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Lucy R. Green
Robert L. Hester *Editors*

Parental Obesity: Intergenerational Programming and Consequences

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Lucy R. Green • Robert L. Hester
Editors

Parental Obesity: Intergenerational Programming and Consequences

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Editors
Lucy R. Green
Institute of Developmental Sciences
University of Southampton
Southampton, Hampshire
UK

Robert L. Hester
Medical Center
The University of Mississippi
Jackson, Mississippi
USA

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Preface

We fat all creatures else to fat us, and we fat ourselves for maggots. Your fat king and your lean beggar is but variable service, two dishes, but to one table; that's the end.
Hamlet. A tragedy by William Shakespeare (1599/1601).

There is more to our state of adiposity than simply what quality of meal we are offering the maggots upon our demise. Obesity brings with it greater risk of non-communicable diseases such as cardiovascular disease, diabetes, musculoskeletal disorders and cancer. It no longer seems likely that the escalating incidence of obesity and these related diseases can be mitigated by just changing adult lifestyle and diet. Now the concept of a developmental origin of health and disease (DOHaD) is firmly part of scientific, clinical and health policy activities aimed at understanding and reducing the risk of non-communicable diseases. But the focus of the field has moved from small babies and maternal undernutrition to the other end of the nutritional spectrum, maternal obesity and the future life of the larger baby. It seems that obesity begets obesity, and so the cycle continues, as is evident from the more than doubling of the worldwide prevalence of obesity since 1980 [1]. The time is ripe for this book.

The chapters are authored by undisputed leading scientists, clinicians and policy makers in this field. In these chapters, the authors set out their ideas and provide an up-to-date synthesis of the current thinking about the problem of parental obesity, the ideas of intergenerational programming, and the physiology behind it. We hope that this book will therefore appeal to a broad readership of students, clinicians, researchers and health policy makers who either seek an introduction to the area of DOHaD or have a specific interest in the pathogenesis of obesity.

In this book, the spotlight is on critical periods in development when obesity might affect offspring physiology, sometimes even before a mother conceives or is aware that she is pregnant. These effects appear to have a legacy across several generations. In Chap. 2, Gaillard and Jaddoe draw upon their considerable experience and data from the observational Generation R study, and in Chap. 3 Patel and Poston write from the perspective of their

recent randomized control trial (RCT) of a diet and physical activity intervention (UPBEAT). In both chapters, the authors call for more RCTs to understand intergenerational programming. The impact of maternal obesity on offspring physiology is multifaceted and linked to disease of the cardiovascular system and metabolism, to allergic diseases (see Chap. 15) and to cancer (Chap. 13). The importance of early critical windows is emphasized by research (Chap. 5) showing the potential for the environment around time of fertilization (pre-implantation) to have a lasting impact on offspring physiology. Indeed many contributors to this book recommend that in order to break the 'intergenerational cycle' of obesity, interventions should target obesity in the preconception period as well as throughout pregnancy. Nevertheless, others argue for a better evidence base, since there may be negative implications of dietary restriction/weight loss or exercise before or around time of conception (Chap. 7). Part of this evidence base is likely to concern the 16 million women 15–19 years old who give birth each year, about 11 % of all births worldwide [2]. The way in which weight gain and obesity during pregnancy in the young, still-growing mother affects offspring is more complex (Chap. 4) and the use of the sheep as a model for this has produced important mechanistic insights.

"Women are responsible not only for the health of their own offspring but also for the cost to the community of an unhealthy future population. . . . Women are caught in a pincer movement between those seeking to protect the fetus and those concerned with the social and economic cost or burden of ill health" wrote Ray Noble (2006) [3]. Therefore, it is timely that research into the influence of paternal obesity on offspring physiology has burgeoned (Chap. 6). Obese fathers are more likely to father an obese child with impaired glucose metabolism, an effect which may then extend into the next generation. These observations have the potential to shift at least some of the burden of responsibility for lifestyle intervention pre-pregnancy from the mother to the father.

To understand causality in human observational studies of maternal obesity and impact on offspring, more sophisticated study designs and detailed maternal-offspring outcome measurements are now needed (Chaps. 2 and 3). However, over the course of this book the reader will discover that substantial advances in understanding the mechanisms and pathways linking parental obesity to offspring physiology are being made using animal models. As with drugs, overeating may involve a chronic cycle of intoxication ('positive reinforcement') and the emergence of withdrawal anxiety over time that perpetuates disordered eating. The physiological evidence described in Chaps. 9 and 10 that pregnancy high fat diet/obesity alters both maternal behaviour towards her offspring and leads to altered food preferences in them, along with heightened risk of mental ill-health, increased anxiety, social behavioural deficits and impaired memory and learning is of real concern.

Current knowledge is expanding on the mechanistic basis of the imbalance between appetite and satiety, and of adipogenesis-lipogenesis in the offspring of mothers with high fat intake during pregnancy (Chap. 11). Leptin, an adipokine peptide hormone produced by fat cells, can cross the blood-brain barrier and in offspring of maternal obesity/high fat pregnancies its action in the hypothalamus is implicated not only in the dysfunction in appetite/satiety pathways (Chap. 11), but also in cardiovascular dysregulation and hypertension (Chap. 14). Furthermore, the mechanisms underlying insulin resistance in offspring of high fat fed and obese mothers are likely to involve changes in insulin sensitivity in skeletal muscle and liver (Chaps. 7 and 8). Nonalcoholic fatty liver disease (NAFLD), whereby fat accumulates in the liver, is the hepatic manifestation of the metabolic syndrome. There is now considerable evidence to suggest that NAFLD in offspring is primed by high fat diet and obesity during pregnancy (Chap. 12). The disease can progress in severity and lead to the development of fibrosis and cirrhosis, and may be linked to hepatocellular carcinoma. The increased risk of malignancy in offspring of obese pregnancies is an emerging field of research and the most persuasive evidence to date is from rodent studies in which the incidence of mammary tumours in female offspring is heightened (Chap. 13).

Throughout the book, and summarized in Chap. 16, contributors highlight epigenetic mechanisms that may help to explain the intergenerational cycle of obesity and physiological dysregulation. It is clear that the advances in the knowledge of epigenetic mechanisms have brought 'environmental sense' to the world of genomics. In addition, the microbiome has provided a new mechanistic perspective on the intergenerational programming of obesity and physiology. Evidence of the susceptibility of the early life microbiome to programming by maternal diet, antibiotic exposure, mode of delivery and breastmilk offers an exciting avenue for understanding how the changes in the early life environment (such as maternal obesity and weight gain) influence the health of the next generation and possible future interventions (Chap. 17).

The importance of parental obesity as a major risk factor for non-communicable diseases is apparent from the outset of this book. The reader gets a sense of the urgency for action if adolescents and young adults are to have a better future, the cost associated with non-communicable diseases is to be reduced, and if the intergenerational programming of obesity is to be halted (Chap. 1). Many of the contributors to this book have synthesized the current state of research into the mechanisms linking parental obesity to altered offspring physiology, and suggest targets for future interventions. Realizing the potential of such interventions is important but enormously challenging and, as the reader will appreciate from Chap. 1, this must be done within a coordinated policy scheme at international, national and local government level.

Southampton, UK — Lucy R. Green
Jackson, MS — Robert L. Hester

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Contents

Chapter 1
Why Obesity in Parents Matters

Mark Hanson

Abstract Promoting the best possible environment for early human development offers one of the greatest missed opportunities today for improving global health, human productivity and longevity. Overweight and obesity in parents and parents-to-be are not only associated with poorer health prospects for this section of the population in the future, but also pass the risk of overweight and obesity to their children. This calls for a new initiative to improve the health of current and prospective parents, commencing with adolescent girls and women of reproductive age, but also their partners.

Keywords Obesity • Parents • Generations • Public health • Education • Childhood • Diabetes • Cardiovascular

1.1 The Challenge

Non-communicable diseases (NCDs), including diabetes, cardiovascular and lung disease, some forms of cancer, mental illness, musculoskeletal disorders and some atopic and allergic conditions now account for almost two-thirds of deaths worldwide and a substantial burden of morbidity. WHO figures show that 38 million people die from NCDs each year, 28 million of these deaths occurring in low-middle income countries [1]. NCDs incur enormous costs in health care, which are challenging even in high-income countries. For example, the McKinsey Global Institute estimates that reversing the rising prevalence of the major NCD risk factor obesity in the UK could save the NHS $1.2 billion/year [2]. The scale of the economic costs is unsustainable, especially in low-middle income countries. NCDs not only cause deaths and shorten lifespan but they can also impair neurocognitive development [3, 4] reducing productivity and well-being. Hence,

M. Hanson (✉)
Institute of Developmental Sciences, University of Southampton, Southampton, UK

NIHR Nutrition Biomedical Research Centre, University Hospital Southampton, Southampton, UK
e-mail: m.hanson@soton.ac.uk

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apart from direct health-care costs, there will be significant economic benefits from reducing the burden of NCDs in all countries.

Until recently, it was widely believed that risk of NCDs in individuals was a combination of fixed inherited genetic risk factors and an unhealthy lifestyle in adulthood. However, genome-wide association studies have not found genetic variants which account for a substantial fraction of risk at the population level (e.g. [5]), and whilst overweight and obesity were estimated to produce 3.8 m deaths globally in 2010, there are as yet no successful national campaigns for tackling the problem [6]. The challenge is to find a new approach.

Overweight and obesity are important and very widely known risk factors for NCDs [1] and so emphasis on preventing them makes much sense. The problem is often thought to commence in childhood, as obesity in children has long-term effects on a wide range of organ systems [7]. The problem of childhood obesity in both high and low-middle income countries led to the establishment in 2014 of a Commission on Ending Childhood Obesity by Dr Margaret Chan, the Director-General of the World Health Organization [8], which will report in early 2016. Obese children have long been known to be more likely to become obese adults [9], and globally, 1.9 billion adults aged 18 years and older were overweight or obese in 2014 [10].

Turning to parental effects, in England in 2013 for example, 48 % of women of reproductive age were overweight or obese [11]. Being overweight or obese will affect their health and increase the risk of complications during pregnancy and delivery [12]. Even more important is the fact that obese women tend to have obese children and maternal obesity is a major factor in the preconceptional and fetal or infant origins of later risk of NCDs in the offspring [13]. This volume attests to the considerable concern about the effects of obesity in parents on their children, especially in mothers, but there is increasing evidence for an increased risk of obesity in children with two obese parents [14, 15] and there is accumulating experimental evidence for a role of paternal effects in transmission across generations [16].

Meeting the challenge posed by obesity in parents also has important social and equity implications, because it is particularly of concern in women with low educational attainment or socio-economic status [17], and in some ethnic and migrant groups [17, 18]. Obesity and the resulting increased risk of NCDs can perpetuate or even widen social inequalities in health [19] adding another level of urgency to finding a new solution to the problem.

The major focus of this chapter is on the biological rather than the social processes by which parental obesity affects the next generation. The distinction between the two is somewhat artificial and should not be taken to imply that aspects of parental behaviour, the family environment, etc., do not play a role in inducing obesity in children and adolescents, or are not areas where potential interventions could have major effects (see for example [20]). There have been some very hopeful initiatives in this respect such as the Family–Nurse Partnership [21] and the Abecedarian project [22] in which wider social considerations about family life and child education have been shown to improve long-term health outcomes including obesity.

1.2 New Insights into the Importance of Healthy Early Development

New research in the field of developmental origins of health and disease (DOHaD) has focused attention on the processes of developmental plasticity, operating during critical periods of early human life to affect growth and development of tissues, organs and physiological control systems [13, 23]. The critical periods of development commence in the early embryo [24], sometimes before the woman knows that she has conceived, and continue through pregnancy [25, 26], infancy and childhood and into adolescence [27]. During these periods the developing individual responds to aspects of their environment, via the mother and placenta before birth, and via breast milk and parental behaviours after birth. Signals relating to maternal nutrition, body composition, physical activity, stress, behaviour and exposure to chemicals and toxins can set the level of the developing individual's responses to later challenges such as living in today's highly obesogenic, increasingly urban, environment [28]. In this way, the effects of unhealthy lifestyle are passed from one generation to the next and can be amplified. This amplification is greater when there is a mismatch between the developmental and adult environments, as happens for example with nutritional and other lifestyle transitions in countries undergoing socio-economic transitions and in migrant groups [29]. These new concepts are fundamental to understanding the growing challenge of NCDs worldwide. They also offer opportunities for promoting future health for the current and future generations at several points in the human reproductive cycle (Fig. 1.1). However, once a critical period has passed intervention is much more difficult, becoming less effective and potentially more expensive. This is one of the reasons why current approaches to reducing the incidence of NCDs in adults may not be achieving the results needed. One of the most important phases in which to prevent and reduce overweight and obesity is during adolescence and the reproductive years, as this will not only promote the health of the woman but also that of her child(ren).

1.3 Obesity in Adolescents and Young People

Apart from the longer-term effects of obesity in increasing the risk of NCDs referred to above, there are a range of short- to medium-term implications which apply specifically to women of reproductive age and to a lesser extent to their partners. These are perhaps not sufficiently emphasised as they could help increase motivation to adopt healthier behaviours. Obesity in women is associated with reduced fertility rates and greater risk of early miscarriage [30] and to reduced sperm counts and morphological abnormalities [31]. The early embryos of obese mothers already show signs of abnormal development and metabolism [32]. Obesity in pregnancy is associated with increased risk of gestational diabetes [33] which, if poorly controlled, can result in the perinatal complications of fetal

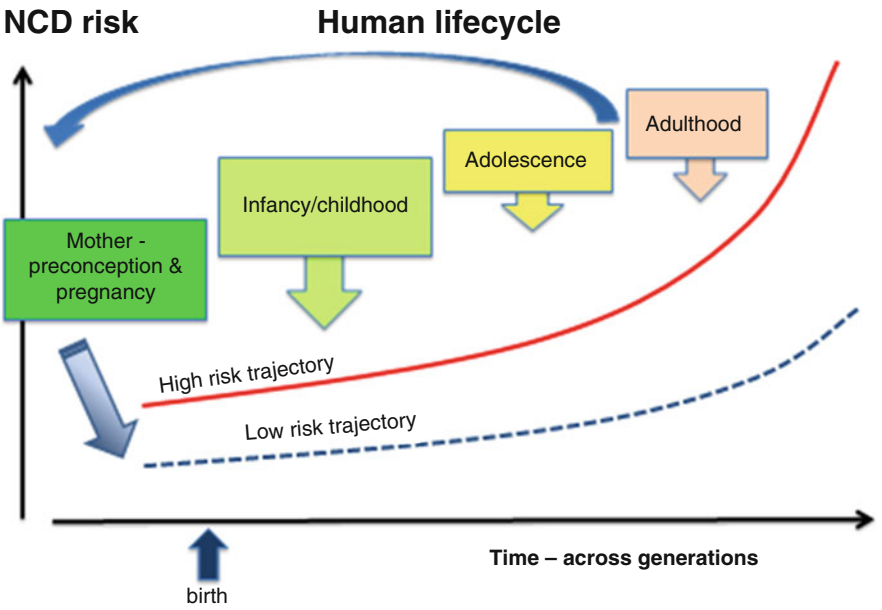

Fig. 1.1 Life-course view of NCD risk. Risk of NCDs increases in a non-linear way throughout life, starting before birth. The trajectory of risk can be affected by interventions at various times, although establishment of a low-risk trajectory must be early in the life course, especially preconception and in pregnancy (broad *blue arrow* on *left*). Risk reduction in adolescents or young adults can not only affect their later health but, as they are future parents, reduce inherited risk in their children (human life cycle *arrow*)

macrosomia, shoulder dystocia, obstructed labour and neonatal hyperinsulinaemic hypoglyceamia [34]. The specific role of pre-pregnancy weight and weight gain in pregnancy in these conditions [35], related to ethnicity [36], may offer new avenues for intervention. Maternal hyperglycaemia is also associated with higher incidence of a range of congenital abnormalities in the baby [37].

It is estimated that there are 1.8 billion people aged 10–24 years in the world today, comprising about one-quarter of the total global population. In some countries, especially in sub-Saharan Africa, they represent an even higher proportion of the population. Adolescence is a time in life when many behaviour patterns become established and is a time when interventions might reverse the effects of earlier poor development [38]. Many adolescent people are overweight or obese (e.g. for England see [39]) and have markers of cardiovascular risk, including elevated blood pressure and lipid or insulin/glucose levels [40]. A high proportion of adolescents and young women and men have an unhealthy lifestyle, with poor diet, low levels of physical activity, smoking, excessive alcohol consumption and use of recreational drugs [41–43]. These lifestyles will have adverse later health implications for the individuals, but will also have repercussions on the development and health of their future unborn children, giving the next generation a poor start to life. Access to health care is fragmentary in this section of the population,

even in high-income countries. Adolescents frequently defer, or discount, any action to improve their health until the future [44, 45] and for those adults with lower educational attainment and socio-economic status poor health can become self-fulfilling prophecy [46].

For those women who become pregnant, contact with health-care services often does not occur until late in the first trimester, by which time the pregnancy is well established and it is too late for modification of risk factors which affect embryonic development. In most high-income countries, prospective parents do not prepare for pregnancy [47]; this is even more true of many low-middle income countries. In the UK, however, it is suggested that more than two-thirds of pregnancies are in fact planned to some degree, at least in the sense that contraception has not been routinely used [48].

1.4 Meeting the Challenge

Most societies do not have in place coordinated schemes to promote the health of adolescents and young people, in particular before conception [49], as this is assumed to be part of routine public health primary care, which is not always the case [50]. This is an important missed opportunity to prepare for pregnancy, to promote healthy pregnancy and to ensure healthy outcomes [51, 52].

Formal educational programmes delivered through schools have only had small effects on, for example, levels of obesity and risky behaviours, and it appears that integration of such programmes more widely into the community and involving parents will improve success [53–55]. New integrated pedagogical approaches are necessary to promote health literacy, for example through linking schools and health researchers through out-of-classroom activities which incorporate continuing professional development for science teachers, hands-on exposure to research methods and ‘meet the scientist’ encounters for school age students [56].

There are three interrelated policy implications in addressing the challenge of parental obesity, which need to be considered simultaneously, both in terms of their implementation and their assessment (Fig. 1.2). While concern about the impact of parental obesity is a global issue, these components will have to be implemented at the level of national and local governments, in order to make them culturally specific. They are:

(a) *Profile and priority*. Make the health of adolescents and young women and their partners a national priority, on a par with events and movements which promote an image of vital, active collective life, e.g. the Olympic Games. This requires establishment of national organisations with professional representation from health, education, communities and local governments, media, sport and the private sector.
(b) *Create demand* by investing in health literacy promotion through education programmes in schools linked to community-based initiatives involving a range

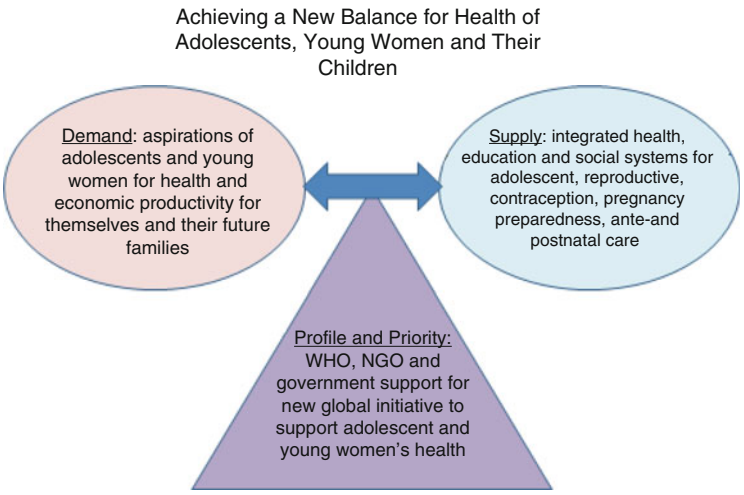

Fig. 1.2 Achieving a new balance for health of adolescents, young women and their children. Promoting health at this time in the life course requires a balance between the supply of health, education and social care services and the demand for such provision based on appreciation of their importance for health and prosperity. This balance needs to be supported through the profile and priority given to it by a range of government and other organisations

of organisations and sponsors. This investment is predicated on the projected return through reduced health care and other costs of reducing parental and childhood obesity, adverse pregnancy outcomes and early markers of NCD risk.

(c) *Supply*. Establish integrated systems for the provision of health care to adolescents and women before conception, throughout pregnancy and delivery and after birth, linked to family planning and sexual health services, primary care and wider community organisations.

1.5 Conclusion

The challenge posed by parental obesity, and obesity in parents-to-be, requires urgent action, because such obesity does not augur well for the health of these adolescents and young adults in the future. As this section of the population have a substantial proportion of their lives ahead of them, the costs of NCDs in terms of their well-being, productivity and longevity as well as the direct health-care costs will be very hard to meet, even in high-income countries. Worse, such ill health passes the risk of overweight and obesity to their children by a range of mechanisms. There is a need to establish a new approach to meeting this challenge, in terms of raising the profile and priority accorded to the issue at the level of national and local governments and in conjunction with organisations such as WHO; creating awareness of the problem and a desire to address it among young people, especially adolescents; and providing an integrated health-care delivery system

linked to education and wider community initiatives to ensure that parents do not miss the opportunity for health promotion at a time in their lives which is critical for them and their children.

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Chapter 2
Maternal Obesity During Pregnancy and Cardiometabolic Development in the Offspring

Romy Gaillard and Vincent W. Jaddoe

Abstract Maternal obesity during pregnancy is a major public health problem worldwide. In Western countries, obesity prevalence rates in pregnant women are estimated to be as high as 30 %. In addition, it is estimated that in these countries approximately 40 % of women gain an excessive amount of gestational weight. An accumulating body of evidence strongly suggests a long-term impact of maternal obesity and excessive weight gain during pregnancy on adiposity and cardiometabolic related health outcomes in the offspring throughout the life course. Maternal obesity during pregnancy may lead to developmental adaptations in the offspring, predisposing to an increased risk of adverse cardiometabolic outcomes in later life. Thus far, it remains unclear whether these associations are explained by causal underlying mechanisms or reflect confounding by various family-based socio-demographic, nutritional, lifestyle-related and genetic characteristics. Further research to explore the causality, underlying mechanisms, and potential for prevention of cardiometabolic disease in future generations by reducing maternal obesity and excessive weight gain during pregnancy is needed.

