	30.6 (30.1, 31.1)	30.6 (30.2, 31.1)	30.7 (30.2, 31.2)	0.490
Pro-inflammatory score				
Model A	0.84 (0.74, 0.95)	0.92 (0.81, 1.02)	0.98 (0.86, 1.09)	0.002
Model B[c]	0.87 (0.77, 0.98)	0.92 (0.82, 1.03)	0.94 (0.83, 1.05)	0.032
Conditional model[d]	0.88 (0.78, 0.97)	0.92 (0.84, 1.02)	0.93 (0.83, 1.03)	0.217
Females	**T1 (n=57)**	**T2 (n=59)**	**T3 (n=62)**	**P_{trend}**
Gestational weight gain (kg)	9 (8, 10)[a]	12 (11, 13)	16 (15, 18)	
Insulin sensitivity (HOMA2-%S) (N=178)				
Model A	69.8 (64.1, 76.0)[b]	67.3 (62.0, 73.0)	62.6 (57.7, 67.8)	0.017
Model B[c]	69.4 (63.9, 75.4)	67.6 (62.4, 73.3)	62.6 (57.8, 67.7)	0.034
Conditional model[d]	68.8 (63.7, 74.3)	67.6 (62.7, 72.9)	63.2 (58.7, 68.0)	0.179
Males	**T1 (n=55)**	**T2 (n=64)**	**T3 (n=49)**	
Gestational weight gain (kg)	9 (7, 10)[a]	13 (12, 14)	17 (15, 18)	
Insulin sensitivity (HOMA2-%S) (N=168)				
Model A	67.8 (61.3, 75.1)[b]	68.4 (62.3, 75.1)	72.6 (65.2, 80.8)	0.713
Model B[c]	67.4 (60.8, 74.6)	69.1 (62.9, 75.9)	72.2 (64.7, 80.7)	0.624
Conditional model[d]	66.5 (60.5, 73.0)	68.8 (63.1, 75.0)	73.7 (66.6, 81.6)	0.127

T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with markers of cardiometabolic risk as continuous variables.

[a] Values are medians (25th, 75th percentiles) of gestational weight gain.

[b] Values are adjusted least squares means (95% Confidence Interval (CIs)) of markers of cardiometabolic risk. For all outcomes apart from HOMA2-%S, Models A adjusted for adult age at blood sampling and sex.

[c] For FLI and HSI, Model B additionally adjusted for gestation duration, early pregnancy weight, birthweight, birth year and maternal overweight or obesity: BMI $\geq$25 kg/m^2 (yes/no). For pro-inflammatory score, Model B additionally adjusted for maternal educational status: high educational status: $\geq$ 12 years (yes/no) and early pregnancy weight. For HOMA2-%S, Model A adjusted for adult age at blood sampling. Model B additionally adjusted for birth year, maternal age at child birth and birthweight by gestation age.

[d] Conditional models additionally adjusted for adult waist circumference.

with adiposity traits but not with insulin or glucose levels [38]. These studies collectively suggest that high maternal weight, BMI prior to or in early pregnancy and GWG are associated with offspring insulin resistance in adulthood similar to our finding. Corroborating our findings, the associations observed in most of these studies were attenuated after adjusting for indicators of offspring adiposity. Thus, suggesting a probable mediation by increased offspring adiposity.

This analysis would have been strengthened by inclusion of adult weight gain; however, whilst this study did follow up participants until adulthood it has not yet collected sufficient data to allow analyses of adult weight trajectories. Therefore, analysis was confined to one adult waist (BMI, height) measurement only. Similarly, complete trajectories of body composition throughout childhood and adolescence are available only for subsets of the sample included in the present analysis. However, previous analyses in this cohort show that fast gains in weight and body fat during mid-childhood and puberty are relevant for adult fat mass [39].

We think early life factors are important for cardiometabolic risk. Our analysis suggests that early life factors may act via influences on adult body composition. Hence, taken together our data do not provide strong support for the latency model, which proposes strong independent effects of early life factors on health emerging later in life [40]. Instead, our data from this and previous analyses rather support the pathways model, according to which early life factors may affect successive trajectories, which in turn affect adult health [40].

Longer breastfeeding duration has been associated with a lower risk of NAFLD [41,42] and T2D [42]. However, these associations were lost after adjustment for adolescent obesity. In our study, we observed a lower adult FLI with full breastfeeding. However, similar to the other studies, the association was no longer significant upon consideration of adult WC. We [43] and others [44–46]

Table 3 Association of breastfeeding categories and markers of cardiometabolic risk in young adulthood.

Breastfeeding categories				
	0–2 wks	2–17 wks	>17 wks	P_{trend}
Breastfeeding, n (%)	95 (29.2)[a]	87 (26.8)	143 (44)	
Fatty liver index (FLI) (N = 325)				
Model A	10.3 (8.6, 12.6)[b]	7.8 (6.6, 9.4)	7.3 (6.4, 8.4)	0.005
Model B[c]	9.8 (8.2, 11.9)	7.8 (6.5, 9.3)	7.6 (6.6, 8.8)	0.037
Conditional model[d]	8.4 (7.6, 9.3)	8.6 (7.7, 9.5)	7.9 (7.3, 8.6)	0.438
Hepatic steatosis index (HSI) (N = 325)				
Model A	31.4 (30.4, 32.4)	30.2 (29.3, 31.2)	30.5 (29.7, 31.4)	0.188
Model B[c]	31.2 (30.2, 32.2)	30.2 (29.3, 31.2)	30.7 (29.9, 31.5)	0.462
Conditional model[d]	30.3 (29.8, 30.9)	30.8 (30.2, 31.3)	30.9 (30.5, 31.4)	0.109
Pro-inflammatory score (N = 325)				
Model A	1.04 (0.91, 1.17)	0.85 (0.73, 0.98)	0.88 (0.78, 0.98)	0.057
Model B[c]	1.02 (0.90, 1.15)	0.83 (0.72, 0.96)	0.90 (0.80, 1.00)	0.149
Conditional model[d]	0.95 (0.84, 1.06)	0.88 (0.78, 0.99)	0.91 (0.83, 1.00)	0.618
Insulin sensitivity (HOMA2 -%S) (N = 323)				
Breastfeeding, n (%)	95 (29.4)[a]	86 (26.6)	142 (44)	
Model A	65.4 (60.8, 70.4)[b]	69.9 (64.9, 75.4)	67.2 (63.3, 71.4)	0.588
Model B[c]	63.9 (59.5, 68.7)	69.2 (64.3, 74.5)	68.7 (64.7, 73.0)	0.155
Conditional model[d]	65.9 (61.5, 70.5)	67.8 (63.3, 72.6)	68.2 (64.4, 72.1)	0.469

Linear trends (P trend) were obtained in linear regression models with type 2 diabetes risk markers as continuous variables.

[a] Values are frequencies (percentages) of breastfeeding durations categories.

[b] Values are adjusted least squares means (95% Confidence Interval (CIs)) of markers of cardiometabolic risk. For all outcomes, Models A adjusted for adult age at blood sampling and sex.

[c] For FLI and HSI, Model B additionally adjusted for gestation duration, gestational weight gain (GWG), birth weight and birth year. For pro-inflammatory score, Model B additionally adjusted for GWG and maternal educational status: high educational status: ≥ 12 years (yes/no). For HOMA2-%S, Model B additionally adjusted for birth year, maternal age at child birth and GWG.

[d] Conditional models additionally adjusted for adult waist circumference.

have also previously shown that breastfeeding is associated with lower waist circumference.

With regards to the low insulin sensitivity observed among our females, emerging evidence from type 2 diabetes studies in young populations i.e. childhood [47,48] and adolescence [49] have demonstrated that females are more insulin resistant compared to males [50]. Studies show that the sex difference may be due to genetic differences and/or in utero programming [50,51]. A higher cord blood insulin levels has been reported in neonate females compared to males, though females weigh less at birth, indicative of intrinsic insulin resistance in females [50] which suggest this may be under genetic control [50,51] and thus sex-associated genes may be responsible for the intrinsic sex disparity [47]. At the onset of conception, both maternal and neonatal metabolic milieu, as well as hormones, are involved in sex specific development of the placenta. The placenta further modulates the sex-specific variations in non-communicable diseases through epigenetic processes which are further influenced by hormonal activities [52]. Taken together differences in placental hormones or genetic susceptibility in some females may be related to vulnerability to insulin resistance [51] yet, the sex difference in insulin resistance could also be due to differences in adiposity [53].

Though the exact mechanisms between early pregnancy BMI or GWG and offspring health outcomes are yet to be elucidated, epigenetics and biological programming have been described as probable underlying complex mechanisms, which may either have persistent adverse effects on later health or may modify DNA methylation and histones of involved organs and/or tissues via further external influences:

Firstly, prenatal factors (pregnancy weight or GWG) can affect the development of organs and organ systems and program permanent organ or tissue structural changes by increasing or decreasing hormone-controlled target values in the child's hypothalamus. Therefore, insulin has been suggested as a central determinant [54]. Fetal insulin production was shown to be stimulated by the availability of glucose and amino acids (offered by the mother), thereby programming the insulin set point [55]. A high glucose and amino acid load due to maternal overnutrition might therefore reflect fetal exposure to an over-nourished environment [56] contributing to a permanent hyperinsulinemia in the offspring, which in turn increases the risk for metabolic disorders [55]. Evidence from a study among participants who underwent a bariatric surgery and later conceived seems to support this concept. A study among two groups of offspring, born before or after maternal bariatric surgery, reported that offspring born after the surgery had decreased HOMA-IR, lower CRP and TG than their siblings born before the surgery. The authors concluded that the favorable cardiometabolic results in those born after the surgery could be attributed to an improved in utero environment after the surgery [57]. The effect sizes observed in our study for early pregnancy BMI (β FLI = 0.414, HSI = 0.167, pro-inflammatory score = 0.256 and HOMA2-%S = −0.316/decade and its association with markers of cardiometabolic risk were higher than those observed for GWG (β FLI = 0.099, HSI = 0.053, pro-inflammatory score = 0.093 and

Table 4 Association of additional early life factors and markers of cardiometabolic risk in young adulthood (N = 348).