Keywords Adverse pregnancy outcomes • Adult obesity • Cardiovascular risk factors • Childhood obesity • Excessive gestational weight gain • Maternal obesity • Paternal obesity • Pregnancy

R. Gaillard, PhD (✉) • V.W. Jaddoe, MD, PhD
The Generation R Study Group (Na 29-15), Erasmus University Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands

Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands

Department of Pediatrics, Erasmus University Medical Center, Rotterdam, The Netherlands
e-mail: r.gaillard@erasmusmc.nl

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2.1 Introduction

Overweight, defined as a body mass index of 25.0–29.9 kg/m^2, and obesity, defined as a body mass index of 30.0 kg/m^2 or higher, are major public health problems worldwide. Over the past decades, the obesity prevalence has strongly increased in both high- and low-income countries. In 2014, the Word Health Organization estimated that more than 1.9 billion adults were overweight, of which over 600 million were obese [1]. The strong increase in obesity prevalence is also present among women of reproductive age. A large study combining data from nine states in the USA showed that from 1993 to 2003 there was a rise of 70 % in the rate of maternal obesity at the start of pregnancy [2]. Currently, obesity prevalence rates among women of reproductive age and at the start of pregnancy are estimated to be as high as 30 % in Western countries [3–5]. Next to prepregnancy obesity, it is estimated that in these countries approximately 40 % of women gain an excessive amount of gestational weight, based on the US Institute of Medicine (IOM) guidelines [6]. The IOM guidelines define optimal ranges of maternal weight gain during pregnancy according to a mother's prepregnancy body mass index and have been established based on evidence from observational studies that relate gestational weight gain to various maternal and offspring outcomes [6] (Table 2.1).

Both maternal prepregnancy obesity and excessive gestational weight gain may adversely affect fetal development through an excessive nutritional in utero environment. An accumulating body of evidence suggests that maternal obesity during pregnancy has persistent effects on various offspring outcomes [7, 8]. This chapter is focused on the associations of maternal obesity and excessive weight gain during pregnancy with cardiometabolic development in the offspring throughout the life course. Results from recent observational studies, with a specific focus on the Generation R Study, the causality, potential underlying mechanisms of the observed associations and challenges for future studies are discussed. This chapter is largely based on our previous reviews on this topic [7, 8].

Table 2.1 Institute of medicine criteria for gestational weight gain[a]

Prepregnancy body mass index	Recommended amount of total gestational weight gain in kg
Underweight (Body mass index $<$ 18.5 kg/m^2)	12.5–18
Normal weight (Body mass index $\geq$ 18.5–24.9 kg/m^2)	11.5–16
Overweight (Body mass index $\geq$ 25.0–29.9 kg/m^2)	7–11.5
Obesity (Body mass index $\geq$ 30.0 kg/m^2)	5–9

[a]Recommended gestational weight gain guidelines according to women's prepregnancy body mass index. Adapted from the IOM criteria [6]

2.2 The Generation R Study

The Generation R Study is a multi-ethnic population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands [9]. The Generation R Study is designed to identify early environmental and genetic determinants of growth, development, and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment was aimed at early pregnancy, but was possible until the birth of the child. In total, 9778 mothers were enrolled in the study, of whom 8879 were included during pregnancy. During pregnancy, multiple assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18–25 weeks of gestation), and late pregnancy (≥25 weeks of gestation) and included parental physical examinations, fetal ultrasound examinations, and self-administered questionnaires. In the preschool period, from birth to 4 years of age, data collection was performed in all children by questionnaires and visits to the routine child health-care centers. All children were invited to a dedicated research center in the Erasmus MC—Sophia Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements at the age of 6 years. Measurements during this visit included anthropometrics, body composition by Dual Energy X-ray Absorptiometry and ultrasound, and measurements focused on cardiovascular development.

In the Generation R Study, the overall prevalence of maternal prepregnancy overweight and obesity is approximately 28 % [10]. There are large ethnic differences in maternal prepregnancy overweight and obesity prevalence. Among Dutch-origin women, the overweight and obesity prevalence is approximately 23 %. Higher prevalences of prepregnancy overweight and obesity are present among Cape Verdean-origin, Dutch Antillean-origin, Moroccan-origin, Surinamese-Creole-origin, and Turkish-origin women [4] (Fig. 2.1). The overall prevalence of excessive maternal gestational weight gain according to the IOM criteria within the Generation R Study is approximately 44 % [10]. As compared to Dutch-origin women, Moroccan-origin women and Surinamese-Hindustani-origin women tend to have a lower risk of excessive gestational weight gain [4].

2.3 Maternal Prepregnancy Body Mass Index

Many observational studies have shown that maternal prepregnancy obesity is an important risk factor for a variety of adverse fetal outcomes (Fig. 2.2). Based on these observational studies, multiple large meta-analysis have been performed. A meta-analysis focused on stillbirth among nine observational studies showed that the odds ratio of stillbirth was 2.07 [95 % Confidence Interval (CI): 1.59, 2.74] among obese pregnant women, as compared to normal-weight pregnant women [11]. In line with this meta-analysis, a large meta-analysis among 38 cohort studies

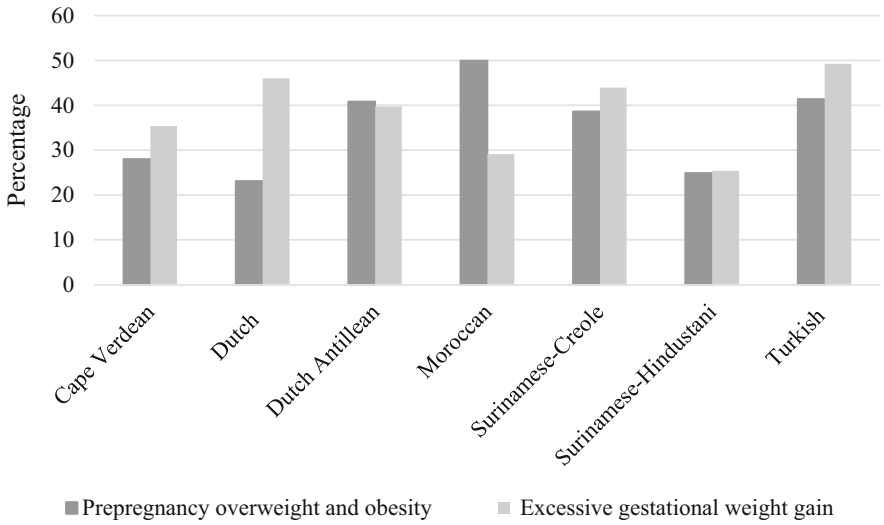

Fig. 2.1 Maternal obesity during pregnancy in the Generation R Study. Percentages of maternal prepregnancy overweight and obesity and excessive gestational weight gain among the largest ethnic groups within the Generation R Study [4]

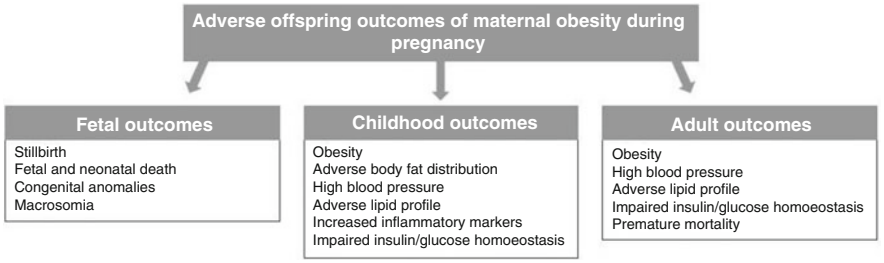

Fig. 2.2 Maternal obesity during pregnancy and adverse cardiometabolic outcomes in the offspring

in total with over 10,147 fetal deaths, 16,274 stillbirths, and 11,294 neonatal deaths showed that the risk of fetal death was 1.21 (95 % CI: 1.09, 1.35), the risk of stillbirth was 1.24 (95 % CI: 1.18, 1.30), and the risk of neonatal death was 1.15 (95 % CI: 1.07, 1.23) per 5-unit increase in maternal prepregnancy or early-pregnancy body mass index [12]. Maternal obesity was associated with an increased risk of a number of congenital anomalies in a meta-analysis among 18 observational studies, including neural tube defects, cardiovascular anomalies, cleft palate, hydrocephaly, and limb reduction anomalies [13]. A recent meta-analysis among 13 studies showed that, as compared to a normal maternal prepregnancy weight, maternal prepregnancy obesity was associated with a twofold higher risk of delivering a large size for gestational age infant [14].

Within the Generation R Study, we assessed the associations of maternal prepregnancy body mass index with fetal growth characteristics in each trimester of pregnancy. Maternal prepregnancy body mass index was not associated with first-trimester fetal crown-to-rump length [15]. Higher maternal prepregnancy body mass index was associated with a higher estimated fetal weight from mid-pregnancy onward, with stronger associations with advancing gestation [16]. Maternal prepregnancy obesity was also associated with an increased risk of cesarean delivery, preterm delivery, delivering a large size for gestational age infant, and a low APGAR score [10].

Maternal prepregnancy obesity is strongly associated with the risk of obesity in the offspring [7, 8]. A meta-analysis among four studies showed that maternal prepregnancy obesity was associated with a threefold higher risk of childhood obesity [17]. Also, multiple studies have shown that a higher maternal prepregnancy body mass index is associated with a higher body mass index in adolescent and adult offspring, independent from socio-demographic and lifestyle-related confounding factors [18–20]. A study among 1400 mothers and their adult offspring showed that offspring of mothers within the highest maternal prepregnancy body mass index quartile had a 5 kg/m^2 higher mean body mass index at the age of 32 years, as compared to offspring of mothers within the lowest maternal prepregnancy body mass index quartile [18].

The associations of maternal prepregnancy obesity with other cardiometabolic outcomes in the offspring have been studied less extensively [7, 8]. Within the Generation R Study, we showed that a higher maternal prepregnancy body mass index was associated with a higher offspring total body fat mass and android/gynoid fat mass ratio measured by Dual Energy X-ray Absorptiometry, and a higher abdominal subcutaneous and preperitoneal fat mass, a measure of visceral fat mass, at the age of 6 years [21]. Also, a higher maternal prepregnancy body mass index was associated with a higher childhood systolic blood pressure and insulin levels and lower HDL cholesterol levels. As compared to children from normal-weight mothers, children from obese mothers had an increased risk of clustering of cardiometabolic risk factors [OR 3.00 (95 % CI: 2.09, 4.34)], a measure of a metabolic syndrome like phenotype. The associations of maternal prepregnancy body mass index with childhood cardiometabolic risk factors were largely mediated by childhood concurrent body mass index [21]. A study among 1090 mother–child pairs participating in a pre-birth cohort in the USA showed that a higher maternal prepregnancy body mass index was also associated with higher mid-childhood leptin, high sensitivity C-reactive protein and interleukin-6 levels, and lower adiponectin levels [22].

Similar associations have been reported in adolescence and adulthood [7, 8]. A study among 4452 mothers and their adolescent offspring in Brazil showed that a higher maternal prepregnancy body mass index was associated with a higher adolescent systolic and diastolic blood pressure in boys and girls [23]. Among 1392 Australian mothers and their adolescent offspring, it was shown that a higher maternal prepregnancy body mass index was associated with a higher adolescent waist circumference, waist to hip ratio, systolic blood pressure, insulin, glucose,

and HOMA-IR levels at the age of 17 years [24]. A study among 1400 mother–offspring pairs in Jerusalem showed that maternal prepregnancy body mass index was positively associated with waist circumference, systolic and diastolic blood pressure, insulin, and triglycerides and negatively with HDL cholesterol in the offspring at the age of 32 years [18]. In line with findings from studies focused on childhood outcomes, these studies focused on adolescent and adult outcomes showed that additional adjustment for offspring concurrent body mass index attenuated the associations of maternal prepregnancy body mass index with offspring cardiometabolic risk factors. A study using birth records from 37,709 participants showed that a higher maternal body mass index at the first antenatal visit was associated with a higher risk of premature all-cause mortality and hospital admissions for cardiovascular events in adult offspring, with a hazard of all-cause mortality in offspring of obese mothers of 1.35 (95 % CI: 1.17, 1.55), as compared to offspring from mothers with a normal body mass index at the first antenatal visit [25]. These associations were not explained by adjustment for maternal age at delivery, socioeconomic status, sex of offspring, current age, birth weight, gestational age at delivery, and gestational age at measurement of maternal body mass index, but no information on concurrent body mass index of adult offspring was available [25].

Thus, maternal prepregnancy obesity is associated with increased risks of adverse fetal outcomes, adiposity, and adverse cardiometabolic development in childhood, adolescence, and adulthood and premature death in adulthood. The associations of maternal prepregnancy body mass index with offspring cardiometabolic risk factors seem to be largely explained by offspring body mass index.

2.4 Maternal Gestational Weight Gain

Next to maternal prepregnancy body mass index, excessive maternal weight gain during pregnancy may be an independent risk factor of adverse fetal development and cardiometabolic development from childhood onwards (Fig. 2.2) [7, 8]. Different measures of maternal weight gain during pregnancy have been studied. Most studies have focused on the associations of excessive maternal weight gain during pregnancy defined according to the IOM criteria. However, from a research perspective, the IOM criteria for excessive gestational weight gain have important limitations [26]. As the IOM criteria for excessive gestational weight gain combine prepregnancy body mass index and gestational weight gain, it is not possible to study the distinct effects of maternal prepregnancy body mass index and gestational weight gain on offspring outcomes [26]. In addition, it is not possible to identify critical periods of maternal weight gain for offspring outcomes. Recently, more studies have therefore also focused on more detailed measures of maternal weight gain during pregnancy.

Excessive maternal gestational weight gain is associated with several adverse fetal outcomes, but associations seem to be weaker and less consistent as compared to the associations of maternal prepregnancy body mass index [7, 8]. Excessive gestational weight gain is most consistently associated with an increased risk of delivering a large size for gestational age infant. A meta-analysis among 15 cohort and case–control studies showed that excessive gestational weight gain based on the IOM criteria was associated with a 2.35 (95 % CI: 1.95, 2.85) higher risk of macrosomia [27]. Thus far, excessive gestational weight gain seems not to be associated with fetal death or stillbirth [28, 29]. A meta-analysis among 24 cohort studies and 14 case–control studies showed that not a high total gestational weight gain but a high weekly gestational weight gain was associated with an increased risk of preterm birth [30]. A study among 20,465 nondiabetic, term, singleton-born infants showed that excessive gestational weight gain according to the IOM criteria was associated with adverse neonatal outcomes, such as a low 5-min APGAR score and neonatal hypoglycemia [31]. Within the Generation R Study, we observed that excessive maternal weight gain during pregnancy according to the IOM criteria was associated with a higher risk of cesarean delivery and large size for gestational age at birth, but a lower risk of preterm birth and small size for gestational age at birth [10]. When we assessed the trimester-specific effects of maternal weight gain during pregnancy, we observed that especially higher second- and third-trimester maternal weight gain was associated with an increased risk of delivering a large size for gestational age infant [10].

A meta-analysis among 12 studies showed that as compared to a recommended amount of gestational weight gain according to the IOM criteria, excessive gestational weight gain was associated with a 33 % increased risk of childhood obesity [32]. A systematic review among seven studies also assessed the associations of total gestational weight gain with the risk of childhood obesity and showed that an additional kilogram increase in total gestational weight gain was associated with a higher child's BMI *z*-score of 0.006–0.06 units and increased the risk of childhood overweight or obesity by 1–23 % after adjustment for potential confounding factors [33]. The associations of excessive gestational weight gain or total gestational weight gain with more detailed childhood fat mass measures, blood pressure, lipid levels, insulin resistance, and inflammatory markers are less consistent and, if present, seem to be largely mediated by childhood body mass index [22, 34–40].

Similarly, increased maternal weight gain during pregnancy has been associated with higher offspring adiposity levels and cardiovascular risk factors in adulthood [18, 20, 41–43]. A study among 1540 Danish mothers and their offspring showed that per kilogram increase in maternal gestational weight gain the odds ratio for obesity at the age of 42 years was 1.08 (95 % CI: 1.03–1.14) [20]. This association was only partly explained by offspring birth weight and body mass index up to 14 years of age [20]. A study among 2432 Australians showed that higher maternal gestational weight gain was independent from maternal prepregnancy body mass index, associated with a higher body mass index, and tended to be associated with a higher systolic blood pressure in the offspring at the age of 21 years [43]. A study among 1400 mother–offspring pairs in Jerusalem showed higher maternal

gestational weight gain was only associated with increased adiposity levels in the offspring aged 32 years, but not with other cardiovascular risk factor [18]. Another study among 308 Danish mother– offspring pairs, which assessed the associations of maternal weight gain among normal-weight women, showed that a higher maternal weight gain was associated with higher insulin levels and leptin levels among male offspring only [41].

Several studies aimed to identify critical periods of maternal weight gain during pregnancy for childhood and adolescent outcomes [7, 8]. Within the Generation R Study, we showed among 5908 mother–offspring pairs that independent from maternal prepregnancy weight and weight gain in later pregnancy, early-pregnancy weight gain was associated with higher adiposity levels and an adverse cardiometabolic profile at the age of 6 years [39]. In line with our findings, a study performed among 5154 UK mother–offspring pairs showed that gestational weight gain in the first 14 weeks of pregnancy was positively associated with offspring body mass index, waist circumference, and fat mass at the age of 9 years [35]. A study among 977 mother–child pairs from Greece showed that maternal first-trimester weight gain was associated with an increased risk of childhood obesity and a higher childhood diastolic blood pressure from 2 to 4 years [44]. A Finnish study among 6637 mothers and their adolescent offspring showed that maternal weight gain of >7 kg in the first 20 weeks of gestation was associated with the risk of offspring overweight and abdominal adiposity at the age of 16 years [45]. A study among 1392 Australian mothers and their adolescent offspring showed that higher maternal weight gain rate in early but not in mid-pregnancy was associated with greater adiposity levels and an increased risk of being in the high-metabolic risk cluster, a proxy measure of the metabolic syndrome at 17 years [24]. These studies suggest that especially maternal weight gain during early pregnancy, when maternal fat accumulation forms a relatively large component of gestational weight gain, may be a critical period for an adverse cardiovascular risk profile in the offspring.

Thus, next to maternal prepregnancy obesity, excessive maternal weight gain during pregnancy may also lead to increased risks of adverse fetal outcomes, adiposity, and adverse cardiovascular risk factors in childhood, adolescence, and adulthood. The adverse effects of maternal weight gain during pregnancy may depend upon the timing of gestational weight gain. Overall, maternal prepregnancy obesity appears to be more strongly associated with adverse offspring outcomes than excessive gestational weight gain. Importantly, both the associations of maternal body mass index and gestational weight gain with offspring outcomes seem not to be only restricted to maternal obesity or excessive gestational weight gain, but are present across the full-range of maternal body mass index and gestational weight gain [7, 8].

2.5 Causality of the Observed Associations

Despite the large number of observational studies reporting these associations, limitations in these studies need to be considered. The most important limitation of these observational studies is confounding of the observed associations [7, 8]. Various family-based socio-demographic, nutritional, lifestyle-related, and genetic characteristics may explain the observed associations of maternal prepregnancy body mass index and gestational weight gain with adverse offspring health outcomes [7, 8]. Multiple more sophisticated study designs can be used to obtain further insight into the role of confounding in the observed associations, including sibling comparison studies, maternal and paternal offspring comparison analyses, Mendelian randomization studies, and randomized controlled trial analyses, as we described previously [7, 8].

2.5.1 Sibling Comparison Studies

The main advantage of sibling comparison studies is their ability to better control for potential confounding factors, such as environmental characteristics as well as maternal genotype, shared within families [46]. A sibling comparison study focused on severe maternal prepregnancy obesity showed among children from mothers who had high levels of prepregnancy weight loss due to biliopancreatic bypass surgery that the risk of overweight and obesity and adverse cardiometabolic risk factors was higher in children born to mothers before surgery than those born to mothers after surgery [47, 48]. A sibling comparison study among 513,501 mothers and their 1,164,750 children showed that children born to mothers who gained more than 24 kg during pregnancy were approximately 148 g (95 % CI: 141.7, 156.0) heavier at birth than were children born to mothers who gained 8–10 kg [49]. A sibling comparison study among 42,133 women who had more than one singleton pregnancy and their 91,045 offspring showed that higher maternal total gestational weight gain was associated with a higher body mass index in childhood, where every additional kilogram of gestational weight gain increased childhood BMI by 0.0220 kg/m^2 (95 % CI 0.0134–0.0306) [50]. This association was only partly mediated by offspring birth weight. A study using a sibling comparison design among 280,866 singleton-born Swedish men showed that a higher maternal body mass index in early pregnancy was not associated with higher offspring body mass index at the age of 18 years within siblings, but only in the whole cohort and between non-siblings [51]. This suggests that the association may be explained by confounding environmental characteristics [51]. However, among the same study population it was also shown that among overweight and obese mothers, higher total gestational weight gain was associated with higher offspring body mass index at the age of 18 years among siblings, which suggests a possible intrauterine effect for gestational weight gain [52]. Findings from these sibling comparison studies

suggest that especially gestational weight gain may affect offspring outcomes through direct intrauterine mechanisms. An important limitation of sibling comparison studies is that next to the major exposures of interest, maternal prepregnancy body mass index and gestational weight gain, also other lifestyle-related characteristics may differ between pregnancies [7, 8].

2.5.2 Parent–Offspring Comparison Studies

As an aid to further disentangle underlying mechanisms, the strength of associations of maternal and paternal body mass index with offspring outcomes can be assessed [53]. Stronger associations for maternal body mass index suggest direct intrauterine mechanisms, whereas similar or stronger associations for paternal body mass index suggest a role for shared family-based, lifestyle-related characteristics or genetic factors [54]. Multiple studies compared the associations of maternal and paternal body mass index with childhood body mass index and have shown conflicting results [55]. However, studies examining these associations with more detailed childhood fat mass measures have shown that maternal prepregnancy body mass index tends to be more strongly associated with childhood total fat mass than paternal body mass index [21, 56, 57]. Within the Generation R Study, we observed that maternal prepregnancy body mass index was more strongly associated with childhood body mass index, total body fat mass, android–gynoid fat mass ratio, and clustering of cardiometabolic risk factors than paternal prepregnancy body mass index [21]. These findings suggest that some of the effects of maternal prepregnancy obesity on offspring outcomes may be through direct intrauterine mechanisms [7, 8].

2.5.3 Mendelian Randomization Studies

Mendelian randomization studies are studies in which genetic variants, known to be robustly associated with the exposure of interest and not affected by confounding, are used as an instrumental variable for a specific exposure [58]. Associations of these genetic variants with the outcomes of interest support causality for these associations. A study among 4091 mother–offspring pairs showed no association of maternal FTO with childhood fat mass at the age of 9 years [57]. Thus far, no other Mendelian randomization studies on these specific associations have been performed.

2.5.4 *Randomized Controlled Trials*

Randomized controlled trials are considered as the golden standard to assess causality. Previous randomized controlled trials have focused on influencing determinants of maternal obesity and excessive weight gain during pregnancy, such as dietary factors and physical activity levels, since directly randomized studies are difficult to perform with maternal prepregnancy obesity and excessive gestational weight gain as major exposures of interest [59]. A meta-analysis of multiple randomized controlled trials showed that dietary and physical activity interventions aimed at reducing maternal weight gain during pregnancy may lead to small reductions in the amount of gestational weight gain and to a slightly lower risk of adverse pregnancy outcomes [59]. In this meta-analysis, dietary interventions appeared to be more effective than physical activity-related interventions [59]. A recent Cochrane review also suggested that interventions during pregnancy focused on diet or exercise, or combined, can reduce the risk of excessive gestational weight gain [60]. However, whether these interventions also have a beneficial effect on long-term offspring health outcomes remains unclear. A small randomized controlled trial among 254 mothers and their children, which provided both dietary advice and exercise during pregnancy to obese women, observed no difference in body mass index or metabolic risk factors in infant offspring, when compared to the control group and an external reference group of normal-weight women [61].

Taken together, results from these studies specifically designed to explore the causality for the associations of maternal prepregnancy body mass index and gestational weight gain with offspring outcomes remain inconclusive [7, 8].

2.6 Underlying Mechanisms

The mechanisms underlying the associations of maternal prepregnancy obesity or excessive gestational weight gain with cardiometabolic disease in the offspring remain unclear. The fetal overnutrition hypothesis suggests that in obese mothers and mothers with high levels of gestational weight gain an increased placental transfer of nutrients to the developing fetus may subsequently affect fetal development, fetal fat deposition, and the development of the hypothalamic–endocrine system that controls appetite and energy metabolism [26, 62, 63]. These adaptations may predispose individuals to a greater risk of adverse cardiometabolic outcomes in later life. Figure 2.3 shows potential mechanisms that might be involved in the associations of higher maternal prepregnancy body mass index and gestational weight gain with adverse cardiometabolic development in the offspring [8]. As described previously, the following maternal exposures and underlying mechanisms may have an important role [8].

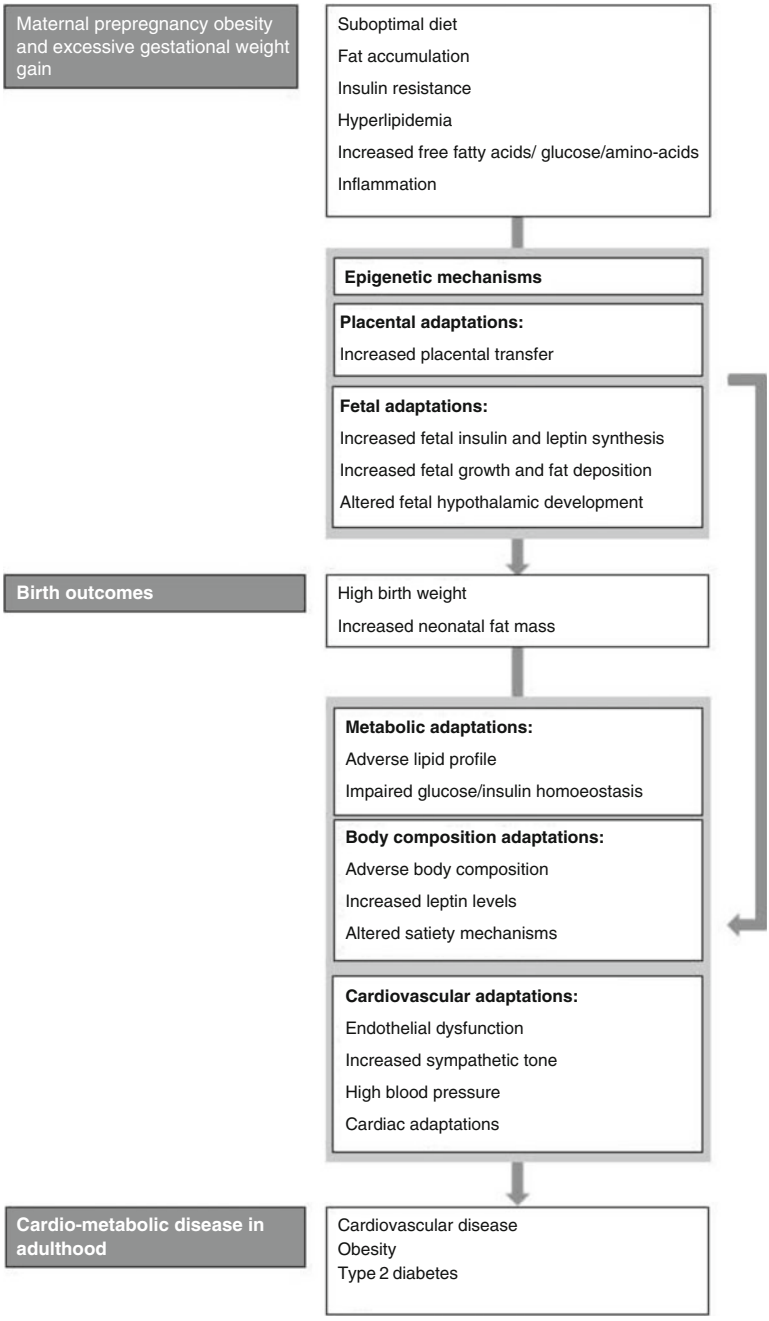

Fig. 2.3 Maternal obesity during pregnancy and offspring developmental adaptations. Conceptual model for potential underlying mechanisms for the associations of maternal obesity during pregnancy with adverse cardio-metabolic health outcomes in offspring. Adapted from [8]

2.6.1 Maternal Exposures

Both maternal prepregnancy obesity and excessive gestational weight gain are complex traits [8]. Maternal prepregnancy obesity not only reflects maternal fat accumulation, but also other maternal characteristics, such as maternal nutritional status, insulin and glucose metabolism, and low-grade systemic inflammation. Similarly, maternal weight gain during pregnancy reflects maternal fat accumulation, but also maternal and amniotic fluid expansion and growth of the fetus, placenta, and uterus [6]. Multiple studies aimed to study the associations of more detailed exposures related to maternal prepregnancy obesity and excessive gestational weight gain with various offspring outcomes [8].

Maternal fat accumulation during pregnancy is important for fetal nutrient supply and fetal development [64]. However, during pregnancy, fat accumulation predominantly occurs centrally [64]. Central fat accumulation is well known to be associated with adverse cardiometabolic outcomes and appears to have similar adverse consequences in pregnant women [65]. These metabolic disturbances may involve insulin resistance and dyslipidemia, which leads to higher maternal circulating levels of free fatty acids, amino acids, and glucose, which affect placental and fetal development [64]. Multiple observational studies, including studies using a sibling comparison design, have shown that gestational diabetes, glycosuria, and higher maternal fasting glucose levels during pregnancy are associated with higher weight and c-peptide levels at birth and higher body mass index, fat mass level, fasting glucose and insulin levels, and the risk of type 2 diabetes in later life [51, 66–71]. Small observational studies have also shown that higher maternal triglyceride and amino acid levels are associated with a higher birth weight and neonatal fat mass [72–75]. Thus, maternal fat accumulation and metabolic factors during pregnancy may have persistent effects on offspring cardiometabolic development [8].

Maternal obesity during pregnancy may be an indicator of a poor quality maternal diet [8]. A Western dietary pattern and macronutrients and micronutrients intake related to a Western diet have been suggested to influence offspring fat deposition, adipocyte function, pancreatic function, and food preference [62, 76]. A maternal diet during pregnancy which is high in saturated fat and sugar intake is associated with an increased risk of obesity in the offspring [77]. Also, a maternal diet with low *Omega-3* and high *Omega-6* fatty acids intake seems to be associated with an increased risk of childhood obesity [78–80]. A study among 906 UK mother–child pairs showed that higher maternal dietary glycemic index and glycemic load in early pregnancy, but not later in pregnancy, were associated with higher fat mass in children at the age of 4 and 6 years [81]. A study among approximately 3000 parents and their children showed that maternal protein, fat, and carbohydrate dietary intake during pregnancy, but not paternal dietary intake, was associated with child's dietary intake of the same macronutrients [82]. The associations of maternal dietary intake during pregnancy with child's dietary intake were also stronger than the associations of maternal postnatal dietary intake, which suggest

that in utero mechanisms may play a role in the programming of offspring appetite [82]. Altogether, these studies suggest that various measures reflecting a suboptimal dietary status in pregnant women are associated with adverse cardiovascular and metabolic outcomes in offspring [8].

Obesity is associated with low-grade systemic inflammation and oxidative stress, also during pregnancy [83–85]. Additionally, pregnancy itself leads to a state of mild maternal systemic inflammation, which may interact with obesity-mediated inflammatory mechanisms [86–89]. Thus far, it has been shown that maternal inflammatory markers during pregnancy correlate with fetal growth and neonatal fat mass [90, 91], but the effects at older ages are less clear and remain to be further explored [92].

2.6.2 Programming Mechanisms

Both maternal prepregnancy obesity and excessive gestational weight gain as well as the correlated maternal exposures may lead to programming effects in the offspring through several pathways [8].

Epigenetic mechanisms, which involve modifications due to early environmental influences to DNA and its associated proteins that regulate gene activity, are likely to play a key role in developmental programming of adverse cardiometabolic outcomes [93, 94]. Thus far, animal studies provide support for epigenetic modifications due to maternal obesity or a high-fat diet, but this has not been explored in large human studies [93]. Small studies among pregnant women showed that maternal obesity and impaired maternal glucose tolerance induced epigenetic changes of placental genes [88, 95–97]. A human study among 88 mother–child pairs suggested that only maternal weight gain in early pregnancy might be associated with epigenetic modifications in offspring cord blood [98]. Epigenetic modifications together with other mechanisms may thus be involved in adiposity and cardiovascular and metabolic developmental adaptations [8].

Offspring from mothers with prepregnancy obesity or excessive gestational weight gain are at increased risk of being born large for their gestational age, which itself is associated with an increased risk of obesity in later life [99]. The associations of maternal obesity during pregnancy with the risk of obesity in childhood and adulthood may thus be explained by tracking of body size and fatness throughout the life course [8]. However, many observational studies have shown that additional adjustment for birth weight does not explain the observed associations [7, 8]. The lack of effect of adjustment for birth weight may partly be explained by birth weight not accurately reflecting neonatal fat mass, but might also suggest that other mechanisms are involved in these associations [62]. Animal studies have suggested that maternal obesity during pregnancy may affect both offspring adipocyte morphology and metabolism, which may influence the development of obesity and insulin resistance in the offspring [8, 63]. Next to altered growth and adipocyte function, altered appetite control may be a key factor in

developmental programming of obesity [8]. A maternal hypercaloric diet during pregnancy and overfeeding in the fetal and early postnatal period may lead to hyperphagia and altered satiety mechanisms through adverse programming of the hypothalamus by high fetal and infant leptin and insulin levels [62, 100].

The associations of maternal prepregnancy body mass index and gestational weight gain with adverse cardiovascular and metabolic outcomes in the offspring appear to be largely mediated through offspring adiposity [8]. However, direct cardiovascular and metabolic programming effects of maternal obesity during pregnancy may also be present [63]. Thus far, mainly animal studies have shown that maternal obesity, a maternal high-fat diet, and increased maternal glucose transport during pregnancy are associated with offspring high blood pressure, endothelial dysfunction, increased aortic stiffness, cardiac hypertrophy, impaired glucose and insulin homeostasis, and measures related to non-alcoholic fatty liver disease [8, 63, 100, 101].

Thus, multiple mechanisms may be involved in the intrauterine pathways leading from maternal obesity and excessive weight gain during pregnancy to long-term adverse offspring health outcomes [8]. These underlying mechanisms have mainly been studied in animal models and remain to be further explored in large human studies.

2.7 Challenges for Future Epidemiological Research

Current evidence from epidemiological studies suggests that maternal obesity and excessive weight gain during pregnancy have important adverse consequences on cardiometabolic development from fetal life onwards, leading to disease in later life [7, 8]. However, there remain important issues to be addressed [7, 8]. These include examining the extent of causality of the observed associations, the underlying exposures and their critical periods, the developmental adaptations, and the potential for development of preventive strategies (Table 2.2) [7, 8].

First, despite extensive adjustment for potential confounding factors in these observational studies, residual confounding may still be an issue [7, 8]. The causality of the observed associations needs to be further addressed. For this purpose,

Table 2.2 Key points for future research[a]

Observational studies using sophisticated study designs to obtain further insight into the causality of the observed associations
Detailed maternal exposures and offspring outcomes measurements to obtain further insight into the specific exposures and their critical periods, and the underlying mechanisms of the observed associations
Long-term follow-up of participants in trials focused on reducing maternal weight throughout pregnancy to assess causality of the observed associations and the effectiveness of maternal lifestyle interventions during pregnancy for improving long-term health outcomes of offspring

[a]Adapted from [8]

large observational studies that are able to conduct sophisticated analyses, such as sibling comparison analyses, parent–offspring comparison analyses, and Mendelian randomization analyses, are needed. In addition, meta-analyses among large numbers of observational studies will provide further insight into the strength, consistency, and independency of these associations. Long-term follow-up of mothers and their children participating in randomized controlled trials focused on reducing maternal weight throughout pregnancy will also provide further insight into the causality of the associations [7, 8].

Second, the mechanisms underlying the observed associations of maternal prepregnancy obesity and excessive gestational weight gain with offspring health outcomes remain to be further explored [7, 8]. Animal studies have identified a number of pathways that may be involved in these associations, but these pathways remain largely unexplored in humans. Maternal prepregnancy obesity and excessive gestational weight gain are complex traits, which reflect multiple biological and lifestyle-related components, which complicates identification of potential underlying pathways [7, 8]. Future studies with more detailed assessments of the maternal exposures and offspring outcomes throughout the life course could provide further insight into potential underlying mechanisms. To obtain further insight into the different maternal components associated with offspring outcomes and their critical periods, detailed repeated measurements of maternal weight and body composition, nutritional status, metabolic measures, inflammatory measures, and pregnancy-related hemodynamic adaptations are needed. Since early pregnancy appears to be a critical period for offspring outcomes, studies are needed with detailed maternal measurements from early pregnancy onward to already assess their influence on placental and embryonic growth and development. For the offspring outcomes, more detailed measurements of fetal and postnatal growth, body composition, and cardiometabolic factors, such as cardiac structures, endothelial function, lipid spectrums, and glucose responses, might also lead to further insight into the underlying growth, vascular, and metabolic mechanisms present in the observed associations. Long-term follow-up of the offspring in observational studies is needed to assess the influence of maternal prepregnancy obesity and excessive gestational weight gain on cardiovascular and metabolic development throughout the life course [7, 8].

Third, further research is needed focused on prevention of adverse health outcomes in offspring through optimizing maternal prepregnancy body mass index, gestational weight gain, and dietary intake during pregnancy [7, 8]. The optimal amounts of maternal weight gain for short-term and long-term maternal and offspring health outcomes need to be examined to improve the IOM recommendations for gestational weight gain [7, 8]. Identification of specific maternal dietary components associated with offspring health outcomes will aid in the improvement of maternal dietary recommendations during pregnancy [7, 8]. Long-term follow-up of mothers and their children participating in randomized controlled trials focused on improving maternal diet and reducing maternal weight throughout pregnancy will provide insight into the effectiveness of these maternal lifestyle interventions during pregnancy for improving long-term health of offspring [7, 8].

2.8 Conclusions

Based on current evidence from observational studies, maternal prepregnancy obesity and excessive gestational weight gain seem to be risk factors for an adverse in utero environment and long-term adverse cardiometabolic outcomes in the offspring. Well-designed studies are needed to identify the extent of causality of the observed associations, the underlying exposures and their critical periods, the developmental adaptations, and the potential for development of preventive strategies to improve long-term health outcomes of offspring.

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Chapter 3
Maternal Obesity and Gestational Weight Gain as Determinants of Long-Term Health

Nashita Patel and Lucilla Poston

Abstract This chapter addresses the prospect that obesity may begin at the earliest stages of life and that one of the determinants may be exposure to maternal obesity in utero or to extremes of maternal gestational weight gain.

Keywords Pregnancy • Obesity • Developmental programming • Gestational weight gain • Offspring

3.1 The Global Prevalence of Maternal Obesity

Obesity is defined as a Body Mass Index (BMI) $\geq$30 kg/m^2. The increase in obesity is a major health concern, having reached epidemic proportions with the prevalence having doubled globally in the last 20 years. The World Health Organization has estimated that 300 million adults of the 1.5 billion adults defined as having a BMI >25 kg/m^2 are obese [1] with this trend continuing to increase. In 2013, an estimated one in five women in the world aged 20 years or above were obese [2]. The rates are greatest in high-income countries, including 33.9 % of women in the United States of America (USA) and 25.4 % from the United Kingdom (UK) [2]. It is forecasted that the prevalence of obesity in the UK will increase by 33 % and of severe obesity by 130 % [3]. The number of obese pregnant women delivering in the UK doubled between 1989 and 2007 [4]. This increasing prevalence of maternal obesity is a significant challenge to pregnancy management, placing a burden on health-care resources.

Despite heightened awareness and public health efforts to address this increasing epidemic, a large and younger population remains affected. Childhood obesity is strongly associated with later obesity and cardiometabolic disease [5, 6]. Globally,

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N. Patel • L. Poston (✉)
Division of Women's Health, Women's Health Academic Centre, Faculty of Life Science and Medicine, King's College London, London, UK
e-mail: lucilla.poston@kcl.ac.uk

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23.2 % of children under the age of 18 years are estimated to be overweight or obese [2]. Obesity is occurring at an increasingly younger age, affecting more than 43 million children aged 0–5 years worldwide [7]. Recent estimates in the UK suggest that 24.4 % of children aged between 2 and 5 years are overweight (>85th centile) [7, 8].

The global increase in obesity over the last one/two generations from 15 to 33.9 % globally suggests that genetic variants are unlikely to play a major role, placing an emphasis on environmental factors on the subsequent influence of the epigenome on the phenotype. While there is little doubt that a high-fat, Western-style diet combined with a reduction in physical activity are strong contributors, other factors including early-life exposures may play a causative role. Maternal antenatal including pre-pregnancy obesity and excessive gestational weight gain (GWG) and early postnatal exposures have been associated with the development of childhood obesity.

Observational data and animal studies have highlighted the associations between alterations in the in utero environment and increased susceptibility to obesity and adverse cardiometabolic profiles in adulthood. Studies have suggested that maternal BMI and excessive GWG independently correlate with offspring adiposity from an early age [9]. This has led to numerous studies exploring these associations and potential mechanisms, with the ultimate objective being the development of interventions in early life, with the aim of reducing the risk of obesity in the offspring.

3.2 Implications of Maternal Obesity on Conception and Pregnancy Outcomes

A full understanding of the implications of maternal obesity on the health of the child in later life requires appreciation of the associated short-term complications for both mother and child.

3.2.1 Preconception and Embryogenesis

Obesity can affect fertility, implying an influence on development of the early embryo or indeed the oocyte. There is an association between obesity and time to spontaneous pregnancy in women with both anovulation and regular menstrual cycles [10, 11]. Increasing BMI is associated with decreased serum adiponectin, increased circulation of leptin, insulin resistance and an unbalanced cytokine profile, critical for central and peripheral regulation of maternal metabolism and subsequent fertility. Diets with high glycaemic index, trans fatty acids and animal proteins are associated with increased risk of ovulatory fertility [12] through resulting hyperglycaemia and lipotoxocity [13].

3.2.2 The Antenatal Period

Maternal obesity is an independent risk factor for several adverse outcomes in pregnancy, including pre-eclampsia, gestational diabetes (GDM) and delivery of a large for gestational age (LGA) infant [14, 15] all of which have been associated with adverse long-term health. A dose–response relationship exists between increasing pre-pregnancy BMI and the risk of these antenatal complications [16, 17]. A high maternal BMI is also an important risk factor for intrauterine growth restriction (IUGR) [18] which is associated with the development of cardiovascular and metabolic disease in the offspring. More obviously, children born to obese or overweight mothers are at increased risk of congenital abnormalities, birth injury, stillbirth and prematurity [19].

3.2.3 Gestational Weight Gain

3.2.3.1 What Is Gestational Weight Gain?

Understanding the different components of GWG in an uncomplicated pregnancy is necessary before we can address the potential of long-term effects on the offspring secondary to suboptimal weight gain.