Early life factors	Fatty liver index (FLI)				Hepatic steatosis index (HSI)				Pro-inflammatory score			
	T1 (n=115)	**T2 (n=117)**	**T3 (n=116)**	**P_{trend}**	**T1 (n=115)**	**T2 (n=117)**	**T3 (n=116)**	**P_{trend}**	**T1 (n=115)**	**T2 (n=117)**	**T3 (n=116)**	**P_{trend}**
Maternal age at child birth (yrs)	27.2 (25.6, 28.1)[a]	30.4 (29.6, 31.3)	34.7 (33.3, 36.4)		27.2 (25.6, 28.1)	30.4 (29.6, 31.3)	34.7 (33.3, 36.4)		27.2 (25.6, 28.1)	30.4 (29.6, 31.3)	34.7 (33.3, 36.4)	
Model A	8.3 (7.1, 9.8)[b]	8.3 (7.1, 9.7)	7.9 (6.7, 9.2)	0.5	30.5 (29.7, 31.4)	30.5 (29.7, 31.4)	30.9 (30.1, 31.8)	0.9	0.95 (0.84, 1.10)	0.89 (0.79, 1.00)	0.89 (0.78, 1.00)	0.4
Model B	8.5 (7.3, 10.1)	8.4 (7.2, 9.8)	7.6 (6.5, 8.8)	0.2	30.7 (29.8, 31.6)	30.5 (29.7, 31.4)	30.7 (29.9, 31.6)	0.5	0.95 (0.84, 1.06)	0.90 (0.80, 1.01)	0.88 (0.78, 0.99)	0.3
	T1 (n= 117)	**T2 (n=115)**	**T3 (n=116)**		**T1 (n= 117)**	**T2 (n=115)**	**T3 (n=116)**		**T1 (n= 117)**	**T2 (n=115)**	**T3 (n=116)**	
Birthweight (g)	3050 (2920, 3150)	3450 (3370, 3550)	3900 (3800, 4100)		3050 (2920, 3150)	3450 (3370, 3550)	3900 (3800, 4100)		3050 (2920, 3150)	3450 (3370, 3550)	3900 (3800, 4100)	
Model A	7.7 (6.6, 9.1)	8.1 (6.9, 9.5)	8.7 (7.4, 10.3)	0.06	30.5 (29.7, 31.4)	30.4 (29.6, 31.2)	31.1 (30.2, 31.9)	0.2	0.89 (0.79, 1.01)	0.90 (0.80, 1.01)	0.93 (0.82, 1.05)	0.5
Model B	7.6 (6.5, 9.0)	8.1 (7.0, 9.5)	8.7 (7.4, 10.4)	0.06	30.5 (29.6, 31.4)	30.4 (29.6, 31.3)	31.1 (30.2, 32.0)	0.2	0.91 (0.81, 1.03)	0.91 (0.81, 1.02)	0.91 (0.80, 1.02)	0.9
Birthweight by gestation age categories												
	Adequate for gestation age	**Large for gestation age**	**Small for gestation age**		**Adequate for gestation age**	**Large for gestation age**	**Small for gestation age**		**Adequate for gestation age**	**Large for gestation age**	**Small for gestation age**	
Birthweight by gestation age	270 (77.6)[c]	39 (11.2)	39 (11.2)		270 (77.6)	39 (11.2)	39 (11.2)		270 (77.6)	39 (11.2)	39 (11.2)	
Model A	8.2 (7.4, 9.2)	6.8 (5.3, 8.9)	9.1 (7.0, 12.2)	0.5	30.8 (30.3, 31.4)	29.7 (28.3, 31.2)	30.5 (29.1, 32.0)	0.7	0.90 (0.83, 0.98)	0.86 (0.68, 1.05)	1.00 (0.81, 1.21)	0.4
Model B	8.2 (7.4, 9.1)	7.0 (5.5, 9.2)	8.8 (6.7, 11.8)	0.7	30.8 (30.3, 31.4)	29.9 (28.5, 31.3)	30.1 (28.7, 31.6)	0.4	0.91 (0.84, 0.98)	0.86 (0.69, 1.05)	0.94 (0.76, 1.14)	0.8

T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with markers of cardiometabolic risk as continuous variables.

[a] Values are medians (25th, 75th percentiles) of maternal age at child birth and birthweight.

[b] Values are adjusted least squares means (95% Confidence Interval (CIs)) of markers of cardiometabolic risk.

[c] Values are frequencies (percentages) of birthweight by gestation age. For all predictors and outcomes, Model A adjusted for adult age at blood sampling and sex. For outcomes FLI and HSI, Model B with the predictor maternal age at child birth, additionally adjusted for gestation duration, birthweight, birth year and gestational weight gain (GWG). Model B with the predictor birthweight additionally adjusted for gestation duration, birth year and GWG. Model B with the predictor birthweight by gestation age additionally adjusted for birth year and GWG. For outcome pro-inflammatory score, Model B with the same predictors additionally adjusted for maternal educational status: high educational status: ≥ 12 years (yes/no) and GWG.

Table 5 Association of additional early life factors and insulin sensitivity in young adulthood (N = 346).

Early life factors	Insulin sensitivity (HOMA2-%S)			
	T1 (n=115)	**T2 (n=116)**	**T3 (n=115)**	**P_{trend}**
Maternal age at child birth (years)	27.2 (25.7, 28.2)[a]	30.4 (29.6, 31.3)	34.7 (33.3, 36.4)	
Model A	65.1 (61.0, 69.5)[b]	70.6 (66.2, 75.3)	67.8 (63.5, 72.4)	0.3
Model B	64.8 (60.8, 69.1)	71.0 (66.7, 75.7)	67.8 (63.6, 72.3)	0.2
	T1 (n=114)	**T2 (n=117)**	**T3 (n=115)**	
Birthweight (g)	3050 (2910, 3150)	3450 (3350, 3550)	3900 (3800, 4100)	
Model A	68.4 (64.1, 73.0)	69.2 (64.9, 73.8)	66.0 (61.8, 70.4)	0.4
Model B	68.3 (64.1, 72.9)	68.7 (64.5, 73.2)	66.5 (62.4, 70.9)	0.5
Birthweight by gestation age categories				
	Adequate for gestation age	**Large for gestation age**	**Small for gestation age**	
Birthweight by gestation age	269 (77.7)[c]	38 (11.0)	39 (11.3)	
Model A	67.6 (64.8, 70.5)	74.3 (66.5, 82.9)	63.5 (56.8, 71.0)	0.3
Model B	67.3 (64.6, 70.1)	74.4 (66.7, 83.0)	65.1 (58.2, 72.7)	0.6

T: tertile, n: sample size in tertile. Linear trends (P trend) were obtained in linear regression models with insulin sensitivity as a continuous variable.

[a] Values are medians (25th, 75th percentiles) of maternal age at child birth and birthweight.

[b] Values are adjusted least squares means (95% Confidence Interval (CIs)) of HOMA2-%S.

[c] Values are frequencies (percentages) of birthweight by gestation age. Model A adjusted for adult age at blood sampling and sex. Model B additionally adjusted for birth year.

HOMA2-%S = −0.128/decade) making early pregnancy BMI or preconception BMI an important indicator for possible offspring adverse outcomes in later life.

Secondly, gene expression can be modified by external influences without changing the base sequence of the DNA itself (epigenetics). The most important epigenetic modifications include DNA methylation (down-regulation of gene expression) and histone modification (changing the compression of DNA histones, which prevents or enables gene activation) [4,58,59]. Maternal obesity during pregnancy can change the general methylation pattern in the offspring [60,61], though it is uncertain how changes in the in utero milieu modifies epigenetic modulation of genes in the offspring, evidence suggests that the surge in obesity and other metabolic disorders e.g. T2D could partly be attributable to occurrences during the fetal period, through epigenetic processes which leads to probable risk of metabolic disorders e.g. T2D and obesity in later life [60]. Maternal bariatric surgery before and after pregnancy has been related with overrepresentation of differently methylated sites in genes associated with cytokine inflammatory and T2D signaling in siblings born before (BMS) and after (AMS) maternal surgery. Genes associated with T2D and obesity e.g. IGF2 (insulin-like growth factor 2), INSR (insulin receptor), FTO (fat mass and obesity associated protein), and TNF (tumor necrosis factor) were hypermethylated or hypomethylated in differently methylated sites in siblings BMS and AMS [60].

In addition to these direct programming mechanisms, there is the possibility of influencing the risk for metabolic disorders via indirect programming. Early pregnancy BMI and GWG are positively associated with birthweight, which is also related to the likelihood of obesity later in life, hence high early pregnancy BMI or GWG could result in tracking of high weight in later life [62–64]. Lastly, shared familial genetics and behaviors (i.e. socioeconomic and physical activity levels) could also link early pregnancy BMI and GWG with offspring health outcomes [65]. Of note adjusting for parameters reflecting social characteristics (maternal overweight, educational status, smoking exposure in the household) in this study did not alter associations of early pregnancy BMI, GWG and markers of cardiometabolic risk.

The strength of this study is the long follow up of participants as well as the assessment of a range of markers of cardiometabolic risk. In addition, careful consideration was given to the prospective assessment of potential confounders.

A limitation of the study is its observational nature; thus caution should be exercised in drawing causal conclusions. The study is also limited by the relatively small sample size and the fact that blood measurements were only taken once in adulthood. The DONALD study participants are from a relatively high educational and socioeconomic status, so the findings cannot be extrapolated to the general population. Another limitation is the unavailability of data on familial dietary and lifestyle habits e.g. maternal diet and physical activity levels. The use of biochemical markers to assess indicators of hepatic steatosis instead of imaging techniques is a limitation of the study.

In conclusion, our study suggests that a higher early pregnancy BMI and GWG among mostly normal-weight mothers are associated with markers of cardiometabolic risk i.e. FLI, HSI, pro-inflammatory score in their offspring. Whilst early pregnancy BMI was associated with a lower HOMA2-%S in the full sample, GWG was associated with a lower HOMA2-%S among females only. Full breastfeeding

was associated with a lower FLI. Of note, a higher early pregnancy BMI emerged as a factor which was particularly associated with all outcomes, thus preconception body composition or BMI is an important modifiable factor for possible prevention of adverse offspring health outcomes. This may call for intensifying preconception counselling, i.e. the need to maintain adequate weight among women in the reproductive age before entering pregnancy.

Data availability

Data from this study are available on request. The DONALD Study is still ongoing and the comparably small sample requires specific precautions to avoid potentially identifying participant information. This has been imposed by the data protection officer Dr. Jörg Hartmann of the University of Bonn as an official ethical restriction. Requests for data access may be sent to the local data protection coordinator Heinz Rinke, email: rinke@uni-bonn.de at the DONALD Study Dortmund of the University of Bonn.

Author contributions

A.E.B. and T.R. conceived the project; J.N. conducted the statistical analysis and wrote the manuscript; J.C. performed initial statistical analyses; J.G.; C.H., M.R., S.A.W. supervised laboratory measurements of blood analytes; Y.H. reviewed the mediation analysis; A.E.B. supervised the project and the drafting process and has primary responsibility for the final content. All authors read and approved the final manuscript.

Declaration of competing interest

A.E.B. is a member of the International Carbohydrate Quality Consortium. All other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2021.03.024.

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Title: Maternal pregnancy weight or gestational weight gain and offspring's blood pressure: Systematic review

Short title: Prenatal weight parameters and offspring's blood pressure

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Abstract

Objective: An increasing number of studies suggest that maternal weight parameters in pregnancy are associated with offspring's blood pressure (BP). The aim of this systematic review – following the updated Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Statement – was to assess and judge the evidence for an association between maternal pregnancy weight/body mass index (BMI) or gestational weight gain (GWG) with offspring's BP in later life.

Methods: MEDLINE, EMBASE, Cochrane Library, CINAHL and Web of Science were searched without limits. Risk of bias was assessed using the "US National Heart, Lung and Blood Institute"-tool, and an evidence grade was allocated following the "World Cancer Research Fund" criteria.

Results: Of 7,124 publications retrieved, 16 studies (all cohort studies) were included in the systematic review. Overall data from 52,606 participants were enclosed. Association between maternal pregnancy BMI and offspring's BP were analyzed in 2 (both "good-quality" rated) studies, without consistent results. GWG and later offspring's BP was analyzed in 14 studies (2 "good-quality", 9 "fair-quality", 3 "poor-quality" rated). Of these, 3 "fair-quality" studies described significant positive results for systolic BP and significant results, but partly with varying directions of effect estimates, for diastolic BP. MAP was analyzed in 1 "poor-quality" congress paper. Overall, based on the small number of "good-quality"-rated studies, the inconsistency of effect direction and methodological weaknesses, no firm conclusion can be drawn.

Conclusion: Evidence for an association of maternal pregnancy weight determinants with offspring's later BP was overall graded as „limited-no conclusion".

Condensed Abstract

Aim was to assess and judge the evidence for an association between maternal pregnancy weight/body mass index (BMI) or gestational weight gain (GWG) with offspring's BP in later life via a systematic review. Of 7,124 publications retrieved, 16 studies were included. Association between maternal pregnancy BMI and offspring's BP were analyzed in 2 (both "good-quality" rated) studies. GWG and later offspring's BP was analyzed in 14 studies (2 "good-quality", 9 "fair-quality", 3 "poor-quality" rated). Overall, based on the small number of "good-quality"-rated studies, inconsistency of effect direction and methodological weaknesses the evidence grade „limited-no conclusion" was chosen.

Keywords

perinatal programming, developmental origins of health and disease, pregnancy weight, gestational weight gain, blood pressure, offspring

Abbreviations

ACC, American College of Cardiology; AHA, American Heart Association; BMI, body mass index; BP, blood pressure; CI, confidence interval; CSB, collider stratification bias; CVD, cardiovascular disease; DAG, directed acyclic graph; DBP, diastolic blood pressure; ESC, European Society of Cardiology; ESH, European Society of Hypertension; GWG, gestational

weight gain; pBMI, pregnancy body mass index; MAP, mean arterial pressure; SBP, systolic blood pressure; WCRF, World Cancer Research Fund;

Main document

Introduction

Cardiovascular diseases (CVD) still exhibit a high disease burden: premature mortality and resulting disabilities contribute to their enormous public health relevance [1, 2]. Therefore, identification and reduction of modifiable risk factors are of major importance. High blood pressure (BP) is the leading (and at the same time a modifiable) risk factor responsible for preventable global CVD burden [3, 4]. An increased prevalence of suboptimal BP is omnipresent in most populations [4]. Of note, elevated BP values have already been observed among children [5, 6], which – combined with a (considerable) probability of tracking into adulthood – is likely to result in an increased risk of CVD in later life [7–9]. Therefore, the prevention of suboptimal BP in children and adolescents represents a major public health challenge.

As of today an increasing number of studies point to a link between risk factors acting already in the early life period and elevated BP in childhood and adolescence [10–12]. The early life period, especially the prenatal time, is regarded as a vulnerable phase: Factors acting during this period on the fetal environment might have long-lasting effects on offspring's later health [13, 14]. Therefore, identification of early factors influencing offspring's later BP is needed. A major hypothesis in this area of research relates to the influence of maternal anthropometric characteristics during pregnancy [15, 16]. So far, some studies described maternal pregnancy body mass index (pBMI) and maternal gestational weight gain (GWG) to be directly associated with offspring's BP later in life [17–19]. However, other study results suggest that maternal weight characteristics are only indirectly – via offspring's anthropometric characteristics, e. g. weight or body mass/fat status – related to BP later in life [20, 21].

The assumption of an association between maternal weight parameters and offspring's BP is based on different possible biological pathways within the concept of perinatal programming. Direct programming of offspring's BP could result from epigenetic modifications of the placental leptin and/or insulin expression or by modulating the offspring's sympathetic nervous system induced by maternal obesity [22, 23]. An indirect association could occur through (1) the influence of maternal pBMI or GWG on offspring's anthropometry characteristics (e. g. birth weight, BMI) [24–26], which (2) in turn impacts on offspring's later BP [27–30]. However, respective clear evidence regarding the mechanisms is lacking.

The aim of this study was to conduct a systematic review to assess and judge the current evidence for a direct or indirect association between maternal pregnancy weight determinants (i. e. weight, BMI or GWG) with offspring's BP (systolic, diastolic or mean arterial) in later life.

Materials and methods

The systematic review was conducted and presented according to the (updated) Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and was registered in the international prospective register of systematic reviews PROSPERO (CRD42020197479) [31]. The PRISMA item checklist for systematic reviews and the PRISMA checklist for abstracts with the reported page-numbers are provided in **Appendix 1** and **Appendix 2**. No review protocol was prepared.

Search strategy and information sources

A 2-step systematic literature search was performed in the data bases MEDLINE, EMBASE (PubMed/EMBASE), Cochrane Library, CINAHL and Web of Science. The 5 databases were searched until July 24, 2020, with the combination of the following search terms:

(1) Terms related to "maternal pregnancy weight or BMI" OR "maternal GWG" AND
(2) Terms related to "offspring's BP" OR "offspring's cardiovascular variables" AND
(3) Limitation to human study populations.

All search terms were searched both as controlled vocabulary terms (Medical Subject Headings or Emtree) and as free words in title and abstract. No limits were set regarding language, age, year of publication or study type. If several publications from the same population or cohort and approximately the same offspring's age were found, only data from the most relevant report were included (e. g. exclusion of congress papers or descriptions of ongoing studies, if the full study paper was also available). Given the importance of socio-environmental factors to weight gain [32], we limited our search to high-income countries for a better study comparability. In addition, reference lists of the included articles were checked for further relevant studies and conference proceedings were screened for relevant submissions. Research progress was monitored in the PROSPERO database. The full search strategies for all five databases are provided in **Appendix 3**.

Study eligibility criteria

The eligibility criteria were defined following the Population-Intervention-Comparison-Outcome (PICO) scheme [33]. To accommodate the fact that it was not expected to retrieve intervention studies, the category "intervention" in this scheme (I) was replaced by "exposure" (E):

1. Population (P): Studies which included the general population; exclusion of studies of ill or institutionalized participants, participants on anti-hypertensive medication, participants from low- and middle-income-countries (classification according to the World Bank Group [34]) and pregnancy impairments (e. g. low birth weight, intrauterine growth restriction, maternal prenatal hypertensive disorders, hyperemesis gravidarum).
2. Exposure (E): Studies with measured or self-reported maternal weight or BMI in pregnancy or GWG.
3. Comparison (C): Not applicable.
4. Outcome (O): Studies with measurement of offspring's (systolic or diastolic) BP or mean arterial pressure (MAP); no restrictions regarding the effect estimates and time points of BP measurement were made.

In case of missing or unclear information, the study authors were contacted for clarification.

Study selection

As suggested by the "Cochrane Handbook for Systematic Reviews" (2020) [33] two reviewers (HLW, JN) screened the databases to identify potentially relevant studies. In a first step titles and abstracts were screened independently and irrelevant studies were excluded. In a second step the full text of the remaining articles was obtained and assessed independently for eligibility according to the study's inclusion criteria. Any discrepancies between the two

reviewers were discussed extensively and, if necessary, resolved by a third author (AK). The reasons for study exclusion in the second step are reported in **Appendix 4**.

Data extraction

Data extraction was conducted by two authors (HLW, JN) using specially developed data collection forms [33]. These forms were pilot tested with a sample of the included studies. Information was collected on study design, characteristics of the study population, details on exposure and outcome assessment and statistical analysis. Any discrepancies between the two reviewers were discussed extensively and, if necessary, resolved by a third author (AK).

Risk of bias assessment

Risk of bias was assessed using a validated checklist published by the US National Heart, Lung and Blood Institute for observational cohort and cross-sectional studies [35], see also **Appendix 5.** This tool comprises 14 categories and classifies studies as poor, fair or good. The defined criteria for each category to assess the included studies are described in **Appendix 6**. For a better comprehensibility, the assessment of 3 categories (measurement exposure, measurement outcome, confounding variables) are briefly presented in the following. Detailed information can be found in the Appendix (**Appendix 6, 7** and **8**).

Exposure measurement can rely on either self-report or transcription from medical records. As self-reported data are susceptible to bias they were considered as low quality (question 9, **Appendix 6**).

To estimate the quality of outcome measurement (question 11, **Appendix 6**) in children and adolescents the criteria of the "Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescent" [36], an updated guideline of "The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents" [37] was used. For assessing the outcome measurement in adults, relevant guidelines (e. g. guidelines by the American College of Cardiology (ACC) and American Heart Association (AHA) [38], guidelines by the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) [39] or guidelines of the National Institute for Health and Care Excellence [40]) were taken as reference. A high rating was selected when BP measurement was carried out according to the recommended auscultatory method or a validated oscillometric method, and when multiple measures of BP were taken in a rested position. A detailed description of the assessment criteria of BP is given in **Appendix 8**.

For assessing potential confounding variables, 2 sets of potential confounders, covariates, and mediators were defined (question 14, **Appendix 6**). An intensive literature search was conducted to designate the most relevant variables to be included into the statistical models. A variable set 1 was defined, including 3 maternal variables (=confounders: maternal age at enrolment [during pregnancy or at delivery], smoking during pregnancy, and maternal socio-economic status) and 2 offspring variables (=covariates: offspring's sex and age). Offspring's and mother's age were included due to the age dependency of BP [41, 42]. In addition, several studies pointed out that smoking during pregnancy [43–46] as well as socio-economic status (e. g., income, education, and occupation) [47–49] affect offspring's later BP. Furthermore, a variable set 2 was defined, which included potential mediators, i.e. birthweight and offspring's anthropometric characteristics (mostly BMI) at outcome assessment. Various studies described a strong relation between birthweight and offspring's later BP [28, 50–52] as well as

offspring's weight status and later BP [7, 53–55]. The included studies were checked for adjustment for variable set 1 and additionally checked for variable set 2.

The selected publications were separately assessed by two independent reviewers (HLW, JN), and disagreement was resolved by discussion with involvement of a third author (AK) where necessary.

Evidence assessment

The final evidence assessment was conducted in accordance with the criteria for grading evidence by two reviewers (HLW, JN), as described in the Project Expert Report 2018 of the World Cancer Research Fund (WCRF) [56]. The assignment of the final evidence grade was primarily based on the appraisal of "good-quality"-rated studies. However, the results of the "fair-quality"-rated studies were also included in the evidence gathering process, as there exist different recommendations for dealing with a heterogeneous quality assessment to generate the overall evidence [57]. The classification tool is provided in **Appendix 5**.

Results

Study selection

In the systematic literature search 7,124 publications were identified. After screening and exclusions, 16 publications met the inclusion criteria. **Figure 1** illustrates the selection process. Reasons for exclusion of the full-text screened studies are described in **Appendix 4**. Of the 16 included studies, 2 studies [17, 58] analyzed the association of maternal pregnancy weight in using pBMI as exposure variable, and 14 studies [18–21, 59–68] examined the association of maternal GWG with later offspring's BP.

Study characteristics

Table 1 and 2 present the summarized study characteristics of the 16 studies. In total, data from 52,606 participants were included. The offspring's age at outcome assessment ranged from 0 years (newborns) to 32 years. From the „Jerusalem Perinatal Study", two publications were included, as offspring's age was 17 years [61] in the first and 32 years in the second publication [21]. Most studies were conducted in Europe and the United Kingdom. Also 1 study from Hong Kong (China) was enclosed, as Hong Kong is categorized as high-income economy by the World Bank Group [34]. The variable pBMI was used as continuous variable and GWG was either used as continuous or as class variable with varying classifications, i. e. categorization according to the revised US Institute of Medicine (IOM) GWG-categories from 2009 [69] in 4 studies and according to the IOM GWG-categories from 1990 in 1 study [70], given in periods (e. g. early, mid, late pregnancy), tertiles or quartiles.