The fetus, placenta, adipose tissue, amniotic fluid, mammary glands and uterus all contribute to GWG. However, the fetus, placenta and amniotic fluid account for only 35 % of total GWG. Maternal fluid volume expansion also makes a major contribution. Fat accrual is another but very variable component of GWG. However, only 5 % of maternal weight gain at 40 weeks' gestation constitutes fat [20]. During pregnancy, fat accumulation is promoted in response to increased vascular and metabolic demands to sustain maternal stores during lactation and to support fetal growth and development. The deposition of adipose tissue occurs within two distinct anatomic locations: subcutaneous and visceral compartments. Visceral adipose tissue is primarily located within the abdominal viscera and differs in its endocrine and lipolytic function in comparison to subcutaneous tissue. In the non-pregnant population, increased visceral adiposity has been shown to increase the risk of type 2 diabetes, dyslipidaemia and accelerated atherosclerosis [21]. Despite its importance, few studies have investigated how the distribution of adipose tissue changes during pregnancy and its relationship with GWG and pregnancy outcomes, including GDM. One recent study has, for example, related differences in maternal fat depots to the risk of GDM [22]. The Rhea Pregnancy Cohort, Greece, assessed 977 mother–child pairs and found that an increased rate of GWG (per 200 g/week) was associated with offspring overweight and obesity at 2 years and significantly increased waist circumference, sum of skinfolds and diastolic blood pressure at 4 years [23]. These findings suggest that not only the

Table 3.1 Institute of Medicine guidelines for maternal weight gain [20]

Pre-pregnancy BMI	BMI (kg/m^2) (WHO)	Total weight gain range (kg)	Rates of weight gain second and third trimester (mean range in kg/week)
Underweight	<18.5	12.5–18.0	0.51 (0.44–0.58)
Normal	18.5–24.9	11.5–16.0	0.42 (0.35–0.50)
Overweight	25.0–29.9	7.0–11.5	0.28 (0.23–0.33)
Obese	≥30.0	5.0–9.0	0.22 (0.17–0.27)

absolute gestational weight gain but also the timing and composition of GWG is a determinant for the development of cardiometabolic risk in the offspring.

Describing the pattern of adipose tissue deposition, rather than measurement of GWG, would provide a more faithful reflection of metabolic exposures encountered by the developing child and provide greater insight into the risk to later health. Surrogate measures of fat mass and distribution such as skinfold thicknesses may provide a simple tool to assess gain in adipose tissue in pregnancy.

Despite these limitations in the measurement of GWG, the Institute of Medicine (IOM) recently published new recommendations for GWG [20]. The recommendations differ depending on the pre-pregnancy BMI (Table 3.1).

The wide range of weight gain for each BMI category reflects the imprecision of the given estimates. The current IOM guidelines were derived from limited data on six main outcomes: SGA, LGA, preterm birth, caesarean birth, maternal postpartum weight retention and early childhood obesity. These criteria were not further stratified on the different classes of obesity during pregnancy as they are for published hypertension and ischaemic heart disease risk stratification guidelines [24]. The clinical usefulness of these criteria continues to be debated, especially in relation to additional patient outcomes including GDM [25]. There is also recognition by the IOM of the lack of evidence among socio-economic and ethnic minority groups, potentially limiting translation to other populations [20].

3.2.3.2 Excessive Gestational Weight Gain

Maternal and perinatal complications have been shown to increase with excessive GWG regardless of whether the mother is obese or not. These include GDM, pre-eclampsia, and for the infant, macrosomia and delivery of an LGA infant. Furthermore, an association between rate of GWG and the incidence of GDM has also been reported [26]. These outcomes are all implicated in the long-term health of the infant. Excessive gestational weight gain is also strongly associated with maternal postpartum weight retention and obesity-related complications in subsequent pregnancies [27].

3.2.3.3 Below Recommended Gestational Weight Gain

The IOM first published in 1990 recommended obese women to gain at least 6.8 kg, in aim to prevent adverse fetal and maternal outcomes [20]. A recent systematic review and meta-analyses utilising 18 cohort studies determined that GWG below recommended guidelines in obese women increased the odds of preterm birth (<37 weeks) (Adjusted Odds Ratio (AOR) 1.46; 95 % CI 1.07–2.00) and delivery of a SGA infant (AOR 1.24; 95 % CI 1.13–1.36) [24], both of which have shown in observational studies to influence long-term health of the child [28, 29]. However, the same study showed that GWG below the recommended guidelines was associated with a reduction in the odds of delivery of an LGA infant (AOR 0.77; 95 % CI 0.75–0.8) in comparison to those who gained weight within the guidelines [24]. This study exemplifies some of the controversies related to these guidelines and the difficulties in assessment of optimal GWG. GDM is one of most important confounders in the association between gestational weight gain and delivery of an LGA or macrosomia infant since treatment for mild GDM has been shown to decrease the delivery of a macrosomic and LGA infant as assessed by population centiles [30].

3.2.3.4 Assessment of Gestational Weight Gain

Several measures of GWG have been used to determine the association with long-term offspring health, for example total, rate and weekly rate of GWG. However, none of these methods have been universally adopted, leading to results which are not comparable due to different assumptions and adjustments made for each [31]. The IOM categories combine pre-pregnancy BMI and GWG. Therefore, it is difficult to distinguish the associations with offspring health within the IOM categories and whether outcomes are contributed to either pre-pregnancy BMI or GWG [32]. A novel approach developed by Fraser et al. utilises repeated measures of weight change during pregnancy by developing a linear spline multilevel model, relating gestational weight to gestational age; thus providing an accurate tool to model GWG on outcomes [32].

The advantage of using repeated measures of weight gain in relation to long-term offspring outcomes enables the detection of subtle changes in maternal GWG in relation to outcomes.

3.3 Effect of Maternal Obesity and Gestational Weight Gain on Long-Term Health of the Child

3.3.1 Developmental Programming Hypothesis

The hypothesis that the early environment could be implicated in the development of adverse health was initially proposed by Barker et al. [33, 34]. Their studies in historical population cohorts identified that low birthweight, implicated in inadequate nutrition in utero, 'programmes' the fetus for future cardiovascular and metabolic disease [34]. Whilst a reduction in birthweight is evident at birth, it is apparent that the influence of an in utero environment can have latent effects of the offspring phenotype. A series of observational studies derived from the Dutch 'Hunger Winter' determined the underlying effects of in utero programming including the observation that famine exposure in utero was associated with a twofold increase in the incidence of obesity at 18 years of age in offspring without a change in birthweight [35].

Intrauterine insults during the critical phases of growth and development have been associated with permanent functional changes in certain tissues, including adipocytes, myocytes, neurons and pancreatic beta cells.

3.3.2 The Influence of the In Utero Environment on Offspring Outcomes

Pre-pregnancy obesity, excessive GWG and GDM have all independently been associated with increased adiposity and adverse cardiometabolic health in the child's later life [36]. These three maternal factors, each associated with fetal overnutrition, are thought to be the most important modifiable in utero risk factors in relation to development of childhood cardiometabolic traits. Undernutrition, overnutrition and hormonal imbalance are thought to be pivotal factors implicated in these processes. During obese pregnancies, the fetal pancreas is often exposed to excessive glucose and amino acids from increased transplacental transport from the mother, which stimulate growth primarily through the resulting overproduction of insulin by the fetal pancreas. Observational studies have demonstrated that offspring of obese mothers are set on a trajectory of increased adiposity and risk of cardiometabolic disorders [37–39].

3.3.3 Pre-pregnancy Obesity

Multiple observational studies have demonstrated that a higher pre-pregnancy BMI is associated with an increased BMI in the adult offspring, independent of socio-

economic and dietary confounding [15]. Examples include a report from a subgroup of The Southampton Women's Survey, a detailed prospective cohort of 216 women with data on maternal weight and skin fold measurements at pre-pregnancy to 34 weeks' gestation. Adiposity was assessed in 9-year-old offspring using the Fat Mass Index (FMI) measured by Dual energy X-Ray absorptiometry (DXA). The study demonstrated that for each standard deviation increase in maternal pre-pregnancy BMI there was an increase in offspring FMI by 0.26 (0.04–0.48) in males and 0.42 (0.29–0.56) in females [40]. Similar findings were reported in the Avon Longitudinal Study of Parents (ALSPAC) in a study of 8234 women using self-reported pre-pregnancy weight. Offspring were followed up to 7 years where obesity was defined as BMI greater than the 95th percentile (equivalent to a standard deviation of $\geq$1.96) adjusted for age and gender. Utilising a large sample size, longitudinal design, advanced multivariate analysis and adjustment for potential confounders (including maternal education, offspring energy intake at 3 years and offspring sex), pre-pregnancy obesity was associated with a significant increase risk of childhood obesity at 7 years (AOR4.25; 95% CI 2.86–6.32) [41]. The effects of maternal obesity are not just limited to childhood. A compilation of the Nurses Health Study II and the Nurses' Mother cohort, a total of 26,506 mothers with a pre-pregnancy BMI $>$29 kg/m^2 and their offspring at 18 years was assessed. Increased maternal pre-pregnancy BMI was associated with 6.1-fold increased risk of obesity in the offspring [42].

To date, few studies have investigated the association between maternal pre-pregnancy obesity and offspring cardiovascular outcomes in offspring, which we have recently reviewed [43]. A study ($n = 4871$) in the Generation R cohort from the Netherlands assessing the individual and combined association of maternal and paternal associations has enabled clarification of a potential causal relationship. Offspring at 6 years from obese mothers had an increased risk of both childhood overweight (OR 3.84; 95 % CI 3.01–4.90) and clustering of cardiometabolic markers (defined as android fat mass percentage $\geq$75th percentile; systolic or diastolic blood pressure $\geq$75th percentile; high-density lipoprotein cholesterol $\leq$25th percentile or triglycerides $\geq$75th percentile; and insulin $\geq$75th percentile) (OR 3.00; 95 % CI 2.09–4.34) in comparison to those of normal weight women [44]. Furthermore, this association was stronger for maternal pre-pregnancy BMI than paternal BMI, providing further support for the intrauterine origins of adverse health in later life. In another study Lemas et al. showed in 753 maternal–infant pairs from a large multi-ethnic observational cohort that maternal weight prior to pregnancy was associated with increased umbilical cord leptin glucose and reduced HDL-c at delivery, independent of neonatal adiposity. This metabolic profile was associated with the development of hypercholesteraemia and subsequent cardiovascular disease in adulthood [45]. Whilst neonatal adiposity was not related to these maternal variables in this study, it provides a measure of in utero nutritional status that cannot be influenced by known causative postnatal exposures and may therefore be a useful index of an adverse in utero environment associated with later disease.

The ABCD study of 3074 maternal–offspring pairs from the Netherlands reported that pre-pregnancy BMI was positively linearly associated with offspring systolic and diastolic blood pressure at 5–6 years. Although this study demonstrated proof of principle of the association of increasing BMI and childhood blood pressure, only 5 % of the total population studied were clinically obese [46]. In a case–control study from Norway, with a small sample size, maternal obesity was related to adverse structural and functional cardiac changes during the first trimester as assessed by ultrasound. These included reduced left and right ventricle global strain at 14 weeks' gestation and increased inter-ventricular thickness (-1.74 ± 0.50/s; $p < 0.001$) at 32 weeks' gestation ($n = 27$) in comparison to lean pregnant women ($n = 24$) [47]. Similar changes in myocardial structure and function have been associated with the development of cardiovascular disease in adults [48].

There is also evidence at the population level for associations between maternal obesity and offspring adulthood cardiovascular function. In a large hospital cohort from Scotland ($n = 37{,}709$), maternal obesity measured in early pregnancy was associated with an increased risk of premature all-cause mortality [Hazard ratio (HR) 1.35; 95 % CI 1.17–1.55] and hospital admissions secondary to cardiovascular events (HR 1.29; 95 % CI 1.06–1.57), following adjustment for maternal age at delivery, socio-economic status, sex of offspring, current age, birthweight, gestation at delivery and gestation at measurement of BMI [49]. Whilst observational studies have demonstrated the association of pre-pregnancy BMI and cardiometabolic health, randomised controlled trials are now required to determine causality of these associations.

3.3.4 Gestational Weight Gain

While elevated pre-pregnancy BMI has been associated with increased risk of adverse long-term offspring health, relationships between excess GWG and offspring outcomes are less readily interpretable, perhaps because of differences in methods of measurement and the variable components of weight which contribute to GWG [50].

Those studies which have assessed relationships between GWG and offspring indicate that excessive GWG is associated with an increased risk of obesity and adverse cardiometabolic health [51–53]. For example, using the Danish Birth cohort, Nohr et al. found that a combination of maternal overweight/obesity and excessive GWG was associated with a significant increase in LGA infants [54]. Utilising a population-based birth cohort ($n = 2432$), Mamun et al. found that greater GWG was associated with an increase of 0.2 mmHg per 0.1 kg of gestational weight gain (95 % CI 0.1–0.4 kg/m^2) and increased BMI in offspring at 21 years of age [55].

A recent systematic review and bias-adjusted meta-analysis ($n = 12$ cohort studies) demonstrated that the offspring of women who gained above the

prescribed IOM weight gain criteria had a 40 % increased risk of obesity over the life course [37]. It is hypothesised that women who gained excess weight during pregnancy are more than likely to have a poor diet quality comprised of high-fat foods, and low levels of physical activity, which have both been independently associated with offspring obesity in animal models and human observational studies [56, 57]. This most recent meta-analysis highlights the independent effects of maternal GWG on the development of offspring obesity over the life course. However, none of the studies included within this analysis assessed women with a pre-pregnancy BMI >30 kg/m^2, but rather grouped the three BMI categories (normal, overweight and obese) together. In other meta-analyses, GWG, defined using the IOM criteria [20], has also been associated with an increase in pre-schooloverweight and obesity (defined as overweight ≥85th percentile and obesity BMI ≥95th percentile) and with the magnitude of effect increasing from 1.5-fold to 4.4-fold, as recently shown by two meta-analyses in offspring after adjustment for sex [37, 58].

An important element often under-recognised is the timing of weight gain. Observational studies have suggested that the first 24 weeks of pregnancy is a crucial time period during which excessive weight gain is a key risk factor for the development of GDM [59] which has also been implicated in offspring obesity. Early weight gain has been shown to be a strong predictor of excessive total pregnancy weight gain [60], rendering it a potential target for future interventions.

3.4 Drawbacks of Birth Cohorts In Interpreting Matenal/ Offspring Associations

Birth cohorts provide detailed data on biological, familial, environmental and socio-demographic characteristics during pregnancy as well as detailed offspring outcome data, and therefore allow assessment of the influence of environmental characteristics on offspring outcomes. The fundamentals of the ‘developmental origins of adult disease’ hypothesis were derived from these observational data, but with focus on exposure of risk factors during early-life periods of developmental plasticity on a given offspring outcome. The longitudinal follow-up of the infants enables prospective time ordering of exposures including exploration of the role of environmental factors on outcomes of interest [61].

Although evidence has accumulated from birth cohorts in support of the ‘developmental origins’ hypothesis, some critics have argued for alternative explanations for the observed associations. These include confounding by genetic factors and socio-demographic characteristics. It has been suggested, for example, that variants of the IRS-2 (Insulin Receptor Substrate-2) gene might account for the observed association between low birthweight and vascular disease, as this is a pleiotropic gene which theoretically could result in two phenotypes, one in the fetus (low birthweight) and the other in the adult (type 2 diabetes and cardiovascular disease)

[62]. Similarly, two others, an ACY5 allele and ADRB1, have been implicated in both infant birthweight and adult-onset hypertension and type 2 diabetes [63]. The social environment may also play a causal role. Socio-economic deprivation has been associated with birthweight, as infants with a lower birthweight are more likely to experience deprivation over the life course in comparison to those born with a higher birthweight [64]. Some but not all studies have adjusted for baseline maternal socio-economic deprivation as assessed by the Index of Multiple Deprivation, at best a crude proxy of socio-economic status, which together with a number of other offspring lifestyle and environmental factors such as diet composition and physical activity are often not reliably measured or absent (residual confounding). Furthermore in observational epidemiology causal inference is limited due to selection bias and reverse causation [65].

A limited number of observational studies have used more sophisticated study design to enable further insight into the role of confounding factors in relation to maternal exposures and offspring outcomes. Lawlor et al. reported that increased maternal weight gain in both overweight and obese mothers was independently associated with higher offspring BMI at 18 years in siblings ($n = 146$, 894 individuals from 136,050 families); by avoiding genetic confounding this approach improves estimation of causal relationships between maternal exposures and offspring outcomes. The association of normal pre-pregnancy BMI on childhood obesity was associated with familial genetic and environmental influences [66]. By using a within and between-non-sibling association study design, the environmental and social confounders are effectively controlled for, thereby providing convincing evidence of a causal relationship of the intrauterine mechanisms of weight gain and later obesity. However, this methodology is not without its limitations. In common with most causal inference studies, sibling comparison studies are formulated on the assumption that the effect of each participant's exposure to a risk factor does not influence other unexposed outcomes [67]. For example, the changing weight status of the mother between pregnancies of one sibling compared to the other is not accounted for, nor the increased risk of offspring obesity with recurring maternal gestational diabetes.

Distinguishing causality from association is essential to identify key early-life modifiable causes of non-communicable disease and for the determination of mechanistic pathways for therapeutic interventions. Few publications state the ordering of exposure variables, and their inter-relationships, both directly or mediated through intermediary variables in association with the outcome measure and therefore unable to distinguish potential 'windows of opportunity' or maternal exposures for targeted interventions [68].

Numerous methods have been developed, taking into account the limitations of observational epidemiology and to further strengthen causal inference. These include maternal- and paternal–offspring comparisons, Mendelian randomisation (MR) and the use of instrumental variables robustly associated with the exposure, thereby controlling for confounding and measurement error [61]. Each method

has recently been employed to good effect; for example the use of MR has supported the notion, with sophisticated statistical methodology, that the maternal genotype [defined as the presence of a variant in the fat mass and obesity associated (FTO) gene] predicted offspring fat mass at 9–11 years, whilst controlling for offspring FTO, providing support for the developmental overnutrition hypothesis as the association may not be present in the preconception period [69]. Although MR enables establishment of causality with a certain degree of certainty, it requires a large sample size and the methodology is not suitable for genetic variants having a direct pleiotropic effect on both exposure and outcome of interest [70].

Another of the limitation of observational studies is the use of categorical definitions, for example GWG within IOM guidelines, which may be more usefully employed as continuous variables. This method has been used previously to determine the association of life course growth trajectories on later health outcomes. Whilst requiring complex statistical modelling, the conclusions have proven valuable in determining the influence of the intrauterine environment on later outcomes [71]. For example, direct measures of fat mass which would allow for longitudinal modelling of weight gain are far preferable to GWG, which as addressed above has multiple components. However, very few studies have undertaken measures of body composition, for example skin fold thicknesses in the mother and offspring.

Despite these limitations, it is of paramount importance to public health that similar studies are undertaken in contemporary cohorts, preferably in the setting of a randomised controlled trial, to determine causal inference.

Observational cohorts have provided a strong association between a suboptimal in utero environment and the development of adverse offspring outcomes. Verification in rodent models, where diet can be tightly controlled can provide further confidence in these associations and also enable the examination of the underlying physiological and biochemical mechanisms behind the nutritional programming of offspring disease, further aiding identification of the mechanisms and critical windows of intervention [72, 73]. However it is critical to appreciate the differences in the stages of development between animals and man to infer generalisability to the human condition. For example, rodents are altricial species, born with an underdeveloped endocrine system and brain and undergo significant maturation of their organs during the weaning period, reflecting the third trimester in human pregnancies. Sheep have a similar rate of pre- and postnatal growth to humans and produce fewer offspring than rodents, comparable to humans. Animal models nonetheless provide many advantages in examining the principles of the DoHaD hypothesis, including independent assessment of the influence of maternal nutrition from potential confounders, including genetic and social factors. Although fully appreciated that there are many biological and species differences, response to interventions in animals can provide valuable insight into the human condition [72]. For example increased physical activity during obese pregnancy has been used

to determine the potential benefits on offspring adiposity. A variety of animal models have been used to assess the effect of maternal nutrition on developmental programming including rodents, sheep, pig and non-human primates. Non-human primates are the most appropriate model, but are limited to due to their long lifespan, resulting cost and ethical considerations. Many researchers choose rodents because of the shorter lifespan and lower costs.

3.5 Dietary Determinants of Associations Between Maternal Exposures and Offspring Outcomes

Optimal maternal nutrition is the primary determinant of normal growth and development of the fetus. Maternal pre-pregnancy obesity is an indicator of a poor quality diet, including consumption of a high-fat or 'Western' diet. Both have been implicated in the programming of adverse cardiometabolic health in the offspring.

The Generation R study ($n = 2695$), a population-based prospective birth cohort, observed that adherence to a 'healthier' dietary pattern in pregnancy was associated with lower offspring body mass index, fat mass index and a reduced risk of being overweight at 6 years of age [74]. However, when the results were adjusted for socio-demographic and lifestyle covariates, none of these measurements remained significant. Using a data-driven approach (for example, principal component analysis or factor analysis) to derive dietary patterns reflects the dietary habits of the mothers. It also allows determination of whether a specific nutrient, independent of the overall dietary pattern is associated with the outcome of interest [75]. The Generation R study highlights that rich data sets which enable adjustment for multiple confounders may provide more precise estimates of associations.

Findings from the Southampton's Women survey have recently demonstrated that the timing of the maternal nutrition determines the magnitude of the outcome in the offspring [57]. Early, but not later pregnancy dietary glycaemic load and index, following adjustment for potential confounders, were positively associated with offspring fat mass at 4 and 6 years ($n = 906$; at mass SDs per 10-unit GI increase: $p = 0.02$ at 4 years; $p = 0.01$ at 6 years, fat mass SDs per 50-unit GL increase: $p < 0.001$ at 4 years, $p = 0.007$ at 6 years). Whilst observational studies such as these have provided some credence to the hypothesist that dietary manipulation could influence childhood adiposity, evidence from randomised controlled trials in pregnancy is needed to establish causality.

Experimental animal studies have been extensively used to determine the mechanistic pathways underlying the association between maternal diet, obesity and offspring outcomes. Whilst there are differences between the studies, most clearly show an influence of maternal diet or obesity on the development of offspring adiposity. Obese pregnant mice consuming a westernised diet, rich in fats, sugar

and salt during gestation and lactation, delivered offspring which later developed increased adipose tissue deposition in comparison to offspring born to chow-fed controls [76]. Furthermore, offspring of obese mice have been shown to have early indications of metabolic syndrome, including increased glucose, triacylglycerols and cholesterol [76]. Other reports have shown that maternal consumption of a Western-style diet is associated with increased weight gain, hepatic hyperlipidaemia, increased liver injury and hepatic expression of inflammatory markers in the offspring [77]. Similar results have also been seen in the offspring of ewes fed a high-fat diet, a model which is more comparable to human gestation [78]. Consumption of a westernised diet during gestation and lactation in the absence of pre-pregnancy maternal obesity has also been associated with increased body weight and peri-renal adipose tissue in the offspring implying an independent role for dietary factors [79]. Furthermore, challenge of the offspring with a westernised diet amplified adiposity in rodents [80]. A westernised diet during gestation and lactation has also been found to increase food intake and high-fat food preference in mice offspring, the pups having a higher daily energy intake, hyperphagia and change in food preference [81, 82]. Further research is warranted in children of obese mothers to determine whether these changes in satiety and food preference are also present and could contribute to childhood obesity.