Risk of bias

Risk of bias of the 16 included studies was assessed using the validated checklist published by the US National Heart, Lung and Blood Institute for observational cohort and cross-sectional studies [35]. The 6th and 7th criteria questions were not applicable to the research question and were therefore excluded. In summary, 4 studies were rated as „good-quality", 9 as „fair-quality" and 3 as „poor-quality". The detailed results of the quality assessment are accessible in

Appendix 7. Due to the heterogeneity in study design, analytical methods and quality a meta-analysis was not possible.

Association of maternal pregnancy weight with offspring's later (systolic or diastolic) BP or MAP

Regarding the association of maternal pregnancy weight (pBMI), with offspring's later BP, 2 studies [17, 58] were included. Both were rated as „good-quality" with adequate variable adjustment. The study of West et al. [58] analyzed associations of pBMI with BP in UK White British and Pakistani children (all born in Bradford, UK). The effect estimates after adjusting for variable set 1 showed a significant positive association with systolic BP (SBP) at 4-5 years of age only in the subgroup of Pakistani children. After additional adjustment for concurrent offspring BMI the effect estimates for all analyzed subgroups were not significant. The study of Cox et al. [17] showed a significant positive association of pBMI with offspring SBP and diastolic BP (DBP) at age 4-5 years after both, adjustment for variable set 1 and variable set 2 (birth weight and BMI). Since the results are inconsistent and the number of studies very limited, the evidence grade „limited-no conclusion" was assigned for the association of pBMI with offspring's later SBP and DBP. No study analyzed the relation between maternal pregnancy weight parameters and offspring's later MAP.

Association of maternal GWG with offspring's later (systolic or diastolic) BP or MAP

The association of GWG with later SBP and DBP in offspring was investigated in 13 studies. 2 studies were rated as "good-quality", 9 as "fair-quality" and 2 as "poor-quality". The "good-quality"-rated studies showed no significant associations of GWG with offspring's SBP and DBP [20, 62]. However, significant associations were reported in 3 "fair"-rated studies with offspring's SBP [18, 21, 59]. In the study by Andersson et al. [59], a significant positive association of maternal GWG with offspring's SBP at age 18 was described in a preliminary, full sample analysis. However, after applying various exclusion criteria no evidence was found for an association between GWG and offspring's BP. Fraser et al. [18] described significant positive associations for (1) a GWG of 0-500 g during gestational week >14-36 and (2) for a GWG over the recommended IOM categories with offspring's SBP at 11 years of age. When assessing possible mediation by birthweight, the association was not substantially altered. However, by examining possible mediation by offspring fat mass, the association was attenuated to non-significance for GWG exceeding the IOM recommendations (no data reported for fat mass adjustment within gestational week periods). In the study by Hochner et al. [21] a significant positive association between GWG and offspring's SBP disappeared after adjustment for offspring BMI at 32 years of age. In the remaining 6 studies rated as „fair-quality" [19, 61, 65–68] and the 2 studies rated as „poor-quality" [60, 64] no further significant associations with offspring's SBP were reported.

A significant association of GWG with later DBP was described in 3 „fair"-rated studies, even after adjustment for possible mediators [18, 19, 66]. However, the direction of the effect estimates varied. Fraser et al. [18] described a significant inverse association between no gestational weight change (≤0 g) during gestational week >14-36 with offspring's DBP at 11 years of age, also after adjusting for birth weight (no data for adjustment for fat mass presented). In the study of Karachaliou et al. [19] greater 1st trimester GWG was associated with higher DBP at 4 years of age, even after adjustment for child's BMI. Tam et al. [66] analyzed the association between GWG, categorized according to the IOM recommendations

[69] with offspring's DBP at 7 years of age. After adjustment for child's BMI at age 7 the significant positive association between GWG exceeding the IOM recommendations with offspring's DBP disappeared. In contrast, for offspring of mothers who gained GWG below the IOM recommendations [69] a significant association with offspring's DBP was only observed after adjusting for child's BMI at 7 years of age. Further analyses of these data with quadratic functions between standardized GWG and offspring's SBP or DBP did not reveal any significant effects. All other studies reviewed, i.e. the remaining 6 „fair-quality" studies [21, 59, 61, 65, 67, 68] as well as the 2 „poor-quality" studies [60, 64], did not find significant associations with offspring's DBP.

Based on the small number of "good-quality"-rated studies, the inconsistency of effect direction and methodological weaknesses (in particular lack of adjustment or adjustment handling) no firm conclusion can be drawn. Therefore, for the association of maternal GWG with offspring's later SBP and DBP the evidence was rated as „limited-no conclusion".

The association of maternal GWG with later MAP in offspring was only analyzed in the study of Marshall et al. [71], published as a congress paper. The results described significant correlations between maternal GWG and later MAP in children 3-9 years of age. Due to the low study number, the insufficient adjustment handling and unclear methods the evidence was graded as „limited-no conclusion".

Discussion

Summary of evidence

To our knowledge, this is the first review which systematically searched and summarized the evidence regarding an association of maternal pregnancy weight or GWG with offspring's BP in later life. Regarding the first exposure (pBMI) only 2 studies could be included. Both studies were rated as „good-quality", however, 1 study [58] showed no significant associations in the fully adjusted model and 1 study [17] showed a significant direct, but relatively small, association with SBP and DBP. For the second exposure (GWG) 14 studies could be included in the systematic review. In 3 „fair quality"-rated studies a significant positive association of GWG with offspring SBP was described [18, 21, 59], but with limitations in the statistical analyses. For DBP, 3 „fair-quality"-rated studies [18, 19, 66] reported significant associations, but partly with varying directions of the effect estimates. MAP was only assessed in 1 congress paper [71] with significant correlations, but methodically limited results. Applying the WCRF evidence classifications [56], the evidence for a direct or indirect association of pBMI or GWG on offspring's later SBP, DBP or MAP was therefore graded as „limited-no conclusion".

Limitations of the included studies

Despite plausible biological pathways the overall results of the reviewed studies did not support the hypothesis of a link between pregnancy related maternal weight parameters and offspring BP. This might be due to imprecise or inadequate exposure measurement. Furthermore, several studies indicated that cardiometabolic outcomes (such as BP) are specifically sensitive to maternal weight gain during pregnancy in certain time windows: especially GWG in early and mid-pregnancy seemed to play a critical role in influencing offspring's anthropometric or cardiometabolic phenotype [18–20, 67, 72] including BP [18, 19]. Hence, especially the first and second trimester might be a critical period for the programming of offspring's later health. However, not all included studies discriminated between anthropometric parameters acting in

different pregnancy periods. In the studies which distinguished different time windows in pregnancy, inconclusive results were obtained [18–20, 67].

Further possible explanations for the insufficient evidence might be that a significant association between pBMI and/or GWG and offspring's BP may be only apparent in (1) the higher ranges of offspring's BP, as described for the association of birth weight and offspring's later risk of hypertension [73, 74] (to investigate this, studies using quantile regression would be needed) and/or (2) later in life, as 2 included studies described significant (but methodically limited) associations at the age of 18 years [59] and 32 years [21]. Consequently, longer observation periods might be needed. The majority of the included studies (56 %) had an observation duration of <11 years of life.

In addition, there are several factors in the early stages of life that could be also related to later BP, as already analyzed by our research group, e. g. maternal prepregnancy BMI or weight and offspring's BP [75]. Other influencing factors could be gestational diabetes [76, 77], prenatal (mental) stress [78–80], prenatal hypertension [81], low birthweight [50, 82], high birthweight [28, 51], breastfeeding [83] or excessive postnatal weight gain [26, 27]. Also, nutrition in pregnancy has been described as an exposure related to offspring BP [84, 85]. Finally, socioeconomic status appears to play a crucial role, as a socioeconomic gradient in elevated BP and hypertension is also evident in children and adolescents [47–49].

A further drawback might be seen in the statistical modelling used in the included studies. Lack of adequate model adjustments was observed in most of the included studies. For 9 of the 16 studies, i.e. in 56%, adjustment was rated as inadequate. For the few studies with adequate adjustment, residual confounding cannot be excluded. In addition, no causal pathway analyses were presented and neither mediating nor colliding effects of variables were considered. Additionally, it should be considered that adjustment for mediators (e. g., birthweight and/or offspring's anthropometric characteristics) can introduce a non-causal association between the prenatal maternal weight characteristics (exposure) and possible unmeasured confounding variables, a so-called collider stratification bias (CSB). This bias may either block the original association or open a backdoor path, thereby inducing confounding [86, 87]. Such pathways are usually illustrated through directed acyclic graphs (DAGs; [86, 87]). However, of the included studies, none considered possible CSB or applied a DAG. Therefore, it cannot be excluded that unmeasured variables biased the analyzed association of pBMI or GWG with offspring's BP.

Finally, the included studies displayed a broad heterogeneity in terms of exposure and outcome assessments. The exposure GWG was often calculated from self-reported pre-pregnancy weight which implies a certain risk of underreporting [88, 89]. In addition, the time point of weight assessment during pregnancy varied considerably. Similarly, the measurement of offspring's BP varied and was not consistently performed according to recommended guidelines.

Limitations of the systematic review

This systematic review has several limitations. First, there is no consented set of variables to be included into the statistical models. The sets of variables defined within this systematic review for assessing the quality of model adjustments were based on a broad literature search attempting to identify the most relevant variables. Therefore, the evaluation of the studies might be different if other requirements for variable control would be applied. Second, pBMI and GWG were used as an indicator for the prenatal metabolic environment. This could be

misleading as also fat distribution, waist circumference, or waist-to-hip ratio might be relevant exposure variables [90–92]. Third, there is also the possibility of publication bias, as there is convincing evidence that statistically non-significant results are less likely to be published than statistically significant results, and hence are less easily identified by systematic reviews [93]. Given the results of this review, no publication bias assessment was performed, as no other conclusion of this review would have been obtained if further statistically non-significant studies had been available. Finally, this review has not considered a possible association in low and middle income countries, as the economic and socio-cultural conditions are not comparable. However, this issue should be addressed in a separate systematic review.

Conclusion

This systematic review found only "limited non-conclusive" evidence for an association of maternal pregnancy weight, as determined by pBMI, or GWG with offspring's later BP. Therefore, more good-quality studies are required for a concluding judgement. These studies should a) follow standardized reporting guidelines (e. g., CONSORT and STROBE), b) use validated assessment instruments and procedures as well as clearly defined exposure and outcome variables and c) pay more attention to the determination of causal pathways as well as to the identification of variables that may potentially confound or mediate the association between exposure and outcome.

Given the high and still rising rates of overweight and obesity, and consequently, rising numbers of pregnant women with a suboptimal weight or weight gain on the one hand, and high prevalences of both suboptimal BP and hypertension on the other hand, this topic remains of high public health relevance.

Figures and Tables

Legend

Figure 1. Flow diagram for the study selection regarding the association of maternal pregnancy weight, body mass index or gestational weight gain with offspring's later blood pressure.

Table 1. Study characteristics of the included studies regarding the association of maternal pregnancy body mass index with offspring's later blood pressure.

Table 2. Study characteristics of the included studies regarding the association of maternal gestational weight gain with offspring's later blood pressure.