3.6 Summary

Modification of maternal obesity, GWG and maternal diet offers potential for a reduction in childhood obesity by improvement of the maternal and fetal metabolic environment.

3.7 Putative Mechanisms Relating Maternal Obesity to Offspring Outcomes

Growing evidence suggests that environmental factors including modifications of diet within early life can alter the epigenome (See Chap. 16). A recent and several independent reports review highlight in different animal models of varying nutritional status how changes in maternal diet are associated with persistent metabolic dysfunction in the offspring, accompanied with epigenetic changes in key genes involved in appetite control and metabolism [73, 83–85]. Relatively few studies of epigenetic marks have been undertaken in offspring from animal models of maternal obesity. These include a study by Godfrey et al. who have shown that alterations in epigenetic biomarkers can be predictive for later disease risk; for example methylation of a single CpG site in the promoter region of the nuclear receptor Retinoid X Receptor-α (RXRA) in cord leucocytes was associated with the development of childhood adiposity in two independent cohorts [86]. Higher methylation

of RXRA, previously lined to increase offspring adiposity, was associated with reduced maternal carbohydrate consumption [86]. A recent report using an epigenome-wide approach (Illumina Infinium® HumanMethylation 450 K BeadChip) in the ALSPAC cohort (1018 maternal–offspring pairs) has provided some of the strongest evidence to date in support of a change in cord blood leucocyte methylation status associated with relationships betweeen maternal obesity and underweight and increased offspring adiposity at a mean age of 9.9 and 15.5 years, in comparison to offspring from normal weight women [87]. On the contrary, GWG during pregnancy had little effect.

Persistent changes in the epigenome offer a final pathway linking early-life exposures such as obesity with offspring health outcomes. Other interactions between environment and genes in fetal development may transiently influence gene expression causing lasting perturbation in organ growth and development.

The following discussion reviews the many different biological pathways which have been implicated in the relationship between maternal obesity and offspring cardiometabolic health which may transiently or permanently alter offspring gene expression, potentially through epigenetic pathways. Multiple mechanisms have been hypothesised, including adverse influences of maternal overnutrition on the developing embryo, overproduction of fetal insulin and maternal inflammatory and metabolic imbalance leading to adaptive responses in the fetal hypothalamus and adipose tissue [37, 88].

3.7.1 Maternal Obesity, Oocyte Quality and Embryogenesis

Obesity may have biological influences in the gamete and at the earliest stages of life. As mentioned above in relation to infertility, obesity influences ovarian function and oocyte quality. Recent data from 218 oocytes from 29 women attending an in vitro fertilisation clinic showed that increasing maternal BMI at conception was associated with phenotypic changes in the early embryo [89]. In another report, obese women ($n = 32$) attending an infertility clinic had higher than normal follicular fluid insulin and lipids, associated with poor quality of the oocytes [90]. Others have shown reduced glucose consumption, increased endogenous triglycerides and abnormalities in amino acid metabolism in oocytes and maternal blastocysts from obese women, again suggesting metabolic impairment associated with obesity before conception [89]. Experimental studies in diabetic and high fat fed mice models have demonstrated oocyte spindle defects, increased rates of follicular apoptosis and mitochondrial abnormalities [91] as well as increased oocyte lipotoxicity, with mitochondrial dysfunction [13, 92]. Our laboratory has also reported mitochondrial abnormalities in oocytes and early blastocysts from obese mice in comparison to lean dams which were associated with increased oxidative stress [93].

Although not as yet established, these influences of maternal obesity on the oocyte and early embryo may have lasting consequences for the health of the

offspring, as has been demonstrated for maternal undernutritional states in the rat [94], emphasising the potential importance of the periconceptional period in life-long health.

3.7.2 Metabolic Stress and the HPA Axis

Obesity is associated with the development of chronic systemic low-grade inflammation in adipose tissue, hypothalamus, liver and muscle [95], and there is suggestion that inflammation in utero predisposes the offspring to metabolic compromise from birth [96] (Fig. 3.1). Low-grade maternal inflammation has been shown to lead to both short- and long-term epigenetic modification of fetal genes involved in regulation of the central HPA pathway [97]. Glucocorticoids readily cross the placenta and enter the fetal brain [98] and it is proposed that downregulation of the fetal HPA axis permanently influences myelination and neurogenesis of the fetal brain and may effect hypersensitivity in the peripheral organ HPA pathway [97]. Sheep models have demonstrated that excess maternal glucocorticoid exert a persisting influence on adult offspring outcomes including increased blood pressure, glucose and insulin levels [99].

A growing body of evidence suggests that early changes in the maternal inflammatory profile are predictive of later cardiometabolic disease [100]. Whether or not this pathway may be of relevance to maternal obesity is not known [101]. Whilst there are reports of raised maternal glucocorticoids in obese rodents [102], there is no evidence of which we are aware of increased glucocorticoids in obese pregnant women.

3.7.3 Adipokines

The principal adipokines implicated in maternal obesity, GWG and offspring obesity and cardiovascular outcomes are leptin and adiponectin (Fig. 3.1). In animal models, there is a well-characterised leptin ‘surge’ in early postnatal life which has been shown to play a critical role in normal neuronal development of the hypothalamus [103]. Maternal obesity is associated with increased magnitude of the leptin surge in rodent offspring [104]. The origin is uncertain, but there is no evidence of an association between maternal milk leptin and offspring serum leptin in rodents [104, 105], whereas neonatal leptin is coincident with increase in Ob gene mRNA expression (the leptin gene) in offspring adipose tissue [104]. Samuelsson et al. have also reported that exogenous leptin administration to normal pups leads to obesity and cardiovascular dysfunction in adult life [106], indicating a casual role in long-term adverse cardiovascular health and hyperphagia. Cord blood leptin is raised in association with obesity in pregnant women, and several mother–

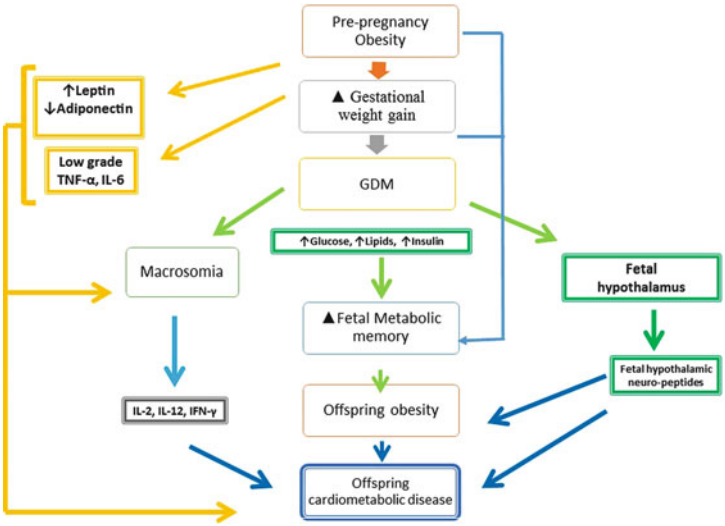

Fig. 3.1 Common biological mechanisms implicated with maternal obesity and excessive gestational weight gain on the developmental origins of health and disease in the offspring

child cohorts are now addressing whether similar associations are present in obese mothers and their children.

3.7.4 The Metabolome

Metabolomics has emerged as tool to examine the human metabolome to detect metabolites, metabolic pathways and their impairments and is recognised as a novel methodological approach likely to provide further insight into the mechanistic pathways associated with DoHaD [107]. Metabolomic profiles signify the cellular response and represent a key link between the genotype and phenotype. This technique has become widely used to identify metabolite pathways modified by disease or adverse exposures.

Few studies have to date addressed whether the neonate metabolome (cord blood) at birth could provide insight into mechanisms of programming by early life maternal exposures. One report examined the metabolome in low birthweight ($n = 20$) and normal birthweight ($n = 30$) neonates, demonstrating differences in the clustering of amino acids and lipids. The metabolic profile associated with low birthweight demonstrated similar patterns to type 2 diabetic adults [108]. A recent study assessed the association of the maternal urinary metabolome with that of the offspring's cord blood, stratified by pre-pregnancy BMI (normal, overweight and obese) in 321 maternal–offspring pairs. Analysis using partial least squares regression-discriminant analysis and logistic regression did not reveal significant

differences in the cord blood metabolic profiles of offspring stratified by maternal BMI group, although infants born to obese mothers had a higher birthweight and lower Apgar Scores [109]. This area of research, which has proved invaluable in understanding the metabolic profile in type 2 diabetes and cardiovascular, is open to investigation in contemporary cohorts of obese mothers and their children.

3.8 Role of Interventions Studies

Studies in obese animals have shown that manipulation of maternal metabolism using diet, physical activity and/or metformin within the antenatal period can potentially alter offspring body composition and adiposity. These provide useful tools to interrogate potential mechanistic pathways.

Randomised controlled trials in obese pregnant women are the 'gold standard' for determining causality and for overcoming the problem of residual confounding. Intervention studies to date in obese women have focused on the prevention of excessive GWG or dysglycaemia and insulin resistance. The primary strategies have included advice to change dietary and physical activity through modification of lifestyle behaviours, and more recently the use of metformin.

In 2014, the LIMIT randomised controlled trial in 2212 overweight and obese pregnant women published its findings. This was one of the first studies, of adequate sample size and study design which randomised overweight or obese women to lifestyle advice or standard antenatal care, with the primary aim to reduce LGA [110]. Although the trial's primary outcome was not reached, the study found fewer infants who were born >4 kg in the intervention arm (15 % vs. 19 %; $p = 0.04$) in comparison to standard care. Despite recent efforts, a Cochrane review of the available evidence from randomised trials (49 RCTs; $n = 11{,}444$ women) suggests that although modification of maternal dietary intake can occur, it has limited success in reducing GWG or GDM. Furthermore, the intervention studies included have not been successful in improving neonatal outcomes, including excessive fetal growth and caesarean sections [111, 112].

We have recently undertaken and published a randomised controlled trial of a dietary and physical activity intervention, the UBEAT trial in 1555 obese pregnant women, the largest study powered for clinical outcomes [113]. Women were randomised to standard antenatal care or an intervention delivered by health trainers over 8 weeks (weekly sessions). The intervention focused on improving glycemic control through a low glycaemic index diet and increased physical activity. This intervention differed from previous studies, in the development and delivery of the intervention. The intervention focused on approaches to achieve Specific, Measurable, Achievable, Relevant, Time-Specific (SMART) goals as well as advice on self-monitoring, social support and problem solving to barriers of behaviours change [114]. Women were advised on reducing saturated fat intake and glycaemic load as well as increase time spent doing low/moderate physical activity. This study did not meet its primary endpoints of reducing maternal GDM or delivery of an

LGA infant. However, significant changes were observed in maternal antenatal diet, physical activity and measures of maternal body composition. These include significant reductions in total energy intake (Mj/day) [−0.70 (95 % CI −0.96 to −0.45); $p < 0.0001$], saturated fat (% energy) [−0.85 (−1.2 to −0.51); $p < 0.0001$] and glycaemic load per day [−21 (−26 to 16); $p < 0.0001$] and increase in physical activity as assessed by the metabolic equivalent of task (min/week) [295 (105–485); $p = 0.0015$] from 15^{+0}–18^{+6} to 26^{+0}–28^{+6} weeks' gestation, in comparison to the control arm. This was associated with changes in maternal body composition in the intervention arm, including a reduction of total GWG (kg) [mean difference −0.55 (1.08 to −0.02); $p = 0.041$] and sum of skin folds throughout pregnancy [mean difference −3.2 (−5.6 to −0.8); $p = 0.0081$] [113]. However, it remains to be determined whether this degree of change observed in the mother has an influence on determining adiposity in later life, and follow-up of the children is ongoing.

The EMPOWaR Study, a randomised double-blinded placebo trial, recruited obese pregnant women at 12–16 weeks' gestation with a normal glucose tolerance test, to receive metformin throughout pregnancy [115]. There were no differences in birthweight or in neonatal or maternal anthropometry. The study interestingly achieved its pharmacodynamic effects of metformin including reduction in fasting glucose, insulin and reduction in inflammatory markers (CRP and IL-6), all which have been implicated in the developmental origins of adverse health and disease. Better pregnancy outcomes for the offspring have been reported in GDM-diagnosed mothers with reduced central or visceral adiposity, due to metformin treatment [116], as well as improved fetal outcomes compared to those treated with insulin. The long-term follow of these pharmacological studies is required to determine the effects of metformin and whether this may be beneficial or not for offspring health.

3.9 Conclusion

Animal studies and observational data have provided robust evidence for the association of maternal obesity and gestational weight gain on offspring risk of obesity. Only recently have randomised controlled trials, with adequate statistical power been attempted in order determine whether strategies for healthy living in pregnancy could influence the obesity epidemic in children. Whilst these studies have not achieved the desired improvement in maternal clinical outcomes, follow-up of the offspring may enrich our understanding on the role and timing of an adverse in utero exposure. Evidence has suggested that preconceptional obesity plays an important role in later cardiometabolic disease for the offspring. However, targeting the preconception period remains problematical requiring a public health approach. A 'two-pronged intervention' during the preconception and antenatal period may ultimately provide an effective strategy to ameliorate the increasing burden of cardiometabolic health and obesity in the next generation.

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Chapter 4
Young Maternal Age, Body Composition and Gestational Intake Impact Pregnancy Outcome: Translational Perspectives

Jacqueline Wallace

Abstract Birth weight is a robust predictor of health and well-being immediately after delivery and throughout the life course. Maternal body composition at conception and gestational intake thereafter impacts prenatal growth velocity and birth weight irrespective of maternal age, but the most pronounced risk of poor outcome is when pregnancy coincides with adolescence and continued or incomplete growth of the mother. Experimental ovine paradigms have helped define the impact of nutrition in mediating pregnancy outcome in young adolescents. Low maternal nutrient status at conception has a modestly negative influence on placental growth and birth weight, but it is gestational intake after conception, particularly during the first third of pregnancy, which has the most profound influence on fetal development. Relative to optimally nourished controls, age-matched adolescents overnourished throughout pregnancy exhibit rapid maternal growth and increasing adiposity at the expense of the conceptus. Placental growth and vascular development, uteroplacental blood flows and fetal nutrient supply are compromised, and premature delivery of low birthweight lambs with a 45 % incidence of marked intrauterine growth restriction (IUGR) ensues. A more modest effect on fetal growth is evident in undernourished mothers (17 % incidence of IUGR). Here preventing maternal growth gradually depletes maternal body reserves and directly lowers nutrient availability in the maternal circulation independent of any change in placental size or gestation length. The maternal and placental adaptations to these diverse gestational intakes and the consequences for the fetus are presented together with the translational implications for detecting and avoiding birthweight extremes in human pregnancy.

Keywords Birth weight • Adolescent • Obese • Underweight • Placenta • Gestational weight change • Growth • Blood flow • IUGR

J. Wallace (✉)
Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK
e-mail: jacqueline.wallace@abdn.ac.uk

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4.1 Introduction

Birth weight is a valuable summation of prenatal fetal nutrient supply and a robust predictor of health and well-being immediately after delivery and throughout the life course. Of the infants born globally in 2013, and weighed at birth, an estimated 16 % were of low weight (<2500 g [1]), and even in relatively affluent countries such as the UK and USA, 8 % of infants were of low birthweight, including 0.9 % and 1.4 %, respectively, with very low birthweight (<1500 g [2–4]). The majority of very low birthweight infants are born prematurely, while those with modestly low birthweight (>1500 to <2500 g) are a mixture of early delivery and fetal growth restriction. Irrespective, relative to normal birthweight individuals, low birthweight infants born in wealthy countries are 25 times more likely to die within the first year of life [2, 4]. Infants that survive, irrespective of their country of birth, experience a range of physical and development issues that can limit their life chances. These include visual and aural impairment, autism, cerebral palsy, stunted growth, immune dysfunction, cognitive delay, behavioural problems and low educational attainment [5, 6]. Furthermore, low birthweight is a risk factor for diabetes, obesity, stroke, cardiovascular disease, immune dysfunction and osteoporosis in later life and several of these effects are exacerbated if the postnatal environment is nutrient rich such as occurs in populations undergoing economic transition and throughout the developed world [7–10]. At the other end of the weight spectrum, high birthweight (>4000 g) currently accounts for 7.4 % and 13.8 % of births in the USA and UK, respectively [3, 11]. Fetal macrosomia is a major risk factor for stillbirth, neonatal mortality (especially due to asphyxia), emergency delivery by caesarean section and infant mortality within the first year of life (especially due to sudden infant death syndrome) and is the predominant cause of birth injuries such as shoulder dysplasia: risks are most pronounced when weight exceeds 4500 or 5000 g [12–16]. High birthweight is strongly linked with the occurrence of an array of cancers throughout childhood, most notably leukaemia [17, 18]. Although less strong and more closely related to other biological factors such as birth length or adult height, positive associations between high birthweight and the incidence of breast, prostate, lymphatic, lung and colon cancer are evident in adult life [19–24]. Moreover, high birthweight is a risk factor for diabetes and obesity in later life and this relationship is variously influenced by maternal and paternal anthropometry and family history of diabetes [25–27].

Accordingly, avoiding birthweight extremes by increasing the proportion of babies born at a healthy weight is a pressing public health objective at individual government and world health organisation levels. The age, body composition and nutritional status of the mother at conception and her gestational intake thereafter plays an important and theoretically modifiable role in determining prenatal growth velocity and hence birth weight and is the focus herein.

4.2 Adverse Pregnancy Outcome: Who Is at Risk?

Across the world, the categories of women most readily identified as being vulnerable to poor pregnancy outcome are young adolescent mothers (<19 years old) and those of all ages who are either underweight or obese at the time of conception. For example, in Scotland these groups of women currently account for 10 %, 3 % and 18 % of all births, respectively [3]. In Sub-Saharan Africa, ~50 % of births are in adolescent mothers [28]. In this region, maternal underweight (17 %) rather than obesity (5 %) dominates in the general obstetric population, although obesity rates are rising [29].

4.2.1 Maternal Body Composition at Conception

Irrespective of the relative proportions in specific geographical locations, women who are underweight at conception are at greater risk of premature delivery, low birthweight and small for gestational age (SGA, birth weight <10th centile after adjustment for gender and gestational age) delivery [30–32]. At the opposite end of the body composition spectrum, maternal obesity is typically associated with a number of risks that generally increase stepwise with degree of overweight, namely hypertensive disorders (including pre-eclampsia), gestational diabetes, thromboembolism, fetal death, stillbirth, premature delivery, high birthweight and large for gestational age (LGA, birth weight >90th adjusted centile: [30, 33–36]). Obese women are also more likely to have an induced labour and to deliver their babies by either elective or emergency caesarean section. More rarely and somewhat paradoxically, obese women are also found to be at greater risk of both actual [37] or relative fetal growth restriction [38] which largely becomes apparent when customised birthweight centiles based on maternal weight, height, ethnicity and parity are used to define SGA [39]. Further, there are known associations between maternal obesity (independent of diabetes) and fetal malformations including spina bifida, anencephaly, congenital heart defects and orofacial clefts: these defects are often challenging to detect prenatally due to poor sonographic visualisation owing to the density of maternal body fat depots [40]. It is unsurprising that this myriad of pregnancy complications associated with a mother's BMI at conception results in a greater number and duration of maternal and neonatal admissions with associated health-care costs [41, 42].

Dietary intake and hence gestational weight change during pregnancy have the potential to ameliorate or exacerbate the risks associated with being under- or overweight at conception and recommended gestational weight gains (GWG) by pre-pregnancy BMI and for each period of pregnancy are available [43]. Although women who are underweight at conception commonly also display inadequate GWG, the converse is not always true. Thus, while ~70 % of obese women exceed current weight gain recommendations, there is also evidence that gestational weight

loss is more common in this group and increasingly prevalent as obesity severity increases [44, 45]. Irrespective, weight gain during pregnancy is an important independent predictor of birth weight [46], and in women with all categories of pre-conception obesity, the incidence of LGA robustly rises with increasing GWG and falls when GWG is inadequate. The converse is also broadly true with a decreased risk of SGA as GWG increased in obese women but with less potential benefit in the morbidly obese [46]. This summary data suggests that it may be safe to advocate GWG below current recommendations in obese women but when systematically examined obese women with GWG below the guidelines had a higher risk of both preterm delivery and SGA negating the benefits associated with a lower risk of LGA, hypertensive disease and caesarean delivery [47].

4.2.2 Young Maternal Age

Above all, the most consistent risk of poor outcome is when pregnancy coincides with adolescence. Relative to adult women, contemporary population-wide and single-centre cohort studies consistently report a higher risk of spontaneous miscarriage, premature delivery, low birthweight and neonatal mortality in adolescent pregnancies. These negative gestational outcomes are observed in low-, middle- and high-income countries and are particularly acute in very young girls who are gynaecologically and biologically immature [48–53]. Indeed, in low resource countries, biological immaturity also predisposes adolescent mothers to serious complications such as obstetric fistula [54], is associated with a plethora of maternal near miss events [55] and is a major factor contributing to the fourfold higher maternal death rate in very young mothers ($\leq$15 years) relative to both older adolescent (16–19 years) and adult (20–24 years) women [56].

Suboptimal dietary intakes are commonplace in the general adolescent population, and therefore many adolescent girls are in danger of becoming pregnant with poor nutrient stores and/or subsequently experiencing inadequate gestational weight gains. For example, relative to adolescents with a normal body mass index (BMI) at pregnancy booking, those classified as underweight (BMI $<$ 19) had a threefold higher risk of SGA birth [57]. In addition, low pregnancy weight gains in adolescent mothers have long been associated with a greater incidence of premature delivery, low birthweight and SGA that is variously dependent on the pattern of weight gain (early versus late versus all gestation) and age ($<$16 years versus 16–19 years) of the mother at conception [58–60].

Obesity has now overtaken underweight prevalence in most adolescent populations throughout the developed world [61] and hence the relationship between periconception obesity, gestational weight gain and pregnancy outcome in adolescent mothers is increasingly relevant. In single-centre and population-wide retrospective cohort studies involving ~700, 4822 and 34,648 adolescents delivering in three contrasting areas of the USA, being overweight or obese was typically associated with an increased risk of pregnancy hypertension, gestational diabetes

and impaired glucose tolerance, labour induction and caesarean section and was protective against premature delivery. Neonatal findings included higher average birthweight, greater incidence of macrosomia and more morbidity, while the prevalence of low birthweight and SGA was less common relative to normal BMI adolescents [62–66]. Some of the negative pregnancy outcomes were exacerbated by high gestational weight gains and influenced by race but on balance the effect of BMI, albeit based on self-reported pre-pregnancy height and weight, dominated. In direct contrast in a prospective observational study of adolescents based in the UK ($n = 368$), having a high BMI was linked to a threefold higher risk of SGA [57].

While the characteristics of adverse pregnancy outcome in adolescent mothers who are either underweight or overweight at conception are broadly similar to those described for adult women, the young adolescent mother is distinguished by the fact that she may still be growing or have the potential to grow at the time of conception. Although skeletal growth peaks before menarche, it can continue albeit at a slower rate into late adolescence. Accordingly, data from the Camden Adolescent Pregnancy and Nutrition Project (based in New Jersey, USA) indicated that continued maternal growth occurs in approximately 50 % of the pregnant adolescent population. This continued maternal growth as measured by sequential changes in knee height was associated with larger pregnancy weight gains and increased fat stores, but in spite of this the babies were 150–200 g smaller than those born to non-growing adolescents and mature women [67]. These effects attributed to a competition for nutrients between the maternal body and her gravid uterus [68] are supported by a large retrospective analysis of subjects ($n = 9694$) with similar pre-pregnancy weight range and term delivery, in which young adolescents (14–17 years) were shown to transfer a smaller proportion of their pregnancy weight gain to their fetuses than older adolescents (17–19 years) and adult (20–25 years) women [69]. A similar maternal–fetal growth competition for nutrients has been observed within a group of Peruvian adolescents (13–15 years). When adolescent growth status at delivery was defined on the basis of achieving parental height, the adolescent mothers who had not achieved their predicted adult height and therefore categorised as 'still-growing' had smaller babies than those who had achieved their expected growth [70]. In a more contemporary multicentre study in two socially deprived areas of the UK, a third of adolescent girls (average age 17.8 years) continued to grow during pregnancy and had higher gestational weight gains and fat accrual than non-growers [57]. Nevertheless and in contrast to earlier studies of younger adolescents, this was not associated with fetal growth restriction but rather an increase in LGA births. Alternatively, comparisons between non-pregnant and pregnant adolescents suggest that normal fetal growth can be maintained if pregnant mothers diminish their resting energy expenditure and cease growing to conserve nutrient supply for the fetus [71].

The relationship between nutritional status at conception, gestational dietary intake and pregnancy outcome is clearly appreciably more complex when pregnancy coincides with the continued or incomplete growth of the mother. It was against this background that a highly controlled sheep paradigm was originally

developed to examine the role of maternal nutrition in mediating pregnancy outcome in the young but still growing adolescent.

4.3 Nutrition, Growth and Pregnancy Outcome in Young Adolescent Sheep

4.3.1 Basic Adolescent Sheep Paradigm

The basic adolescent paradigm as developed in my laboratory involves assisted conception procedures to establish singleton pregnancies in peripubertal adolescent ewes of equivalent age, live weight and adiposity at conception. Adult ewes of known reproductive history and in prime breeding condition are superovulated and intrauterine inseminated by a single sire and act as embryo donors. Within individual studies the resulting grade 1 embryos for any given embryo donor are then distributed evenly across the study groups: this controlled approach minimises the impact of the main peri-conceptual factors known to influence feto-placental growth and maximises the genetic homogeneity of the resulting fetuses [72]. Adults are preferentially used as embryo donors as prior reciprocal embryo transfer studies revealed that embryos derived from adolescent ewes have inherently low viability following transfer into either an adolescent or adult uterus [73, 74]. Nutritional treatments typically commence immediately after embryo transfer and involve offering the young still-growing adolescent recipient varying quantities of the same complete diet to manipulate gestational weight gain and thereby growth and adiposity. In the overnourished model, this involves offering the adolescent mothers a high dietary intake throughout gestation (~2 × maintenance requirements) to promote rapid maternal growth and is designed to mimic pregnancy in adolescent girls who continue to grow significantly while pregnant. In contrast in the second and to date less well-studied undernourished model, the adolescent dams are prevented from growing while pregnant (low intake, ~0.7 × maintenance). The control group for both models involves a moderate dietary intake designed to facilitate a small amount of maternal growth (maintenance) and calculated to maintain maternal adiposity at a consistent level throughout gestation: this allows the estimated nutrient requirements for optimum conceptus growth to be met and is achieved by modest step-wise increases in maternal intake of control dams during the final third of gestation.

4.3.2 Pregnancy Outcome in Overnourished Adolescents

The overnourished model has proved extremely robust over many years and a summary analysis of pregnancy outcome in relation to maternal nutrition during

gestation was published a decade ago [75]. This revealed that high dietary intakes to promote rapid maternal growth were associated with an increased incidence of miscarriage and stillbirth in late gestation, and while mean placental and fetal growth were significantly reduced relative to controls, the degree of compromise was variable with 52 % of pregnancies classified as intrauterine growth restricted (IUGR). A summary analysis for the new trials carried out in the intervening period is presented in Table 4.1 together with indices of maternal anthropometry. A live born fetus spontaneously delivered at term was categorised as markedly growth restricted if its birth weight was two standard deviations below the mean birth weight of fetuses in the control group. As control group male fetuses were on average 287 g heavier than females, the categorisation used sex-specific cut-offs (IUGR birth weight, <4108 g for males and <3798 g for females) and on this basis 98 of 218 high intake pregnancies (45 %) were classified as growth restricted. Using this approach to subdivide the high intake (overnourished) pregnancies reveals that in the growth restricted category, placental weight and fetal cotyledon weight were reduced by 46 % and 58 %, respectively, relative to the control group, and associated with a 45 % reduction in birth weight. In contrast in the non-growth-restricted group, placental weight and fetal cotyledon weight were reduced by 17 % and 31 %, respectively, and lambs were on average 12 % smaller: although much less perturbed, feto-placental weights were still statistically lower than in the control group. The positive relationship between placental mass and birth weight is emphasised in Fig. 4.1a and is appreciably stronger in the IUGR pregnancies suggesting less of a functional reserve when the placental growth trajectory has been severely compromised. Another consistent feature of the overnourished pregnancies is a major reduction in gestation length with viable lambs being born as early as day 135 of gestation (term = 145 days, Fig. 4.1b). Although the average reduction in gestation length is slightly greater in the growth restricted compared with the non-IUGR pregnancies (~4.5 and 3.7 days, respectively, Table 4.1), it is the dam's nutritional intake and associated reduction in placental hormone concentrations (progesterone and oestradiol-17ß) which dominates and most likely underlies early delivery relative to the control group [76, 77]. Importantly, when birth weight is adjusted to a standard gestational age, the large differences in birth weight between groups remain. As sheep tolerate prematurity poorly, even small reductions in gestation length can have profound consequences for the smallest lambs. These are exacerbated by a major reduction in the initial yield (Table 4.1), nutrient composition and IgG content of colostrum in overnourished dams [78–80] and by the delayed formation of an adequate ewe–lamb bond. The colostrum yield at parturition is positively related to placental mass and thereby reflective of previously documented reductions in lactogenic hormones including those secreted by the placenta predominantly during the second half of gestation (placental lactogen, progesterone, oestradiol-17ß [76, 77, 81]). Lambs which fail to ingest sufficient quantities of quality colostrum in the early neonatal period are vulnerable to hypothermia and infection and more than 65 % of overnourished pregnancies detailed (Table 4.1) were deemed potentially at risk. Initially neonatal mortality rates were unacceptably high [72] and thus a proactive regimen of intensive

Table 4.1 Maternal anthropometry and pregnancy outcome in singleton-bearing adolescent sheep offered a moderate (control) or high nutrient intake (overnourished) throughout gestation and categorised according to fetal growth status after spontaneous delivery[¥]

	Maternal nutrient intake and fetal growth status[¥]			
	Control—normal	High—IUGR	High—non-IUGR	Significance[ß]
No. of pregnancies	97	98	120	
Weight at conception (kg)	44.2 ± 0.39	43.0 ± 0.63	45.1 ± 0.63	$P = 0.042$
GWG, d4 to d50 (g/day)	48 ± 3.2^{a}	286 ± 6.5^{b}	255 ± 6.0^{c}	$P < 0.001$
GWG, d50 to d95 (g/day)	109 ± 2.9^{a}	319 ± 6.3^{b}	316 ± 5.2^{b}	$P < 0.001$
Weight after delivery (kg)	56.9 ± 0.4^{a}	77.3 ± 0.70^{b}	77.7 ± 0.64^{b}	$P < 0.001$
*Adiposity at conception	2.3 ± 0.02^{a}	2.3 ± 0.02^{b}	2.4 ± 0.02^{b}	$P = 0.031$
Delta adiposity, d4 to d50	0.0 ± 0.0^{a}	0.3 ± 0.03^{b}	0.2 ± 0.01^{b}	$P < 0.001$
Delta adiposity, d50 to d95	0.0 ± 0.0^{a}	0.6 ± 0.02^{b}	0.6 ± 0.02^{b}	$P < 0.001$
Adiposity pre-delivery	2.3 ± 0.02^{a}	3.1 ± 0.03^{b}	3.2 ± 0.03^{b}	$P < 0.001$
Gestation length (days)	145.2 ± 0.18^{a}	140.7 ± 0.23^{b}	141.5 ± 0.17^{c}	$P < 0.001$
Birthweight (g)	5427 ± 76^{a}	3007 ± 69^{b}	4769 ± 55^{c}	$P < 0.001$
Male:Female	46:51	55:43	60:60	NS
[þ]Adjusted birthweight (g)	5405 ± 72^{a}	3166 ± 70^{b}	4989 ± 54^{c}	$P < 0.001$
Placental weight (g)	442 ± 12^{a}	238 ± 6^{b}	365 ± 9^{c}	$P < 0.001$
Fetal cotyledon weight (g)	146 ± 4.4^{a}	61 ± 2.1^{b}	101 ± 2.6^{c}	$P < 0.001$
Birth wt : cotyledon wt	39.6 ± 1.00^{a}	52.1 ± 1.16^{b}	49.9 ± 1.13^{b}	$P < 0.001$
Birth wt : Maternal wt. gain d4 to d95	859 ± 39.9^{a}	109 ± 3.4^{b}	182 ± 4.3^{c}	$P < 0.001$
Colostrum yield (ml)	497 ± 40^{a}	113 ± 10^{b}	202 ± 13^{c}	$P < 0.001$
[ɣ]No. with inadequate colostrum/kg fetus	24 of 91[a]	63 of 91[b]	77 of 117[b]	$P < 0.001$

Values are mean ± sem. Data from seven studies ([80, 82, 86, 107, 116] plus unpublished)

[¥]Lambs from overnourished pregnancies were classified as intrauterine growth restricted (IUGR) if birthweight was < two standard deviations below the mean sex-specific birthweight of the optimally nourished control group, i.e. <3798 g for females and <4108 g for males

[ß]From Anova followed by Tukey comparison. Within rows where superscript letters (a,b,c) differ, $P < 0.01$. Sex distribution and number of ewes with inadequate colostrum compared by binary logistic regression

*Based on external body condition score (5 point scale where 1 = emaciated and 5 = morbidly obese) and assessed by a single experienced operator across all studies

[þ]Individually adjusted to a standard gestation of 145 days on the basis of the formula; adjusted birthweight = weight at birth/1.01305 per day of gestation

[ɣ]Defined on the basis of requirement of 50 ml per kg fetal weight

GWG gestational weight gain

neonatal care including supplementary feeding and prophylactic antibiotics is required to ensure that the most premature and growth-restricted individuals survive.

Retrospective analysis of maternal anthropometry in adolescent dams fed ad libitum to promote rapid growth was used to identify the antecedents of fetal growth

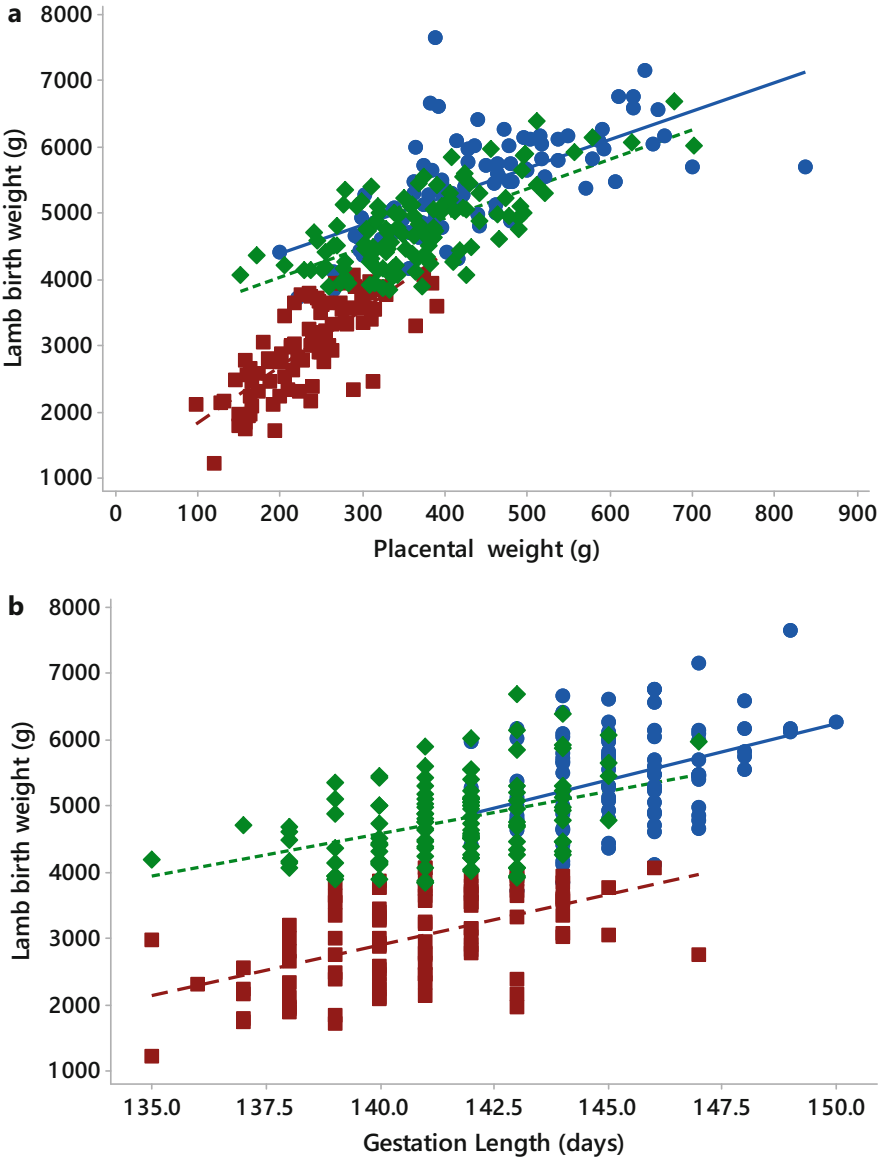

Fig. 4.1 Relationship between lamb birthweight and (**a**) placental weight and (**b**) gestation length in singleton bearing adolescent dams offered a control dietary intake to maintain maternal adiposity (*blue circles*) or a high dietary intake to promote maternal growth and adiposity throughout gestation. The latter pregnancies were categorised as intrauterine growth restricted (IUGR, *red squares*) or non-IUGR (*green diamonds*) using sex-specific birthweight cut-offs derived from the control group birthweight data as defined in the text

restriction. From an equivalent weight and adiposity at the time of conception, the dams allocated to the high intake group and subsequently delivering a growth-restricted fetus were very slightly lighter and leaner at conception than dams delivering a non-IUGR fetus (Table 4.1). However, the most striking difference between these groups was in the rate of weight gain during the first third of pregnancy (High-IUGR > High-Non-IUGR > Control). This is consistent with an early impact of maternal dietary intake on the development of the placenta (see below). Furthermore, the birth weight to maternal weight gain ratio serves to illustrate that the adolescent dams that grow fastest during the first two-thirds of gestation transfer a lower proportion of that gain to their fetus (Table 4.1). This alteration in the hierarchy of nutrient partitioning between the maternal body and her gravid uterus is independent of the protein content of the diet [82] and is unique to the adolescent growth period as it does not occur in identically treated primiparous adult ewes [83].

4.3.3 Pregnancy Outcome in Undernourished Adolescents

When adolescents are prevented from growing while pregnant the impact on pregnancy outcome is less pronounced. Accordingly in undernourished adolescents, placental weight and gestation length are equivalent to control pregnancies and no incidences of miscarriage, stillbirth or neonatal death have been recorded. By maintaining maternal body weight at conception levels, maternal nutrient reserves (mainly fat) are progressively depleted as gestation proceeds. This directly limits nutrient availability in the maternal and hence fetal circulation and leads to a slowing of fetal soft tissue growth. By late gestation and at term, the fetus is mildly growth restricted (10–17 % smaller than controls [84–86]), and using the same definition as above, only 14 % of these undernourished lambs were classified as IUGR. Furthermore, although the quantity of colostrum produced immediately after parturition was reduced it largely met the minimum requirement for IgG content and nutrient composition [86]. Thus, while both high and low dietary intakes during pregnancy negatively influence fetal growth in young adolescents, it is the overnourished model which most closely replicates the human with respect to the greater risk of miscarriage, preterm delivery, low birth weight and neonatal mortality.

4.3.4 BMI at Conception Versus Gestational Intake

The basic adolescent paradigms originally focused on manipulating dietary intake and maternal growth status immediately after pregnancy had been established and great care was taken to ensure that the adolescents were of equivalent age, weight and adiposity at conception. However, in the real world, adolescent girls have

diverse nutritional histories and enter pregnancy with varying nutrient reserves which may interact with subsequent gestational intake and growth status to influence pregnancy outcome. To partly model this scenario, two groups of adolescent ewes of the same age but with different weight and adiposity were selected 4 weeks before the application of assisted conception procedures and nutritionally managed to maintain their initial weight. In reality, this represented a 10 kg (20 %) differential in weight, and a 5 % differential in estimated body fat between groups and adolescent ewes were hence classified as having a relatively good or poor BMI at conception. Thereafter ewes were overnourished, undernourished or fed a control intake throughout gestation to drive maternal growth and gestational weight gain in contrasting directions as described previously. BMI at conception did not influence gestation length but did influence placental size and lamb birth weight (good > poor, $P < 0.001$ and $P = 0.031$, respectively). Indeed, although the initial differences in maternal weight and adiposity between groups were relatively small, ewes with a poor BMI at conception gave birth to lambs that were on average 500 g lighter than those with a good BMI. In spite of this, gestational intake still had the most marked effect on lamb birth weight (control > undernourished > overnourished, $P < 0.001$) and the percentage of lambs classified as IUGR, irrespective of baseline BMI was greatest in the overnourished group (55 % versus 4 % in control and 12.5 % in undernourished groups, $P < 0.001$ [80]).

4.3.5 Donor Ewe Adiposity Versus BMI at Conception

Pregnancy outcome may also be influenced by nutrition before conception and studies in adult sheep and rodent models report effects of varying nutrition during the pre and peri-conception periods on fetal growth and physiology [87–89]. Interpretation of these data is complex in that nutritional treatments may have carry-over effects which influence the metabolism of the dam and her early uterine environment. The assisted conception procedures used to derive the adolescent pregnancies described herein potentially offer a cleaner approach and hence the impact of maternal obesity during oocyte development and its putative interaction with nutrient reserves at conception on pregnancy outcome have been assessed. Adult donor ewes were nutritionally managed to achieve a control and obese phenotype corresponding to a 12 % differential in body fat, and these adiposity levels were maintained for 6 weeks prior to superovulation and embryo recovery. Embryos were then transferred into adolescent recipients with either a relatively good or poor BMI at conception and all were subsequently overnourished throughout gestation (2×2 factorial). A fifth group of recipients with standard BMI at conception received embryos from control donors and were fed a control intake throughout and studied in parallel: these acted as the reference point for optimal adolescent pregnancy outcome (Table 4.2). Embryo donor adiposity did not influence ovulation or embryo recovery rates, and somewhat contrary to expectation, we

Table 4.2 Impact of embryo donor adiposity during oocyte development and embryo recipient weight and adiposity at conception on pregnancy outcome in young adolescent sheep

Embryo donor adiposity	Control	Control	Obese	Obese	Two-way ANOVA *P*-value[ß]			Control*
Embryo recipient BMI	Poor	Good	Poor	Good	Donor Adiposity	Recipient BMI	Interaction	Standard
Gestational Intake	Overnourished	Overnourished	Overnourished	Overnourished				Control
Recipient wt. at ET, kg	38.2 ± 0.37^{a}	57.6 ± 0.71^{b}	38.0 ± 0.33^{a}	55.8 ± 1.01^{b}	0.141	<**0.001**	0.266	46.7 ± 0.30^{c}
[¥]Recipient adiposity at ET	2.0 ± 0.00^{a}	2.7 ± 0.02^{b}	2.0 ± 0.00^{a}	2.6 ± 0.03^{c}	**0.028**	<**0.001**	**0.028**	2.3 ± 0.01^{d}
Wt change, ET to term, kg	36.0 ± 1.27^{a}	26.9 ± 0.91^{b}	35.8 ± 1.30^{a}	25.9 ± 2.27^{b}	0.706	<**0.001**	0.781	12.7 ± 0.49^{c}
Adiposity change, ET to term	0.9 ± 0.03^{ab}	0.6 ± 0.05^{b}	1.0 ± 0.03^{a}	0.7 ± 0.11b	0.286	<**0.001**	0.801	0.1 ± 0.02^{c}
Gestation length, days	140.7 ± 0.53^{a}	141.2 ± 0.54^{a}	139.4 ± 0.59^{a}	140.6 ± 0.35^{a}	0.060	0.106	0.506	143.9 ± 0.34^{b}
Birthweight, g	3634 ± 292^{a}	4499 ± 337^{ab}	3802 ± 357^{a}	4259 ± 315^{ab}	0.912	**0.047**	0.533	5425 ± 166^{b}
[¥]Proportion IUGR	9 of 14	4 of 14	8 of 13	4 of 14	0.883	**0.035**		1 of 14
Fetal cotyledon weight, g	75 ± 9.1^{a}	107 ± 11.0^{a}	78 ± 10.6^{a}	99 ± 11.7^{a}	0.799	**0.017**	0.620	156 ± 8.2^{b}
Fetal:cotyledon weight	52 ± 3.3^{a}	44 ± 2.4^{ab}	51 ± 2.6^{a}	47 ± 3.0^{ab}	0.786	**0.036**	0.491	36 ± 1.4^{b}

Values are mean ± sem. JM Wallace, RP Aitken, JS Milne unpublished data

[ß]Four group comparison by two-way ANOVA with significant P-values in bold.