Table 3. Reported associations between the maternal pregnancy body mass index and offspring's later blood pressure.

Table 4. Reported associations between the maternal gestational weight gain and offspring's later blood pressure.

Figure 1. Flow diagram for the study selection regarding the association of maternal pregnancy weight, body mass index or gestational weight gain with offspring's later blood pressure.

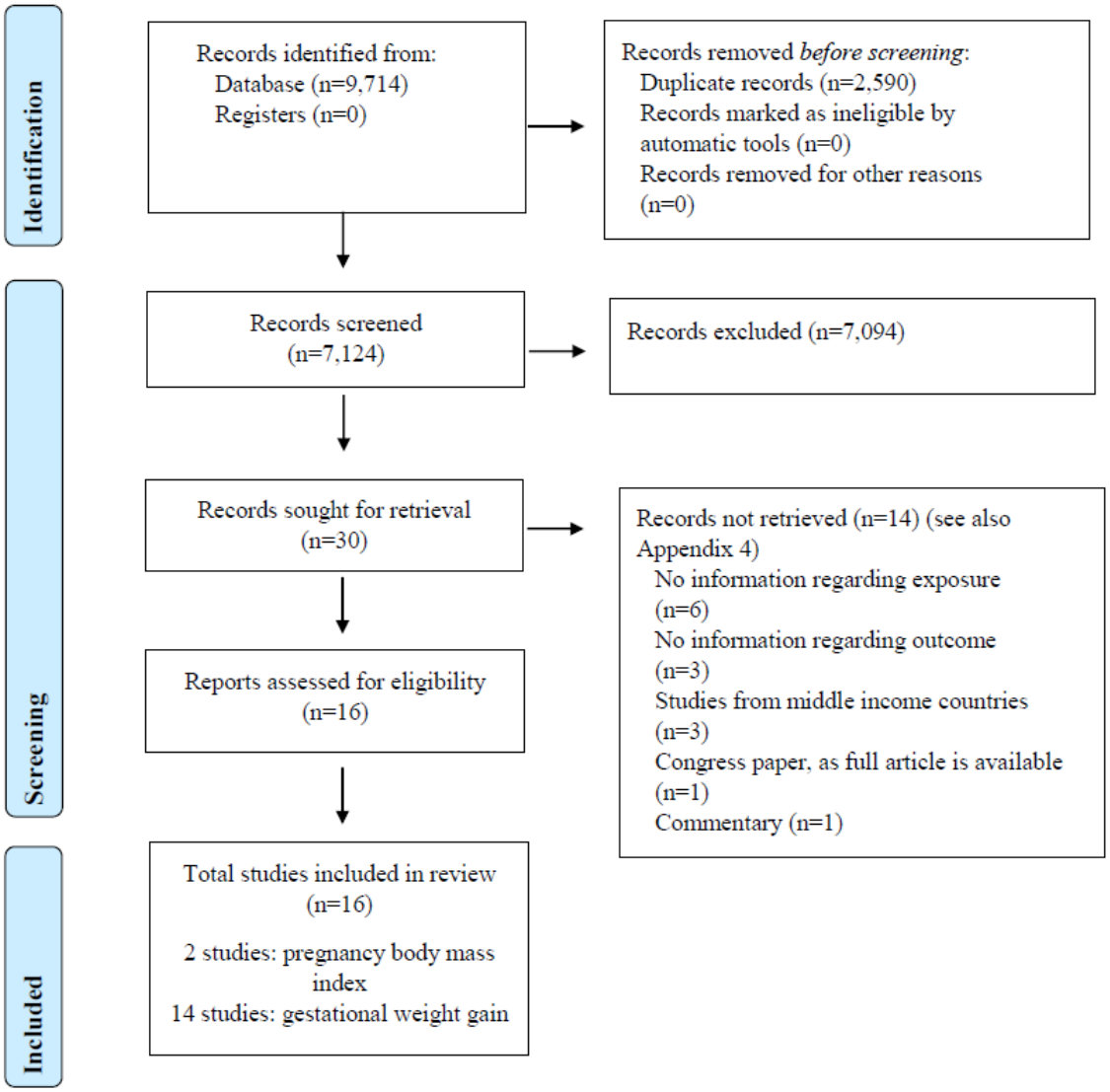

Table 1. Study characteristics of the included studies regarding the association of maternal pregnancy body mass index with offspring's later blood pressure.

			Maternal characteristics		Offspring's characteristics	Outcome measurement's characteristics	
First author; Year; Country	**Study setting; Population**	**Sample size (% female offspring)**	**Age at enrollment in pregnancy (AP) or at delivery (AD), years**	**Type of pregnancy BMI; measurement**	**Age at outcome measurement, years**	**Method (company name), validation for children/ adolescents**	**Technique of outcome measurement**
Cox et al.; 2020; Belgian [17]	Birth cohort; Environmental Influence on Early Aging (ENVIRONAGE) study	240 mother-child pairs (52.5)	NR: mean±SD; 29.9±4.2	Pregnancy BMI (continuous); at first antenatal visit (7-9 pregnancy week), abstracted from medical hospital records	4-6	Oscillometric method (Omron 750IT, HEM-759-E), validated by Stergiou et al. (2006) [94]	5 consecutive measurements with 1 minute intervals, averaging the last 3 measurements, seated position, upper arm at heart level, using right arm, appropriate cuffs.
West et al.; 2018; United Kingdom [58]	Pregnancy and birth cohort study; Born in Bradford (BiB) study	3,468 mother-child pairs: 1,644 White British and 1,824 Pakistani (NR)	AD: mean±SD; 27.4±5.6	Pregnancy BMI (continuous); at first antenatal assessment (median 12 pregnancy week), abstracted from medical records	4-5	Oscillometric method (Omron HEM-907), validated by El Assaad [95], but not for children	1 measurement, 2 minute rest, using left arm.

AD, age at delivery; AP, age in pregnancy; BMI, body mass index; BP, blood pressure; NR; not reported; SD, standard deviation;

Table 2. Study characteristics of the included studies regarding the association of maternal gestational weight gain with offspring's later blood pressure.

			Maternal characteristics		Offspring's characteristics	Outcome measurement's characteristics	
First author; Year; Country	**Study setting; Population**	**Sample size (% female offspring)**	**Age at enrollment in pregnancy (AP) or at delivery (AD), years**	**Type of gestational weight gain (GWG); measurement**	**Age at outcome measurement, years**	**Method (company name), validation for children/ adolescents**	**Technique of outcome measurement**
Andersson et al.; 2015; Sweden [59]	Prospective cohort study; recruitment of women with at least two male children (full brothers)	4,908 mother-son pairs (0)	AD: mean±SD; 1st son: 26.7±3.9; 2nd son: 29.4±4.0	Total GWG as continuous variable; retrieved from medical birth register	mean±SD; 1st son: 18.3±0.3, 2nd son: 18.2±0.3	NR	1-2 measurements, 5-10 minutes rest, supine position, cuff at heart level.
Clark et al.; 1998; United Kingdom [96]	Study of nutrition in pregnancy; women attending the antenatal clinic had been invited to take part	296 children (NR)	NR	GWG between 18 and 28 week of gestation, NR whether continuous or categorized variable; weighing at each visit on the antenatal clinic	mean±SD; 11.0±0.8	Oscillometric method (Dinamap 1846 SX), NR	Mean of 3 measurements, 5minute rest, seated position, room temperature and time of day were recorded.
Fraser et al.; 2010; United Kingdom [18]	Prospective population based cohort study; Avon Longitudinal Study of Parents and Children	5,154 mother–child pairs (50.5)	AD: mean±SD: 29.2±4.5	Total GWG categorised in US Institute of Medicine (IOM) criteria [69] and GWG-variables for 3 periods (0-14 week, >14-36 week, >36 week); abstraction from obstetric medical records	~9	Oscillometric method (Dinamap 9301 Vital Signs Monitor), NR	Mean of 2 measurements, seated position, arm supported at chest level on a table.

Table 2. *continued*

			Maternal characteristics		Offspring's characteristics	Outcome measurement's characteristics	
First author; Year; Country	**Study setting; Population**	**Sample size (% female offspring)**	**Age at enrollment in pregnancy (AP) or at delivery (AD), years**	**Type of gestational weight gain (GWG); measurement**	**Age at outcome measurement, years**	**Method (firm name), validation for children/ adolescents**	**Technique of outcome measurement**
Gaillard et al.; 2015; Netherlands [20]	Population-based prospective cohort study; Generation R Study	5,908 mother-child pairs (50.1)	AP: mean±SD: 30.3±5.1	Total GWG, GWG-variables for 3 periods (early, mid and late) and IOM-criteria [69]; self-reported pre-pregnancy weight and measured maternal pregnancy weight by study staff	median (95% range); 6.0 (5.6, 8.0)	Oscillometric method (Datascope Accutor Plus), validated by Wong et al. (2006) [97]	4 measurements, 1 minute intervals, mean measure of the last 3 BP measurements, measured at the right brachial artery.
Gaillard et al.; 2016; Australia [67]	Population-based prospective cohort study; Western Australian Pregnancy (Raine) Cohort	1,392 mother-children-pairs (49.3)	AD: mean±SD; 29.0±5.8	Total GWG as continuous variable and GWG-variables for 2 periods (early and mid-pregnancy); self-reported pre-pregnancy weight and GWG obtained from medical records at 16 and 34 weeks of gestation	median (95% range); 17.0 (16.7; 17.7)	Oscillometric method (Dinamap 8100), NR	5 measurements, 2 minute intervals, rested supine for 5 minutes, average values (exclusion of the first measurement).
Hochner et al.; 2012; Israel [21]	Population-based cohort; The Jerusalem Perinatal Family Follow-Up Study	1,256 offspring (50.5)	AD: mean±SD; 28±5.47	Total GWG as continuous variable and GWG quartiles; self-reported pre-pregnancy weight and self-reported end of pregnancy weight	~32	Oscillometric method (Omron M7), validated by Coleman et al. 2008 [98]	Mean of 3 consecutive measurements, sitting position, 5 minute rest, right arm.