*Five group comparison analysed by one-way ANOVA (all parameters $P < 0.001$) followed post-hoc by Tukey's Method to differentiate between groups; thus, within rows values with a different superscript letter (a,b,c,d) differ at $P < 0.01$

[¥]External adiposity score determined by single experienced operator

[¥]Lambs were classified as intrauterine growth restricted (IUGR) if birthweight was < two standard deviations below the mean birthweight of the optimally nourished control group, i.e. <3939 g. Incidence of IUGR compared by binary logistic regression

Wt. weight, *ET* embryo transfer (single embryos, all from one sire)

found no evidence that embryo donor obesity (equivalent to ~33 % body fat) negatively influenced conception rate or prenatal conceptus growth following embryo transfer. The caveat is that by selecting only those oocytes that had been fertilised and developed appropriately to day 4 of the cycle, the study design avoided transferring embryos that were potentially nutritionally perturbed. Irrespective, the previously reported impact of low nutrient reserves at conception (poor BMI) on average birth weight and the incidence of IUGR was replicated here, but once again comparison with the optimally nourished control group demonstrates that high gestational intakes to promote rapid maternal growth remain the main determinant of fetal growth in young still-growing sheep.

4.3.6 Maternal Adaptations to Diverse Gestational Intakes

The endocrine responses to diverse levels of dietary intake and their putative role in nutrient partitioning during adolescent pregnancy have been extensively studied. Briefly, relative to the control group, high gestational intakes are associated with increased insulin and insulin-like growth factor 1 (IGF-1) concentrations from early in pregnancy providing a sustained anabolic stimulus to maternal tissue deposition. Metabolic challenges demonstrate that overnourished dams are insulin resistant [80] and circulating glucose levels are raised throughout gestation [90, 91]. High maternal leptin concentrations reflect that internal fat depots are elevated as early as day 50 of gestation and that maternal carcass fat content progressively increases from mid to late pregnancy [75, 92, 93]. This rapid maternal growth and increased adiposity is linked with early depletion of maternal liver iron stores during the first two-thirds of gestation and with a failure of the normal blood volume expansion of pregnancy between mid and late gestation [94]. The associated increase in maternal haematocrit, haemoglobin and plasma protein concentrations may in turn impact blood viscosity and thereby uteroplacental blood flow and fetal nutrient supply. Indeed the blood from overnourished dams at day 130 of gestation is more viscous than that of controls (1.471 ± 0.0111 units versus 1.406 ± 0.0139 units, respectively, $P < 0.001$; unpublished data).

In contrast, undernourished adolescent dams are characterised by low circulating insulin, IGF-1 and leptin concentrations: by late gestation maternal glucose concentrations are reduced and high non-esterified fatty acid concentrations reflect depleted maternal fat stores [84]. Relative blood volume expansion is unperturbed and low availability of nutrients in the maternal circulation is the main cause of the modest reduction in fetal growth velocity. This differs markedly from the situation in overnourished adolescents where in spite of excess nutrients in the maternal circulation, fetal growth restriction is mediated by major alterations in placental growth and function.

4.3.7 Placental Adaptations to Diverse Gestational Intakes

While cross-sectional studies at key stages of development demonstrate that placental mass is not significantly perturbed until the beginning of the final third of gestation [95], the adaptations that underlie the placental programming of fetal growth restriction in overnourished adolescents can be detected from early in pregnancy. Accordingly, reduced proliferative activity was measured in both placental compartments at day 50 of pregnancy [96], and, in a separate study at the same stage, vascular development (i.e. vessel size) within the fetal cotyledon was already impaired [93]. In addition, there was a delay in the onset and magnitude of placental lactogen and pregnancy-specific protein-B concentrations indicative of reduced trophoblast cell migration [76, 81] and by the beginning of the second third of gestation placental steroid secretion was lower than in control-fed dams [76, 77, 97]. At mid-pregnancy and the apex of placental growth, angiogenic growth factor ligand and receptor mRNA expression was attenuated and indices of proliferation and apoptosis perturbed [98, 99]. These adaptations preceded the change in placental mass and together indicate that the placentae were already on a different developmental and haemodynamic trajectory. In support, uterine blood flow was reduced and umbilical artery Doppler indices increased in overnourished pregnancies at mid-gestation and both predicted the reduced fetal growth velocity observed during the final third of gestation [100, 101]. By late gestation (~day 133) placental weight was ~45 % lower, and similarly uterine and umbilical blood flows, uteroplacental glucose and oxygen consumption and lactate production, and placental glucose transport were all reduced by 35–40 % relative to control pregnancies. However, all the aforementioned parameters were equivalent between groups when expressed on a placental and or fetal weight-specific basis [102, 103], indicating that it is the small size of the placenta rather than altered nutrient uptake, metabolism and transport which mediates reduced fetal growth velocity in the final third of gestation in these rapidly growing adolescents.

Initial nutritional switch-over studies indicated that the placental growth trajectory could be rescued by reducing maternal intakes from a high to a control level at day 50 of gestation, thereby restoring birth weight to the same level as in dams fed control rations throughout. In contrast an abrupt increase in dietary intake at this time inhibited placental and fetal growth to the same degree as in continuously overnourished dams [91]. In a more recent study when the dietary intake of overnourished dams was radically reduced sufficient to induce major maternal catabolism during the final third of pregnancy (high to low intake from day 90–130, HL), placental expression of five angiogenic genes including vascular endothelial growth factor (VEGF) was upregulated in the fetal cotyledon. This is commensurate with blood vessel remodelling, but in spite of this presumed adaptation, placental mass and fetal weight could not be rescued and were equivalent in high versus HL groups [104]. Thus, unsurprisingly the placenta is most sensitive to abrupt changes in maternal nutrition during its main proliferative growth phase.

The precise mechanisms underlying the nutritionally induced suppression of placental growth have however remained elusive. Attempts to reverse the negative effects of overfeeding by restoring circulating maternal oestrogen or progesterone concentrations to control levels during early-mid gestation have failed to influence placental vascularity or rescue placental weight as assessed at late gestation or at term, respectively [97, 105]. In contrast overnourished pregnancies are also characterised by attenuated growth hormone (GH) secretion and when dams were treated with exogenous GH during the period of rapid placental proliferation (day 35–80) an initial study indicated nutrient partitioning was altered in favour of uteroplacental and fetal growth as assessed at day 81 of gestation [106]. In a subsequent study, maternal GH treatment either targeted the period of rapid placental growth or the period after placental growth was complete and fetal nutrient demand was high. These early (day 35–65) and late (day 95–125) pregnancy GH treatments both had a major influence on maternal metabolism, resulting in insulin resistance, decreased lipogenesis and a threefold increase in maternal glucose concentrations. For the late pregnancy group this resulted in a modest stimulation of fetal growth and a major increase in fetal adiposity at day 130 of gestation which was independent of any change in placenta size, suggesting that while GH has a major impact on nutrient partitioning within the still-maturing somatotrophic axis of the pregnant adolescent it does not act directly on the placenta [107].

In undernourished adolescents neither placental proliferation nor mass differed from control pregnancies at mid-late gestation or following spontaneous delivery at term [85, 86]. Irrespective, vascular changes within the placenta may play a role in mediating the reduction in nutrient supply between the dam and her fetus in these pregnancies. A robust 20 % decrease in capillary area density within the maternal caruncular component of the placenta was measured at both day 90 and 130 of gestation and could not be reversed by re-alimentation to control intakes between these two stages [85]. Contemporaneous assessments of uterine blood flow (UtBF) in vivo in undernourished compared with control dams suggest that the reduction in capillary development is mirrored by a decrease in average flow of similar magnitude [average daily UtBF between day 88 and 135 of gestation $= 418 \pm 43$ and 326 ± 23 ml/min in control ($n = 9$) and undernourished ($n = 11$) pregnancies, respectively, $P = 0.08$]. This modest reduction in uterine blood flow may in part be secondary to mild maternal anaemia as low intakes are associated with a decrease in maternal haematocrit and haemoglobin content relative to control dams by late gestation (30 ± 0.4 % versus 35 ± 0.6 % and 9.6 ± 0.13 g/dl versus 10.6 ± 0.17 g/dl, $n = 21$ and 16 per group respectively, $P < 0.001$; unpublished).

There is a paucity of placental data in relation to growth and nutrition in human adolescents. Path analysis in a large cohort of Peruvian women suggests that the contribution of placental weight to birth weight was less in girls who were still growing [70], and similarly umbilical artery Doppler indices were elevated indicating reduced flow in growers versus non-growers in the Camden Study [67]. In contrast, in more contemporary studies, placental weight and morphometry were independent of adolescent growth status, but adolescents per se had inherently reduced placental transport of amino acids compared with adults [108, 109]. Unlike

in our sheep paradigms, it is important to emphasise that non-growers may comprise girls who are skeletally mature and those whose growth is constrained by poor nutrient intakes making the data complex to interpret.

4.3.8 Fetal Consequences of Diverse Gestational Intakes

Regular ultrasound examination allows fetal growth velocity to be monitored non-invasively throughout gestation and accordingly in the overnourished adolescents various indices of fetal size including abdominal circumference (AC), renal volume (RV) and femur and tibia lengths were reduced from around day 100 of gestation onwards compared with normally growing control fetuses [101]. This relatively late-onset fetal growth restriction is asymmetric in that growth of the brain and adrenal glands were preserved at the expense of the visceral organs [102, 110, 111]. The access to the placental and fetal circulation offered in sheep models additionally allows interrogation of fetal endocrine and nutrient status, nutrient uptakes and metabolism. So in late gestation the growth-restricted fetuses are characterised by absolute reductions in umbilical (fetal) uptakes of glucose, oxygen and amino acids which are equivalent to normally growing control fetuses when expressed on a fetal weight-specific basis [75, 102, 103, 111]. Moreover, even though the growth-restricted fetus increases glucose extraction in an attempt to offset diminished glucose supply, the concentrations of glucose, insulin and IGF-1 in the fetal circulation remain low. The fetal sensitivity to insulin and glucose has been examined during fetal hyperinsulinaemic–euglycaemic and hyperglycaemic–euinsulinaemic clamps and reveals normal body weight-specific responses to short-term experimental increases in plasma insulin and/or glucose [112]. This is indicative of maintained mechanisms of insulin action and glucose uptake/utilisation capacity allowing the fetus to preserve essential metabolic functions at the expense of body growth. If such adaptations persist, these IUGR offspring may be vulnerable to increased fat deposition postnatally when nutrient supply is no longer limiting. Indeed, there are indications that the growth-restricted fetuses of overnourished dams may already have a relatively fat phenotype prior to birth. Thus, while absolute perirenal adipose tissue (PAT) mass is reduced, fetal weight-specific PAT mass and carcass fat content are greater [113] and plasma cholesterol and LDL levels at birth are elevated [86]. This increase in relative adiposity may have its origins earlier in gestation prior to placental limitation of absolute fetal glucose supply. Thus, greater glucose concentrations in the amniotic fluid at day 50 and in the fetal plasma at both day 77 and 90 of gestation [93] may drive an increase in adipocyte proliferation in early-mid pregnancy and thereby increased potential for fat accumulation in late pregnancy and beyond. While definitive evidence to support such a hypothesis is lacking, it is noteworthy that appetite regulatory genes in the fetal hypothalamus (primarily anorexigenic neuropeptides) are responsive to fetal hyperglycaemia at mid and late gestation [114, 115]. Furthermore, relative to normal birthweight controls, both male and female IUGR offspring

of overnourished dams display rapid fractional growth rates particularly during the neonatal period, have more body fat at weaning at 3 months of age and show altered metabolic responses to exogenous glucose from juvenile through to adult life [116].

In direct contrast, fetuses of undernourished adolescent dams have a thin phenotype. Key genes that regulate fetal adipocyte proliferation and function are active at mid-gestation when they are sensitive to maternal undernutrition: this leads to reduced fetal adiposity by late pregnancy while skeletal growth is preserved [117]. In this instance, the fetal hypothalamus is sensitive to presumed fetal hypoglycaemia and orexigenic neuropeptides are upregulated in the fetuses of undernourished dams. Crucially the expression of these genes can be normalised by realimenting undernourished dams to a control intake between mid and late pregnancy [118]. At birth, plasma lipids in the modestly growth-restricted offspring are equivalent to normally growing controls and thereafter there is little evidence of altered growth, perturbed metabolism or body composition [86].

4.3.9 Translational Perspectives

Essential differences between these ruminant-based experimental studies and human pregnancies are fully appreciated. Nevertheless, the information obtained from these highly controlled paradigms has implications for both young adolescents and for women at risk of adverse pregnancy outcome irrespective of maternal age. For young adolescent girls, it is clear that both nutrient reserves at conception and gestational dietary intake thereafter are likely to be a powerful determinant of fetal growth particularly if maternal growth per se is ongoing or incomplete. In cultures where early marriage soon after menarche and hence pregnancy during young adolescent life is normal, girls with a low BMI should be encouraged to gain weight and achieve a normal BMI before conception. Thereafter, dietary intakes should be sufficient to maintain maternal nutrient reserves throughout gestation and meet fetal nutrient requirements particularly during the rapid growth phase in the final third of gestation. Measuring changes in skinfold thickness in addition to monitoring weight gain may be a simple and beneficial tool in this respect. Determining the growth status of individual adolescents at pregnancy outset is likely to be challenging, and while measuring, biomarkers of growth and nutrient status may be helpful in predicting the risk of poor outcomes, studies verifying such an approach are currently lacking. Where adolescent pregnancies are unplanned and calorie intakes likely to be high, the mother should be advised of the dangers of overeating and excessive weight gain during pregnancy, particularly during the period spanning placental proliferation. Indeed, as the placenta is central to mediating poor pregnancy outcome in young adolescents, early diagnosis of deficiencies in uteroplacental growth and/or blood flow is likely to be beneficial for identifying those at risk of fetal growth restriction.

Similarly for pregnant women irrespective of age, placental size plays a pivotal role in determining the fetal growth trajectory and birthweight extremes. Recent analysis of pregnancy complication risk reveals that placental weight in the lower tertile for a given population is a risk factor for pre-eclampsia, spontaneous preterm delivery, stillbirth and low birthweight while a placental weight in the upper tertile is associated with a higher risk of caesarean section and high birthweight [119]. Placental weight increases with increasing maternal BMI at conception through underweight to morbidly obese categories (4 g per BMI unit), and relative to women with a normal BMI, underweight women are more likely to experience placental growth restriction, while obese women have a twofold higher risk of having a large placenta: thus, the growth and final size of the placenta offers an explanation for the aforementioned relationship between the extremes of maternal BMI and pregnancy outcome. In addition, the placenta is sensitive to changes in maternal weight between consecutive pregnancies and appears to lie on the causal pathway between both inter-pregnancy BMI loss and BMI gain leading to a greater risk of SGA and LGA, respectively, at the second delivery [120]. The implication is that weight change between pregnancies impacts maternal nutrient reserves at the start of the second pregnancy and hence the placental growth trajectory as shown in the aforesaid sheep studies. Again placental screening in the first trimester may help early identification and appropriate management of those at risk [121].

Finally, as uteroplacental insufficiency and low uterine blood flow are the main underlying cause of most severe fetal growth restriction, there is a requirement to develop therapies to improve fetal nutrient supply, maintain growth and extend gestation until the baby can be delivered safely and survive without handicap. A potential therapy involving local uterine artery adenovirus (Ad.)-mediated overexpression of vascular endothelial growth factor (VEGF) in the putatively growth-restricted pregnancies of overnourished adolescent dams has been evaluated. Ad.VEGF administration in mid-pregnancy robustly increased fetal growth velocity as measured at 3 and 4 weeks after treatment, reduced the incidence of IUGR and increased birth weight by 20 % [122]. Proof of concept that this gene therapy is safe and efficacious in sheep now paves the way for clinical trials.

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Chapter 5
Maternal Obesity and Programming of the Early Embryo

J.J. Eckert, M.A. Velazquez, and T.P. Fleming

Abstract Obesity is on the increase and becoming one of the biggest health concerns worldwide due to associated non-communicable diseases such as type 2 diabetes and cardiometabolic dysfunction. Epidemiological and experimental evidence shows that obesity does not only impact on the individual but also on progeny across generations, implying contributing causal factors other than post-natal lifestyle. A wealth of studies have confirmed that maternal obesity is linked to offspring BMI and non-communicable diseases in later life through developmental programming in utero. This is mediated by developmental plasticity whereby the developing organism adapts to prevailing conditions. Developmental plasticity and its consequences are detectable as early as preimplantation, before the mother is aware of her pregnancy. Significantly, embryo transfer and developmental studies indicate the adult non-communicable disease phenotype can be traced back to the periconception period with poorer quality oocytes and embryos. Here, we give an overview of our current understanding of mechanisms involved linking preimplantation embryo morphogenesis and metabolism through to gene expression and epigenetic regulation in response to adverse environments such as obesity. Potential upstream mediators such as embryonic environmental sensors and maternal inducers are considered, including the impact of the reproductive tract at the maternal–embryonic interphase at a time preceding the formation of a functional placenta.

J.J. Eckert (✉)
Faculty of Medicine, Human Development and Health, University of Southampton, Southampton General Hospital, Mailpoint 840, Level D Laboratories and Pathology Block, Tremona Road, Southampton SO16, 6YD, UK
e-mail: jje@soton.ac.uk

M.A. Velazquez
Centre of Biological Sciences, Southampton General Hospital, University of Southampton, Tremona Road, Southampton SO16, 6YD, UK

School of Agriculture, Food and Rural Development, Newcastle University, Agriculture Building, Newcastle upon Tyne NE1 7RU, UK

T.P. Fleming
Centre of Biological Sciences, Southampton General Hospital, University of Southampton, Tremona Road, Southampton SO16, 6YD, UK

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Abbreviations

AMPK	Adenosine monophosphate-activated protein kinase
ART	Assisted reproductive technologies
CVD	Cardiovascular disease
DOHaD	Developmental origins of health and disease
EGA	Embryonic genome activation
Epi	Epiblast
ER	Endoplasmic reticulum
ET	Embryo transfer
ICM	Inner cell mass
mTORC1	Mammalian target of rapamycin complex 1
NEFA	Non-esterified fatty acids
NCD	Non-communicable diseases
PE	Primary endoderm
PPAR	Peroxisome proliferator-activated receptor
TE	Trophectoderm

5.1 Introduction

Obesity is a global threat of increasing public health concern worldwide, contributing to non-communicable disease (NCD), notably cardiovascular disease (CVD), type 2 diabetes and hypercholesterolaemia [1]. In the UK, 25 % of the population are obese which poses a huge economic burden, ~£16 billion per annum from health costs, lost production and premature morbidity [2]. Besides being associated with NCDs, obesity and overweight are also detrimental to reproductive function, including anovulation and delayed spontaneous conception [3] and reduced success in assisted reproductive treatment (ART) with lower clinical pregnancy and higher miscarriage rates [4]. Perhaps even more importantly, maternal obesity can lead to poor offspring health. Children of obese mothers tend to become obese themselves and develop hyperinsulinaemia and glucose intolerance leading to increased NCD risk in later life [5]. Animal models substantiate this. For example, in the mouse, maternal high-fat diet leads to heavier offspring with increased adiposity, fatty liver disease and metabolic and CV dysfunction [6–8]. This fits within the broader Developmental Origins of Health and Disease (DOHaD) concept originating from human epidemiological studies linking experiences during prenatal life to postnatal disease risk and constitution, including body weight and fat mass. Such prenatal programming of postnatal events is widespread across the animal kingdom and is

believed to be a natural mechanism to permit adaptations to prevailing conditions, or developmental plasticity [9, 10]. Human observational studies cannot separate genetic, pre- and postnatal contributions to programming of the offspring. Experimental animal models have furthered our understanding of the underlying mechanisms, including transgenerational inheritance through epigenetic modifications [11]. Such insight can now, in turn, inform human cohort studies [10].

5.2 Periconception Period, Long-Term Programming and Maternal Obesity

Most women with a high body mass index (BMI) before conception will continue to gain more weight throughout pregnancy than women of normal pre-pregnancy BMI and will also impact on the postnatal lifestyle of their children. Thus, it is difficult to distinguish between mechanisms involving maternal BMI, gestational weight gain and maternal metabolic control and to elucidate critical developmental windows sensitive to programming. Animal models have been instrumental in identification of vulnerable prenatal periods. Thus, it is now clear that developmental programming becomes detectable very early on, within hours or days after fertilisation, before the embryo implants into the uterus and the mother is aware of her pregnancy [10, 12, 13]. This suggests the periconception period as a time suitable to identify potential biomarkers for programming but also as a critical window of sensitivity. In support of the latter, a large body of literature has demonstrated that manipulations and challenges experienced *exclusively* during the first few days after fertilisation, the preimplantation period, can have profound long-term consequences for offspring health. Best characterised and shown across mammalian species are the adverse postnatal phenotypes induced by brief removal of the preimplantation embryo from its natural environment, the reproductive tract, and in vitro culture as used in ART. Changes in offspring growth, body composition, physiology, metabolic and cardiovascular health as well as behaviour and cognitive function have all been linked to in vitro environments in rodents, livestock or human [14–20]. Similarly, brief exposure in vivo to acute maternal sickness around fertilisation [21] or low protein diet preimplantation [12, 22] is sufficient to increase postnatal disease risk in mice. With regard to maternal obesity, embryo transfer (ET) and developmental studies indicate the adult NCD phenotype can be traced back to retarded fetal growth and ultimately the periconception period with poorer quality oocytes/embryos [23–25]; similar outcomes come from rat [26] and sheep [27] models. Collectively, this suggests that the preimplantation embryo itself is highly susceptible to its environment but also highly plastic in adapting its physiology and developmental trajectory in an attempt to compensate for adverse experiences and ensure survival [12, 22, 28, 29].