Table 2. *continued*

First author; Year; Country	Study setting; Population	Sample size (% female offspring)	Maternal characteristics: Age at enrollment in pregnancy (AP) or at delivery (AD), years	Maternal characteristics: Type of gestational weight gain (GWG); measurement	Offspring's characteristics: Age at outcome measurement, years	Outcome measurement's characteristics: Method (firm name), validation for children/ adolescents	Outcome measurement's characteristics: Technique of outcome measurement
Karachaliou et al.; 2015; Greece [19]	Population-based prospective cohort study; Pregnancy cohort "Rhea"	977 mother-child-pairs (50), blood pressure measurements were available for 518 offspring.	AD: 47% <30 years; 53% ≥30 years	Total GWG as continuous variable and GWG-categories for 2 periods (1st trimester and 2nd&3rd trimesters GWG); self-reported pre-pregnancy weight, measured pregnancy weight within the study period, self-reported total weight gain	~4	Oscillometric method (Dinamap Pro Care 400), NR	Average of 5 consecutive time measurements, 1 minute interval, sitting position, 5 minute rest, right arm.
Laor et al.; 1997; Israel [61]	Population-based cohort; The Jerusalem Perinatal Study (JPS)	10,833 offspring (38.6)	NR	Total GWG as continuous variable; self-reported pre-pregnancy weight and end of pregnancy weight	~17	Auscultation method (Baumann), recommended method [36]	Sitting position, right arm, appropriate cuff size, end point for diastolic blood pressure was the disappearance of the Korotkoff sounds (phase V).
Mamun et al.; 2009; Australia [62]	Population-based birth cohort; Mater-University Study of Pregnancy and its Outcomes (1981-1983)	2,432 mother-child-pairs (50)	NR	GWG by IOM-criteria [70], self-reported pre-pregnancy weight and abstracted maximum weight from medical records	~21	Oscillometric method (Omran HEM-703C), NR	Mean of 2 measurements, 5 minute intervals, rested, arm supported at chest level, appropriate cuff size.
Marshall; 2011; USA [71]	Mixed-longitudinal study; NR	71 children (49.3)	NR	NR	Range; 3.4-8.8	NR	NR

Table 2. *continued*

			Maternal characteristics		Offspring's characteristics	Outcome measurement's characteristics	
First author; Year; Country	**Study setting; Population**	**Sample size (% female offspring)**	**Age at enrollment in pregnancy (AP) or at delivery (AD), years**	**Type of gestational weight gain (GWG); measurement**	**Age at outcome measurement, years**	**Method (firm name), validation for children/ adolescents**	**Technique of outcome measurement**
Morrison; 2013; Canada [99]	Longitudinal cohort study; Family Athero-sclerosis Monitoring In earLY life (FAMILY)	901 mother-newborn-pairs (49.6), sample size for BP-analyses: 488	AD: mean±SD; 32.1±5.2	Total GWG as continuous variable; self-reported pre-pregnancy weight and last clinic visit weight prior to delivery	~2.6 days after birth	Oscillometric method (Dinamap Pro100 V2), NR	3 measurements, baby was sleeping or lying quietly, 2 minute intervals.
Perng; 2014; USA [68]	Prospective cohort study; recruitment of pregnant women in Massachusetts (USA) to examine prenatal diet and other factors in relation to maternal and child health (Project Viva)	1,090 mother-child-pairs (50.3), sample size for BP-analyses: 1,084	AP: category, n, age category; I, 106, 15-24; II, 647, 25-34; III, 337, 35-44	GWG by IOM-categories; self-reported pre-pregnancy weight and clinically-measured weight prior to delivery	Median; 7.7 Range: 6.6-10.9	Oscillometric method (NR), NR	5 measurements, 1 minute interval, mean value.
Russo; 2013, 8 European countries [65]	Epidemiological multicentred cohort study; Identification and prevention of Dietary- and lifestyle-includes health Effects In Children and infantS (IDEFICS project)	12,775 children (49)	AD: GWG-tertiles, n, median (interquartile range), AD (95% CI); I, 5,330, 10 (8-11 kg), 29.7 (29.6-29.9); II, 3,440, 14 (13-15 kg), 29.3 (29.2-29.5); III, 4,005, 20 (18-24 kg), 28.4 (28.2-28.6)	GWG-tertiles; self-reported GWG	GWG-tertiles, n, median, interquartile range; I, 5,330, 10, 8-11 kg, II, 3,440, 14, 13-15 kg; III, 4,005, 20, 18-24 kg	Oscillometric method (Welch Allyn 4200B-E2), NR	2 measurements, 2 minute interval, sitting position, 5 minute rest.
Tam; 2018; China [66]	Multicentered prospective birth cohort study; Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study	905 mother-child-pairs (48.1)	AP: mean ±SD; 31.3±4.6	GWG by IOM categories and standardised GWG; self-reported pre-pregnancy weight and abstracted weight at delivery from medical records	~ 7	Oscillometric method (Omron T5 BP monitor), NR	Mean of 3 measurements, 1 minute interval, 5 minute rest, all subjects advised to abstain from smoking and drinking alcohol, tea or coffee.

AD, age at delivery; AP, age in pregnancy; BMI, body mass index; BP, blood pressure; GWG, gestational weight gain, NR; not reported; SD, standard deviation;

Table 3. Reported associations between the maternal pregnancy body mass index and offspring's later blood pressure.

First author; Year; Country;	Statistical analysis	Variable adjustment	Outcome	Adjusted effect estimate	Note
Cox; 2020; Belgian [17]	Linear regression models	Model with variables from variable-set 1: Maternal variables: parity, age, education, smoking weight gain, date of follow-up visit, season of follow-up. Offspring variables: sex, gestational age, race/ethnicity, age at follow-up.	SBP	ß [95%]; 0.35 [0.12, 0.57]; p=0.003	Estimate represent differences (95% CIs) in offspring BP associated with an increase of 1 kg/m² in maternal pregnancy BMI
			DBP	ß [95%]; 0.18 [-0.01, 0.36]; p=0.07	
		Model with additional variables from variable-set 1&2: Child's birth weight and BMI at 4-6 years of age.	SBP	ß [95%]; 0.27 [0.03, 0.51]; p=0.03	
			DBP	ß [95%]; 0.26 [0.06, 0.45]; p=0.01	
West; 2018; United Kingdom [58]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age at delivery, parity, education, pregnancy smoking, family housing tenure, and receipt of benefits. Offspring variables: sex and age at blood pressure (BP) measurement.	SBP, White British (WB) sub cohort	ß [95%]; 0.26 [-0.17, 0.70]; p≥0.05	Estimate represent differences in means (95% CIs) in offspring BP associated with an increase of 5 kg/m² in maternal pregnancy BMI
			SBP, Pakistani (P) sub cohort	ß [95%]; 0.56 [0.08, 1.05]; p<0.05	
			DBP, WB sub cohort	ß [95%]; 0.02 [-0.41, 0.46]; p≥0.05	
			DBP, P sub cohort	ß [95%]; 0.31 [-0.21, 0.82]; p≥0.05	
		Model with additional variables from variable-set 1&2: Additional adjustment for: child's BMI at BP measurement (4-5 years of age).	SBP, WB sub cohort	ß [95%]; -0.27 [-0.71, 0.17]; p≥0.05	
			SBP, P sub cohort	ß [95%]; -0.23 [-0.73, 0.27]; p≥0.05	
			DBP, WB sub cohort	ß [95%]; -0.28 [-0.73, 0.17]; p≥0.05	
			DBP, P sub cohort	ß [95%]; -0.27 [-0.81, 0.28]; p≥0.05	

BMI, body mass index; BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; P, Pakistani; SBP, systolic blood pressure; WB, white British;

Table 4. Reported associations between the maternal gestational weight gain and offspring's later blood pressure.

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Andersson et al.; 2015; Sweden [59]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age (delivery), birth year, gestational age, early-pregnancy body mass index, maternal education, and parity. Model with additional variables from variable-set 1&2: Additional adjustment for: age of conscription and conscription center (adding sons' concurrent BMI [measured at 18 years of age] did not change the results).	SBP DBP SPB DBP	ß [95%], 0.03 [-0.08, 0.14]; p≥0.05 ß [95%], -0.03 [-0.12, 0.05]; p≥0.05 ß [95%], 0.03 [-0.08, 0.14]; p≥0.05 ß [95%], -0.03 [-0.11, 0.05]; p≥0.05	Difference in offspring BP (95% CIs) in mmHg per 1-kg difference in gestational weight gain
Clark et al.; 1998; United Kingdom [96]	Multivariable regression	NR	SBP DBP	ß [95%], 2.0 [-4.5, 8.5]; p≥0.05 NR	Difference in offspring SBP (95% CI) in mmHg per 1-kg difference in gestational weight gain per week
Fraser et al.; 2010; United Kingdom [18]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age at birth, smoking in pregnancy, GWG in previous period, parity, head of household social class, mode of delivery, and pre-pregnancy weight. Offspring variables: sex and age.	SBP, GWG 0-14 week SBP, GWG >14-36 week SBP, GWG >36 week	GWG ≤0g: ß [95%], 0.40 [-0.85, 1.64]; p≥0.05 GWG 0-500g: ß [95%], 0.46 [-0.29, 1.21]; p≥0.05 GWG >500g: ß [95%], -0.22 [-1.54, 1.10]; p≥0.05 GWG ≤0g: ß [95%], -3.82 [-9.08, 1.50]; p≥0.05 GWG 0-500g: ß [95%], 1.7 [0.13, 3.28]; p<0.05 GWG >500g: ß [95%], 0.86 [-0.41, 2.13]; p≥0.05 GWG ≤0g: ß [95%], 0.48 [-1.03, 1.98]; p≥0.05 GWG 0-500g: ß [95%], -0.48 [-2.04, 1.09]; p≥0.05 GWG >500g: ß [95%], 0.37 [-0.66, 1.39]; p≥0.05	Mean difference in offspring BP (95% CI) per 400-g/week estimated gestational weight gain

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
continued Fraser et al.; 2010; United Kingdom [18]			DBP, GWG 0-14 week	GWG ≤0g: ß [95%], -0.27 [-1.13, 0.59]; p≥0.05 GWG 0-500g: ß [95%], 0.39 [-0.13, 0.91]; p≥0.05 GWG >500g: ß [95%], 0.35 [-0.57, 1.26]; p≥0.05	
			DBP, GWG >14-36 week	GWG ≤0g: ß [95%], -4.48 [-8.13, -0.83]; p<0.05 GWG 0-500g: ß [95%], 1.00 [-0.09, 2.09]; p≥0.05 GWG >500g: ß [95%], 0.20 [-0.68, 1.07]; p≥0.05	
			DBP, GWG >36 week	GWG ≤0g: ß [95%], 0.49 [-0.56, 1.53]; p≥0.05 GWG 0-500g: ß [95%], 0.18 [-0.91, 1.26]; p≥0.05 GWG >500g: ß [95%], 0.07 [-0.64, 0.78]; p≥0.05	
		Model with additional variables from variable-set 1&2: Offspring variables: birthweight.	SBP, GWG 0-14 week	GWG ≤0g: ß [95%], 0.39 [-0.87, 1.65]; p≥0.05 GWG 0-500g: ß [95%], 0.51 [-0.25, 1.27]; p≥0.05 GWG >500g: ß [95%], -0.15 [-1.48, 1.17]; p≥0.05	
			SBP, GWG >14-36 week	GWG ≤0g: ß [95%], -3.63 [-8.90, 1.64]; p≥0.05 GWG 0-500g: ß [95%], 1.85 [0.25, 3.44]; p<0.05 GWG >500g: ß [95%], 1.12 [-0.16, 2.40]; p≥0.05	
			SBP, GWG >36 week	GWG ≤0g: ß [95%], 0.41 [-1.11, 1.92]; p≥0.05 GWG 0-500g: ß [95%], -0.45 [-2.02, 1.12]; p≥0.05 GWG >500g: ß [95%], 0.36 [-0.67, 1.34]; p≥0.05	

Table 4. *continued*

First author; Year; Country; *continued*	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Fraser et al.; 2010; United Kingdom [18]			DBP, GWG 0-14 week	GWG ≤0g: ß [95%], -0.30 [-1.17, 0.57]; p≥0.05 GWG 0-500g: ß [95%], 0.40 [-0.13, 0.92]; p≥0.05 GWG >500g: ß [95%], 0.37 [-0.55, 1.29]; p≥0.05	
			DBP, GWG >14-36 week	GWG ≤0g: ß [95%], -4.33 [-7.97, -0.68]; p<0.05 GWG 0-500g: ß [95%], 0.95 [-0.16, 2.05]; p≥0.05 GWG >500g: ß [95%], 0.30 [-0.59, 1.19]; p≥0.05	
			DBP, GWG >36 week	GWG ≤0g: ß [95%], 0.42 [-0.63, 1.47]; p≥0.05 GWG 0-500g: ß [95%], 0.26 [-0.83, 1.35]; p≥0.05 GWG >500g: ß [95%], 0.07 [-0.64, 0.78]; p≥0.05	
		Model with variables from variable-set 1: Maternal variables: age at birth, smoking in pregnancy, GWG in previous period, parity, head of household social class, mode of delivery, and pre-pregnancy weight. Offspring variables: sex and age.	SBP, GWG < IOM categories [70]	ß [95%], -0.37 [-0.97, 0.23]; p≥0.05	Mean difference in offspring BP (95% CI) by IOM categories of maternal gestational weight gain for BMI
			SBP, GWG > IOM categories [70]	ß [95%], 1.25 [0.60, 1.90]; p<0.05	
			DBP, GWG < IOM categories [70]	ß [95%], -0.23 [-0.64, 0.18]; p≥0.05	
			DBP, GWG > IOM categories [70]	ß [95%], 0.23 [-0.22, 0.67]; p≥0.05	
		Model with additional variables from variable-set 1&2: Additional adjustment for offspring's variable: birthweight.	SBP, GWG < IOM categories [70]	ß [95%], -0.32 [-0.93, 0.28]; p≥0.05	
			SBP, GWG > IOM categories [70]	ß [95%], 1.25 [0.60, 1.90]; p<0.05	
			DBP	NR	

Table 4. *continued*

First author; Year; Country; *continued*	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Fraser et al.; 2010; United Kingdom [18]		Model with additional variables from variable-set 1&2: Additional adjustment for offspring's variable: fat mass, height and height-squared.	SBP, GWG < IOM categories [70] SBP, GWG > IOM categories [70]	ß [95%], 0.17 [-0.37, 0.71]; p≥0.05 ß [95%], 0.21 [-0.38, 0.79]; p≥0.05	
			DBP	NR	
Gaillard et al.; 2015; Netherlands [20]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age, education level, ethnicity, pre-pregnancy BMI, parity, smoking and alcohol consumption during pregnancy, folic acid supplement use, total calorie intake during pregnancy, and delivery mode. Offspring variables: sex, age, breastfeeding duration, average duration of TV watching and timing of introduction of solid food.	SBP, total GWG DBP, total GWG	ß [95 %]; 0.01 [-0.03, 0.04]; p≥0.05 ß [95 %]; 0.01 [-0.03, 0.05]; p≥0.05	Values are regression coefficients (95% CI) from regular linear regression models that reflect the difference in childhood BP per SDS change with total GWG
		Model with variables from variable-set 1: Maternal variables: gestational age at maternal weight measurement, age, education level, ethnicity, pre-pregnancy BMI, parity, smoking and alcohol consumption during pregnancy, folic acid supplement use, total calorie intake during pregnancy, and delivery mode. Offspring variables: sex, age, average weight at birth, breastfeeding duration, average duration of TV watching, and timing of introduction of solid food.	SBP, GWG early pregnancy SBP, GWG mid pregnancy SBP, GWG late pregnancy DBP, GWG early pregnancy DBP, GWG mid pregnancy DBP, GWG late pregnancy	ß [95 %]; 0.03 [0, 0.06]; p≥0.05 ß [95 %]; 0.02 [-0.01, 0.05]; p≥0.05 ß [95 %]; -0.01 [-0.04, 0.03]; p≥0.05 ß [95 %]; 0.01 [-0.02, 0.04]; p≥0.05 ß [95 %]; 0 [-0.03, 0.03]; p≥0.05 ß [95 %]; -0.02 [-0.05, 0.02]; p≥0.05	Values are regression coefficients (95% CI) from regular linear regression models that reflect the difference in childhood BP per SDS change in GWG in early, mid and late pregnancy

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
		Model with additional variables from variable-set 1&2: Additional adjustment for: pregnancy complications, birth characteristics, infant growth, and childhood BMI.	SBP, GWG early pregnancy	ß [95 %]; 0.01 [-0.02, 0.04]; p≥0.05	
			SBP, GWG mid pregnancy	ß [95 %]; 0.02 [-0.01, 0.04]; p≥0.05	
			SBP, GWG late pregnancy	ß [95 %]; -0.01 [-0.05, 0.02]; p≥0.05	
			DBP, GWG early pregnancy	ß [95 %]; 0.01 [-0.03, 0.04]; p≥0.05	
			DBP, GWG mid pregnancy	ß [95 %]; 0 [-0.03, 0.03]; p≥0.05	
			DBP, GWG late pregnancy	ß [95 %]; -0.02 [-0.06, 0.02]; p≥0.05	
		Model with variables from variable-set 1: Maternal variables: age, education level, ethnicity, parity, smoking and alcohol consumption during pregnancy, folic acid supplement use, total calorie intake during pregnancy, and delivery mode. Offspring variables: sex, age, breastfeeding duration, average duration of TV watching and timing of introduction of solid food.	SBP, excessive GWG compared with no-excessive GWG (IOM categories [69])	ß [95 %]; 0.01 [-0.07, 0.08]; p≥0.05	Values are regression coefficients (95%) from regular linear regression models that reflect the difference in childhood BP for mothers with excessive GWG as compared to mothers with non-excessive GWG
			DBP, excessive GWG compared with no- excessive GWG (IOM categories [69])	ß [95 %]; 0.05 [-0.03, 0.13]; p≥0.05	

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Gaillard et al.; 2016; Australia [67]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age, ethnicity, education level, household income, parity, pre-pregnancy BMI, smoking during pregnancy, gestational hypertension disorders, gestational diabetes, caesarean delivery. Paternal covariate: BMI. Offspring variables: sex, age, gestational age at birth, weight and length at birth, breast-feeding duration, infant length and weight growth, adolescent Tanner stage, smoking, alcohol consumption, dietary intake, physical activity, sedentary behavior.	SBP, GWG early pregnancy	ß [95%], 0.04 [-0.02, 0.09]; p≥0.05	Values are regression coefficients (95%) form linear regression models that reflect differences in adolescent BP in SDS per SD change in maternal early, mid and total pregnancy weight gain rate
			SBP, GWG mid pregnancy	ß [95%], 0.02 [-0.04, 0.07]; p≥0.05	
			SBP, total GWG	ß [95%], 0.05 [-0.01, 0.10]; p≥0.05	
			DBP, GWG early pregnancy	ß [95%], 0.02 [-0.04, 0.08]; p≥0.05	
			DBP, GWG mid pregnancy	ß [95%], 0.02 [-0.04, 0.09]; p≥0.05	
			DBP, total GWG	ß [95%], 0.04 [-0.03, 0.10]; p≥0.05	
		Model with additional variables from variable-set 1&2: Additional adjustment for offspring's variable: offspring BMI (~17 years of age).	SBP, GWG early pregnancy	ß [95%], 0.01 [-0.04, 0.07]; p≥0.05	
			SBP, GWG mid pregnancy	ß [95%], 0.02 [-0.03, 0.07]; p≥0.05	
			SBP, total GWG	ß [95%], 0.03 [-0.03, 0.08]; p≥0.05	
			DBP, GWG early pregnancy	ß [95%], 0.02 [-0.04, 0.08]; p≥0.05	
			DBP, GWG mid pregnancy	ß [95%], 0.02 [-0.04, 0.09]; p≥0.05	
			DBP, total GWG	ß [95%], 0.04 [-0.03, 0.10]; p≥0.05	

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Hochner et al.; 2012; Israel [21]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: age, ethnicity, smoking, parity, years of education, socioeconomic status, medical condition. Offspring variables: sex, birth weight, gestational week, physical activity, smoking status, years of education. Model with additional variables from variable-set 1&2: Additional adjustment for offspring's variables: offspring BMI (~ 32 years of age).	SBP DBP SBP DBP	ß [95%], 0.26 [0.003, 0.41]; p=0.047 ß [95%], 0.17 [-0.004, 0.35]; p≥0.05 ß [95%], 0.06 [-0.14, 0.26]; p≥0.05 ß [95%], 0.06 [-0.11, 0.23]; p≥0.05	Coefficients (95% CI) indicate increment (positive or negative) in BP per 1-unit increase in maternal GWG (kg)
Karachaliou et al.; 2015; Greece [19]	Multivariable regression	Model with variables from variable-set 1&2: Maternal variables: age, education, parity, smoking in pregnancy, pre-pregnancy BMI, gestational length (for models using total GWG as an exposure). Parental variables: BMI. Offspring variables: sex, age, current BMI (~4 years of age).	SBP, GWG 1st trimester SBP, GWG 2nd&3rd trimesters SBP, total GWG DBP, GWG 1st trimester DBP, GWG 2nd&3rd trimesters DBP, total GWG	ß [95%], 0.09 [-0.68, 0.48]; p≥0.05 ß [95%], 0.05 [-0.68, 0.78]; p≥0.05 ß [95%], -0.15 [-0.38, 0.07]; p≥0.05 ß [95%], 0.43 [0.00, 0.86]; p<0.05 ß [95%], -0.06 [-0.61, 0.49]; p≥0.05 ß [95%], 0.00 [-0.15, 0.17]; p≥0.05	ß coefficients (95% CI) from linear regression models for the association between offspring BP and GWG per 200-g/week

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Laor et al.; 1997; Israel [61]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: ethnic origin, BMI. Offspring variables: birth weight, weight at age 17.	SBP	Women: ß [95%], 0.02 [-0.06, 0.11]; p≥0.05 Men: ß [95%], -0.03 [-0.09, 0.04]; p≥0.05	Mean regression coefficients (95% CI) for the association of offspring's BP with GWG in kg
			DBP	Women: ß [95%], 0.02 [-0.03, 0.08]; p≥0.05 Men: ß [95%], -0.01 [-0.06, 0.04]; p≥0.05	
Mamun et al.; 2009; Australia [62]	Multivariable regression analyses	Model with variables from variable-set 1: Maternal variables: maternal age, education, parity, cigarette smoking, and pre-pregnancy BMI. Offspring variables: age, sex.	SBP, GWG adequate	ß [95%], 0.2 [-1.0, 1.4]; p≥0.05	Mean difference (95% CI) in offspring's BP by IOM categories for GWG
			SBP, GWG excessive	ß [95%], 0.4 [-0.9, 1.7]; p≥0.05	
			DBP, GWG adequate	ß [95%], 0 [-0.9, 0.9]; p≥0.05	
			DBP, GWG excessive	ß [95%], 0 [-1.0, 0.9]; p≥0.05	
		Model with additional variables from variable-set 1&2: Additional variables: gestational hypertensive disorder, birth weight, breast-feeding, and BMI at 21 years.	SBP, GWG adequate	ß [95%], 0.1 [-1.1, 1.4]; p≥0.05	
			SBP, GWG excessive	ß [95%], 0.2 [-1.1, 1.6]; p≥0.05	
			DBP, GWG adequate	ß [95%], -0.2 [-1.1, 0.6]; p≥0.05	
			DBP, GWG excessive	ß [95%], -0.6 [-1.5, 0.3]; p≥0.05	
Marshall et al.; 2011; USA [71]	Correlation	Not reported	MAP	r=0.35-0.53; p<0.05	Correlation coefficient

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Morrison et al.; 2013; Canada [99]	Multivariable regression	Model with variables from variable-set 1: Offspring variable: sex, gestational age, newborn's age at birth visit.	SBP	Data not reported; p≥0.05	Not reported
			DBP	Data not reported; p≥0.05	Not reported
Perng et al.; 2014 USA [68]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: pre-pregnancy BMI, age, race/ethnicity, smoking habits during pregnancy, parity, annual household income. Paternal variable: BMI. Offspring variables: sex, age at mid childhood examination, height z-score.	SBP	ß [95%], -0.11 [-0.59, 0.38]; p≥0.05	Difference (95% CI) of offspring's BP per 5 kg maternal GWG
		Model with additional variables from variable-set 1&2: Additional adjustment for offspring's variable: DXA total fat mass index.	SBP	ß [95%], -0.47 [-0.99, 0.04]; p≥0.05	
Russo; 2013, 8 European countries [65]	Multivariable regression	Model with variables from variable-set 1: Maternal variables: current BMI, parental education level, gestational age, age at delivery, alcohol and smoking during pregnancy, gestational diabetes, gestational hypertension. Offspring variables: sex, age, practice of sport, birth weight, breastfeeding duration and child BMI z-score.	SBP, I GWG-tertile	mean [95%]; 100.3 [100.0; 100.5]; p≥0.05	Mean (95% CI) of the association between offspring's BP and GWG-tertiles
			SBP, II GWG-tertile	mean [95%]; 100.4 [100.1; 100.8]; p≥0.05	
			SBP, III GWG-tertile	mean [95%]; 100.6 [100.3; 100.9]; p≥0.05	
			DBP, I GWG-tertile	mean [95%]; 63.0 [62.8; 63.2]; p≥0.05	
			DBP, II GWG-tertile	mean [95%]; 63.1 [62.9; 63.4]; p≥0.05	
			DBP, III GWG-tertile	mean [95%]; 63.2 [63.0; 63.5]; p≥0.05	

Table 4. *continued*

First author; Year; Country;	Statistical analysis	Adjustment	Outcome	Adjusted effect estimate	Note
Tam; 2018; China [66]	Multivariable regression analyses	Model with variables from variable-set 1: Maternal variables: pre-pregnant BMI, current hypertensive status, parity, age, AUCs for glucose during pregnancy, mode of delivery, gestational age at delivery. Offspring variables: sex and age at childhood, childhood height, history of breastfeeding and childhood exercise level.	SBP, comparison of GWG < IOM criteria with GWG = IOM criteria	NR; p=0.732	Effect estimates are not reported for the individual models
			SBP, comparison of GWG > IOM criteria with GWG = IOM criteria	NR; p=0.273	
			DBP, comparison of GWG < IOM criteria with GWG = IOM criteria	NR; p=0.072	
			DBP, comparison of GWG > IOM criteria with GWG = IOM criteria	NR; p=0.009	
		Model with additional variables from variable-set 1&2: Additional adjustment for offspring variables: birthweight and childhood BMI.	SBP, comparison of GWG < IOM criteria with GWG = IOM criteria	NR; p=0.817	
			SBP, comparison of GWG > IOM criteria with GWG = IOM criteria	NR; p=0.718	
			DBP, comparison of GWG < IOM criteria with GWG = IOM criteria	NR; p=0.045	
			DBP, comparison of GWG > IOM criteria with GWG = IOM criteria	NR; p=0.064	

BMI, body mass index; BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; GWG, gestational weight gain; IOM, Institute of Medicine; NR, not reported; SBP, systolic blood pressure; SDS, standard deviation score;

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APPENDIX 4

Table 4 Studies on associations between selected early life factors and cardiometabolic outcomes

Breastfeeding and IMT

Citation, Country	Study design, population, Follow-up	Exposure (assessment, average baseline values), Covariates	Outcomes (average values)	Results
Evelein 2011 Utrecht city [406]	Prospective cohort study WHISTLER-Cardio 300 2001-2007	Exclusively formula fed (EF) vs. exclusively breastfed (EB) <3 months (mths) vs 3-6 mths vs >6 Assessed by monthly parental questionnaire % of EF =76, % EB <3 mths (n = 145) 3–6 mths (n = 42) >6 mths (n = 37) **Covariates** Age, sex, gestational age, birth weight, maternal smoking during pregnancy, weight at 3 mths, BMI, height at 5yrs, WC, SBP, DBP prepregnancy BMI, SES etc.	CIMT+ Distensibility Elastic modulus at 5 yrs **average values** Mean CIMT was 386.1 ± 37.4 µm.	At age 5, children EB for 3 to 6 mths had a CIMT that was 21.1 lm greater than that of EF children (95% CI: 5.0, 37.2 lµm; P =0.01. CIMT was not significantly different between children EB for either <3 or >6 mths and EF children.
Linhares Brazil 2015 [407]	Prospective Cohort Study, 3,188 adults 1982-2013	Information on breastfeeding (BF) duration (mths) childhood **Covariates** Family income at birth; maternal skin color; maternal age at child birth; maternal education; maternal smoking; sex; & skin color	IMT	Positive association between duration of BF and IMT up to 11.9 mths, IMT (2.97 (–0.94 to 6.87); a lower IMT among those breastfed for >12 mths (–1.01 (–3.30 to 1.27). A non-significant association after adjustment for confounders
Ja ¨rvisalo 2009 Finland [177]	cross-sectional studies, 1667 adults 1980-2001	BF duration, formula feeding. **median BF duration** 4 months (range <1–36 months). **Covariates** birth weight, WC and brachial artery baseline diameter; LDL & HDL-cholesterol, smoking	CIMT, brachial artery flow-mediated dilatation (FMD), carotid artery compliance (CAC) or carotid artery elasticity, BP **average IMT for breastfed and formula fed** men: 0.59±0.10 vs 0.60±0.09 women: 0.57±0.08 vs 0.59±0.10	Breastfed women had a trend for lower IMT, (P=0.06); SBP (P=0.06), and higher CAC (P=0.05) compared to formula fed women. Maximal FMD was higher in breast-fed compared to formula fed men (P=0.029).

				Breast-fed men have better endothelial function later in life in young adulthood than formula-fed men. Association lost after controlling for confounders.
Leeson 2001 Cambridge [168]	331 adults born between 1969 and 1975 and assessed at age 20 and 28 years.	Type and duration of infant feeding **Covariates** Pulse pressure, age, sex, resting vessel, cholesterol concentration, BMI, and socioeconomic factors	Distensibility of brachial artery, and other cardiovascular risk factors.	Longer BF duration led to a less distensible artery wall in early adulthood, (regression coefficient = -3.93 µm/month, 95% CI -7.29 to -0.57, P = 0.02). BF in infancy linked to reduced arterial function 20 years later. Vascular changes were not explained by alterations in plasma cholesterol concentration in adult life.

Birthweight and IMT

Citation, Country	Study design, population, Follow-up	Exposure (assessment, average baseline values), Covariates	Outcomes (average values)	Results
Evelein, 2013 Utrecht city [408]	Prospective cohort study 333 children 2001-2007	Weight gain & length gain -weight gain rate for length gain rate (WLG), as a measure of excess weight gain in 1st 3 mths of postnatal growth. **Covariates** Age, gender, current height, observer	CIMT & vascular stiffness **Average** CIMT, were 385.8 ± 38.2 μm	For each 1 SD increase in postnatal WLG, CIMT was 5.1 μm higher (95% CI, 1.0–9.2; P = .01). The thinner the children were at birth, the stiffer the arteries were with increasing WLG
OREN 2003 Utrecht [409]	Prospective Cohort Study	Birth weight, birth length, gestational age **Covariates** Reader and gender	IMT **Average** CIMT was 0.49 (0.05) mm	In the lowest tertile of birth length there was an inverse association between birth weight and CIMT. Low birth weight was significantly associated with increased CIMT in those who showed exaggerated postnatal growth.
Skilton 2014 Australia [410]	Young Finns Study 696	Birth weight, length, BMI at birth, term born LGA **Covariates** Age, sex, study center, employment status, marital status, LDL–cholesterol, HDL–cholesterol, systolic blood pressure, triglycerides, glucose, C-reactive	CIMT, brachial flow–mediated dilatation, & cardiovascular risk factors	CIMT > in LGA (0.60 mm [SD 0.09], versus normal birth weight 0.57 mm [SD 0.09], P=0.003, independent of CVD risk factors (P=0.001 after adjustment)

Maternal pregnancy weight and cardiometabolic outcomes

Citation, Country	Study design, population, Follow-up	Exposure (assessment, average baseline values), Covariates	Outcomes (average values)	Results
Tam 2018 Hong Kong [379]	Prospective multicentre study 905 mother–child pairs From pregnancy to follow up visit when child was 7 years	Maternal weight at delivery Pre-pregnancy BMI maternal GWG classified as weight below, within or exceeding the 2009 IOM guidelines. **Covariates** sex and age of child, maternal pre-pregnancy BMI, maternal hypertension, maternal and paternal diabetes, parity, maternal age, mode of delivery, gestational age, breastfeeding, birthweight, childhood BMI	Hypertension, insulin resistance (IR), adiposity	A U-shaped association between GWG and increased risks of IR and hypertension, higher DBP in children whose mother had gained more or less weight than IOM recommendations during pregnancy Adjustment for child's current BMI attenuated associations
Gaillard 2016 Western Australia [132]	Population-based prospective cohort study 1392 mother –child pairs From early pregnancy onwards, children assessed at 17years	Maternal prepregnancy BMI, rates of early-pregnancy, mid-pregnancy and total GWG **Covariates** adolescent sex and age at outcome measurements, maternal age, educational level, smoking during pregnancy, total GWG; gestational hypertensive disorders, gestational diabetes, caesarean delivery, gestational age at birth; breastfeeding, adolescent current WC and height.	Adolescent BMI, WC, BP, total and HDL-cholesterol, insulin, glucose and HOMA-IR.	Higher prepregnancy BMI was related with higher adolescent BMI, WC, SBP, insulin, glucose and HOMA-IR levels (P-values <0.05). Higher weight gain in early-pregnancy, but not mid-pregnancy led to higher adolescent BMI, WC (P-values <0.05). Higher prepregnancy BMI and early-pregnancy weight gain were associated with high risks of the high-metabolic risk cluster (OR 1.57, 95% CI 1.33, 1.85 and OR 1.23, 95% CI 1.03, 1.47 per SD increase in prepregnancy BMI and early-pregnancy weight gain. Adjustment for adolescent current BMI attenuated the associations
Hochner 2012 Jerusalem [136]	Population-based cohort study 1,400 offspring from 1974-1976 birth cohort, examined in 2007 and 2009 at 32 years old	Maternal prepregnancy BMI (mppBMI) GWG **Covariates** ethnicity and gender, maternal age, parity, education, gestational week, birthweight, offspring BMI at 32 years	SBP and DBP, glucose, insulin	mppBMI was positively linked with offspring SBP (p=0.003), DBP (p=0.017), insulin (p=0.007) GWG adjusted for mppBMI and all confounders was also positively associated with offspring BMI (p=0.0001) and WC (p=0.024), and with TG (p=0.04)