A wealth of evidence connects maternal obesity with long-term programming and postnatal health (discussed elsewhere in the book). However, the impact of

maternal obesity on the preimplantation embryo is less well characterised, perhaps due to technical challenges [30]. Even scarcer is our understanding of the relative vulnerability to maternal obesity of oocyte/early embryo compared to postimplantation/fetal development. Here, we will (i) summarise some key events during preimplantation embryo development, (ii) give examples of the range of early embryonic phenotypes associated with maternal obesity at morphological, physiological and molecular level, (iii) discuss how the reproductive tract may be affected in maternal obesity, (iv) consider the relative contribution of the oocyte, preimplantation embryo and reproductive tract to programming through maternal obesity, (v) discuss some current intervention strategies and (vi) suggest future areas of scientific interest.

5.3 Key Processes in Early Preimplantation Development

Early embryo development consists of dynamic, well-orchestrated processes driving developmental progression. Intricate intrinsic and extrinsic signalling networks cooperate to coordinate this developmental progression sensitive to environmental conditions which have been detailed recently [31, 32]. Here, we will briefly summarise some of the key features with focus on rodent models (Fig. 5.1).

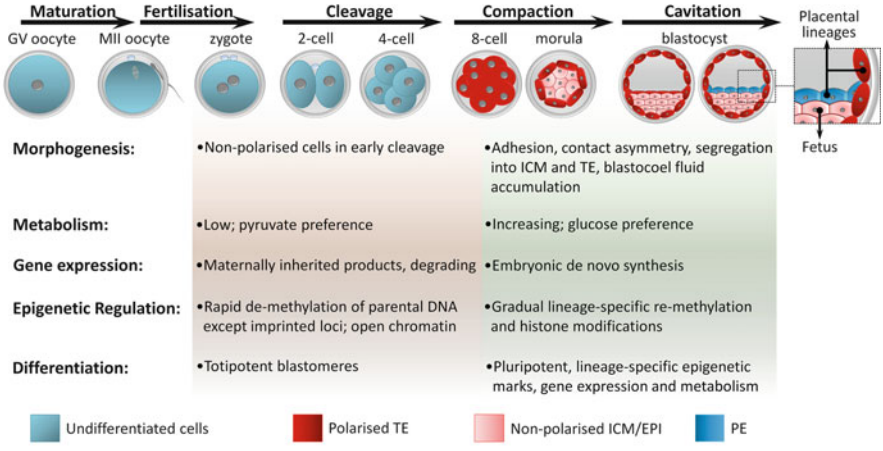

Fig. 5.1 Schematic summary of key processes involved in regulating preimplantation embryo development. Morphogenesis, metabolism, gene expression, epigenetic regulation and differentiation events are interconnected and dynamic, collectively coordinating blastocyst biogenesis. *TE* trophectoderm, *ICM* inner cell mass, *EPI* epiblast, *PE* primitive endoderm

5.3.1 Morphogenesis

At fertilisation, meiotic progression of the oocyte is activated and a diploid biallelic genome is re-established, essential for developmental success. Upon entry into the oocyte, the sperm releases phospholipase C-zeta (PLCζ) into the egg cytoplasm responsible for regulating Ca^{2+} oscillations via the phosphoinositide signalling pathway. Cortical granule exocytosis alters zona pellucida chemistry establishing a block to polyspermy. Ca^{2+} oscillations contribute to extrusion of the second polar body, pronuclear formation and syngamy culminating in resumption of cell cycling and embryonic genome activation (EGA). Cleavage is characterised by asynchronously dividing blastomeres. These blastomeres consistently communicate with each other to regulate embryo morphogenesis and cell lineage differentiation. This process is initiated during compaction when blastomeres not only tightly adhere to each other but also polarise into apical and basolateral domains, permissive of cell asymmetry, differentiative divisions and epithelial phenotype. These events are regulated by a complex network involving a variety of structural and signalling proteins and enzymes amongst others. Lineage diversification culminates at the blastocyst stage when an outer trophectoderm epithelium (TE; progenitor of extra-embryonic chorio-allantoic placenta) separates from an eccentric inner cell mass (ICM). The TE generates the blastocoelic cavity through sodium/potassium-transporting ATPase (Na^+-K^+-ATPase)-driven transport processes across the epithelium with gradually establishing tight junctions. The ICM segregates into epiblast (Epi; adjacent to TE; progenitor of the embryo proper and all fetal lineages) and primitive endoderm (PE; adjacent to blastocoel; progenitor of extraembryonic parietal and visceral endoderm and yolk sac placenta) during blastocyst expansion. After hatching from the zona pellucida, the late blastocyst implants into the uterine wall through TE signal interaction with the uterine endometrium [31, 33].

5.3.2 Gene Expression and Epigenetics

Both maternal and embryonic control mechanisms are involved in regulating development of the blastocyst. Maternal factors encoded by the maternal genome are accumulated during oogenesis and facilitate EGA critical for developmental progression. Maternal factors play key roles during processing of the male genome, degradation of maternally inherited RNAs and proteins, early cell divisions and initiation of cell lineage diversification [34]. EGA occurs at species-specific time points, for example at the two-cell stage in the mouse. Mechanisms that regulate EGA are debated, but it is likely to be a combination of maternally inherited messages and chromatin structure. These maternally inherited compounds are gradually degraded whilst embryonic de novo synthesis takes over. Studies have shown consistently high numbers of genes changing expression levels at EGA demonstrating a major regulatory switch towards embryonic control of developmental progression [34–36].

Gene expression patterns and epigenetic remodelling are closely linked. Reliable analysis of the epigenetic landscape in early embryos is technically challenging due to the limited material available. However, recent advances in technology are beginning to give new insights into the substantial chromatin remodelling taking place when a new diploid biallelic organism is formed. At fertilisation, maternal and paternal genomes display an asymmetrical chromatin organisation. This includes DNA methylation patterns and posttranslational histone modifications, two major epigenetic players. During cleavage, methylation marks on both parental genomes are largely erased before gradually re-establishing at the blastocyst stage, most likely in a lineage-specific manner. Similar dynamics and parental asymmetries are observed with regard to histone variants and their posttranslational modifications, also establishing a lineage-specific pattern at the blastocyst stage. In short, the main characteristics of the epigenetic landscape unique to the early embryo are extensive re-organisation and a relatively open chromatin structure accessible for transcription factors and permissive for gene expression, at least around EGA [34, 37]. Coinciding with first lineage differentiation into TE and ICM, both epigenetic marks and gene expression patterns become more restrictive and lineage specific [14, 36, 38–41]. HIPPO signalling is critical for TE and ICM segregation whilst further differentiation of the ICM into Epi and PE involves the FGF4/MAPK signalling cascade influencing gene expression. Some key transcription factors involved in initiating and stabilising cell lineage diversification include Tead4/Yap/Cdx2/Eomes (TE), Oct4/Nanog/Sox2 (ICM), Nanog (Epi) and Gata6/Sox17/Gata4 (PE). Their mutually exclusive, lineage-specific expression pattern is gradually established through reciprocal suppression and feedback loops [33, 40]. Epigenetic mechanisms also contribute to maintenance of pluripotent ICM identity. Several specific epigenetic regulators important in chromatin remodelling and histone modifications are involved by suppressing differentiation [41].

5.3.3 Metabolism

The developing preimplantation embryo is gradually establishing its metabolic capacity and ability to control utilisation of nutrients [14, 42]. Mechanisms include gradual expression and functionality of the nutrient transporter machinery [43–47]. Therefore, the first few days of development are not only dynamic in demand and highly metabolically adaptable to prevailing conditions but also potentially vulnerable to adverse nutrient environments. For example, due to its inability to utilise glucose as energy substrate, the early embryo utilises pyruvate before switching to glucose preference after compaction [32]. Nevertheless, glucose can be used through the pentose-phosphate pathway in early cleavage for nucleic acid synthesis and NADPH production and its presence is required for activation of stress responses [32]. Protein and total amino acid turnover and uptake of specific amino acids is dependent upon developmental stage as well as nutrient environment

[42, 48]. A similar scenario has been suggested for fatty acid profiles across species where embryos display lipid signatures indicative of membrane synthesis during cleavage and membrane specialisation by the blastocyst stage [47, 49, 50]. Thus, uptake and metabolism of specific fatty acids in the preimplantation period are dynamic processes [51]. Furthermore, chemical inhibition has proven β-oxidation as essential for oocyte maturation and early embryo development [51–53].

Although discussed separately, it is important to consider that the above processes interact in an orchestrated effort to optimise the early stages of development in life which, collectively, signifies developmental plasticity [14, 42]. Thus, it is not surprising that environmental challenges experienced during this short time of development can have profound and widespread impact on many cellular, physiological and molecular systems. Given that almost all cells present at this stage will give rise to an entire body including the gametes strengthens the implications adverse experiences may have on developmental trajectory and health of the new organism and its progeny.

5.4 Maternal Obesity and the Preimplantation Embryo

Across mammalian species, maternal obesity can impair reproductive function due to anovulation, delay in spontaneous conception and reduced pregnancy rates and more miscarriages after ART. Reduced or delayed blastocyst formation, altered developmental kinetics, compromised morphology and changes in gene expression patterns, metabolic activity and blastocyst cell lineage allocation/differentiation have all been reported in the human [54] and in animals from rodent to livestock [24, 30]. Below we discuss some examples (Fig. 5.2).

5.4.1 Morphogenesis and Morphokinetics

Cell lineage allocation in the blastocyst is a dynamic process with considerable plasticity. Alterations in cell numbers and relative allocation to either TE or ICM are common consequences of a wide variety of environmental influences during early development including short maternal diets, sickness and in vitro culture conditions. Thus, adjusting the relative number of cells populating ICM and TE seems critical in ensuring embryo survival, even if at a cost for later health. Maternal obesity is often seen to reduce blastocyst cell numbers, implying less proliferation and/or increased apoptosis [55–58]. Dysregulated maternal glucose/insulin homeostasis often linked to obesity may be critical in inducing such embryonic adaptations. Treatment of obese mothers with insulin sensitisers such as rosiglitazone could restore developmental capacity and normalise blastocyst cell lineage allocation [58]. It is also worth noting that the ICM lineage may be better protected from adverse environments since, for example, maternal obesity impacts

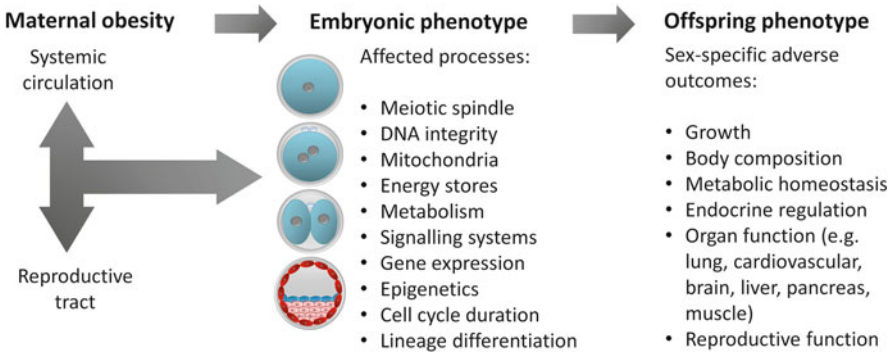

Fig. 5.2 Schematic summary of the impact of maternal status such as obesity on preimplantation embryo phenotype and postnatal consequences. Obesity-related alterations within the maternal systemic circulation and the reproductive tract environment induce adaptive responses at multiple levels in the preimplantation embryo through developmental plasticity. Exposure to an adverse environment such as maternal obesity around conception is sufficient to increase the risk of impaired postnatal health and organ function in the offspring

preferably on the TE lineage [57, 58]. This may, at least in part, explain reduced implantation rates and an increase in pregnancy complications in obese pregnancies as it is the TE that will mediate implantation and give rise to the placenta. Interestingly, some studies show two populations of embryos derived from obese mice: those that do develop to blastocysts comparable to controls and those that do not [55, 59]. Whether this could be due to sex differences has not been explored in detail to date, but sexual dimorphism in embryonic responses to maternal programming has been suggested as early as preimplantation [13, 60]. The duration of specific cell cycles early in development is another key process affected by embryonic environment. Detailed time-lapse studies have suggested a longer duration of the cell cycle during early cleavage in embryos derived from obese mice whilst timing of blastocyst formation was similar to controls (unless both parents were obese) [61]. In the human, the effect of maternal obesity on morphokinetics is less clear. Whilst one study did not find altered morphokinetics in embryos derived from mothers with high BMI [62], another reported accelerated compaction [57]. It is worth noting though that the former [62] detected retarded development up to at least the five-cell stage in embryos from all infertility patients irrespective of maternal BMI compared to embryos from healthy oocyte donors with normal body weight. In the human, the only ethically accessible material are embryos generated by ART in connection with infertility treatment. The impact of infertility may mask more subtle influences of maternal BMI. The fact that in the second study only early development (up to morula) was accelerated, and only within the developmentally competent cohort, may support this idea [57]. This demonstrates the difficulty in drawing general conclusions from human embryo studies and makes animal models invaluable.

5.4.2 Physiology and Metabolism

Embryonic metabolic activity has been related to sex and developmental competence across species [42, 48, 60]. For example, developmental arrest prior to blastocyst formation can be predicted by increased amino acid turnover during early cleavage, and reduced blastocyst glucose consumption relates to the male gender and reduced pregnancy rates [48, 63, 64]. In cattle, intermediate levels of pyruvate consumption in early cleavage predict the highest chance of blastocyst development [65]. Preimplantation carbohydrate and fatty acid metabolism (glycolysis, β-oxidation) and amino acid turnover (often featuring branched-chain amino acids such as leucine) have been shown environmentally sensitive in different species [25, 31, 47, 51–53, 57, 61, 66–68]. Mechanisms involve altered transport [46] and downstream effectors such as adenosine monophosphate-activated protein kinase (AMPK; energy homeostasis) and mammalian target of rapamycin complex 1 (mTORC1; biosynthesis regulation) signalling [31, 69].

In obese mothers, disturbed glucose/insulin homeostasis and elevated levels of blood glucose, lipids and their metabolites are commonly found either alone or in combination [70]. This can upregulate specific energy-sensing and stress signalling pathways affecting embryo physiology and coincides with reduced developmental competence. A number of adaptations in embryo physiology have been linked with maternal obesity. For example, evidence from diet-induced obesity mouse models implies increased O-linked glycosylation of proteins as a result of an upregulated hexosamine biosynthetic pathway together with increased endoplasmic reticulum (ER) stress, possibly mediated by elevated glucose levels [32, 71]. ER stress is one mechanism affecting developmental competence derived from exposure of the developing oocyte to obesity [71, 72]. In ER stress, protein misfolding and reactive oxygen species (ROS) production initiate downstream signalling cascades impacting on a number of physiological mechanisms including gene expression, autophagy or apoptosis which have been implicated in embryo demise [73]. Altered expression of genes involved in fatty acid metabolism and mitochondrial structure and function has also been linked to maternal obesity and fat feeding affecting embryonic developmental capacity, their lipid stores and metabolism via β-oxidation across species [46, 51, 67, 74–76]. Both diet-induced and genetic rodent models of obesity indicate embryonic AMPK activity, lipid handling and fatty acid oxidation as key mediators of adaptive responses to maternal obesity, possibly in a sex-specific manner [77, 78]. In view of the current obesity epidemic, the role of lipid handling including the role of specific fatty acids in preimplantation has gained more scientific interest recently. A recent small study employing metabolomics on spent culture media from human day 3 embryos revealed distinct fatty acid profiles generated by embryos from obese compared to normoweight women implying maternal BMI as one factor impacting on fatty acid turnover [68]. Moreover, new highly sensitive technologies allowing single embryo analysis have shown that lipid composition and metabolism of the embryo itself are stage specific and environmentally sensitive, e.g., in in vitro culture across different

species [47, 49, 79]. In vitro culture models where specific fatty acids either alone or in combination were supplemented have improved our mechanistic understanding. For example, exposure of the oocyte to suboptimal fatty acid levels either alone or in combination reduces developmental competence of the resulting embryo [23, 51, 66, 67, 80].

5.4.3 Gene Expression and Epigenetics

Gene expression patterns in early embryos are dynamic, stage specific and highly susceptible to environmental challenges. For example, maternal obesity can impact on expression of genes involved in glucose/insulin homeostasis and signalling and lipid droplet markers in mouse and rabbit models [23, 30, 81, 82]. In the rat, blastocysts derived from obese mothers show gene expression patterns mirroring a pro-inflammatory phenotype in which nuclear factor-kB-regulated pro-inflammatory genes (CCL4 and CCL5) are increased and expression of antioxidant (GPx3) and mitochondrial (TFAM and NRF1) genes is decreased [83]. In total, over 350 genes were up- or downregulated in male periimplantation blastocysts from obese mothers including developmental and epigenetic regulators. Moreover, maternal high-fat diet-induced obesity can affect DNA methylation patterns in oocytes, a pattern that was still visible in offspring livers displaying corresponding responses in gene expression, and was partially transmitted to the gametes of the next generation [84]. Epigenetic analysis in preimplantation embryos is technically challenging. Thus, it is not surprising that very few data are available investigating the effects of environmental experiences on preimplantation embryo phenotype with a link to adult phenotype postnatally. A recent study in mice using an in vitro fertilisation model has shown persistent aberrant histone modifications in blastocysts and in offspring adipose tissue. Aberrant H4 acetylation in the promoter region of thioredoxin-interacting protein, a gene involved in integrating cellular nutritional and oxidative states with metabolic response, translated into dysregulated mRNA expression [85]. Similarly, a maternal weight loss strategy can induce gene-specific changes in DNA methylation patterns in oocytes which persist into blastocysts and offspring livers [86].

5.4.4 Concerted Adaptations: Morphokinetics, Physiology and Gene Expression

Metabolic activity, for example amino acid turnover, glucose uptake or oxygen consumption, has long been linked to embryo viability, but reliably defining criteria has been challenging. This may not be surprising considering the number of confounding factors that can influence metabolic activity. For example, in the

human, developmental stage, ploidy, gender and mitochondrial activity can influence amino acid balance [87]. Recently, morphokinetics using time-lapse microscopy have become another focus of attention to determine embryo developmental capacity, at least up to the blastocyst stage. However, developmental speed is also linked to sex [60] and metabolic activity, albeit with some ambiguity. For example, developmental speed has been linked to glucose consumption, glycolytic rate and amino acid turnover [56, 57, 88] across species. Collectively, such non-invasive measurements continue to be hotly debated in the quest to find biomarkers for embryo quality to improve selection criteria of the most viable embryos for transfer in ART [89–91]. To date, published data do not yet allow consensus to define such criteria or understand underlying mechanisms linking developmental speed, metabolic activity and embryo competence. A better understanding of how parental body status can influence embryonic characteristics may help to reach consensus [89, 91]. For example, in the mouse, maternal obesity is linked to reduced developmental speed pre-compaction and increased glucose consumption in blastocysts [56], possibly through upregulated glucose transporter 1 (GLUT1 or SLC2A1) expression [61]. This suggests that increased glucose consumption indicates metabolic stress within the embryo as a consequence of maternal BMI compromising fertility. However, another study by the same laboratory has linked increased glucose consumption to embryos whose first cleavage occurred earlier in a non-obese environment. Such faster developing embryos resulted in higher fetal survival after transfer to foster mothers compared to their slower developing counterparts [88]. Such apparently contradictory findings indicate that the ability to succeed developmentally may depend on matching the different embryo characteristics such as gender, developmental speed and metabolic activity to the maternal environment experienced. It may, therefore, be most informative to assess embryo characteristics in context to each other rather than individually. It is worth noting these two studies used different time points for defining developmental speed (compaction versus first cleavage) which could contribute to the opposing conclusions. Similarly, extrinsic factors such as maternal body composition may have a specific 'best match' adaptation of developmental processes for developmental progression. For example, in the human, blastocysts from high BMI mothers take up less glucose compared to their counterparts from non-obese mothers. However, such blastocysts reach the morula stage faster and have less cells suggesting a compensatory earlier differentiation [57]. Blastocysts from obese mothers also displayed altered amino acid metabolism and increased triglyceride content compared to non-obese counterparts, confirming metabolic, morphokinetic and differentiation adaptations of the conceptus to maternal BMI as early as preimplantation [57]. In cattle, short exposure to elevated non-esterified fatty acids (NEFA) as observed during disorders linked to lipolysis exclusively during oocyte maturation not only reduced subsequent embryo viability but also reduced blastocyst cell numbers. Expression of developmentally important genes such as DNA (Cytosine-5-)-Methyltransferase 3 α (DNMT3A), Insulin-Like Growth Factor 2 Receptor (IGFR2), GLUT1 (SLC2A1) and genes related to lipid and carbohydrate metabolism was disrupted and metabolic regulation altered profoundly akin to a

glucose-intolerant state [51, 67, 80, 92]. Elevated oocyte β-oxidation was suggested key for these alterations seen by the blastocyst stage since inhibition of β-oxidation during oocyte maturation exposed to elevated NEFA restored developmental competence [66]. Recent studies in the mouse have revealed DNA methylation changes in oocytes in maternal obesity [84]. Once again, metabolic regulation was a major target. Both genes investigated with a critical role in metabolic control were affected, leptin and peroxisome proliferator-activated receptor (Ppar) α, whilst all five imprinted genes analysed remained unaltered suggesting better protection from an adverse environment such as maternal obesity [84].

5.5 Maternal Obesity and the Reproductive Tract

Whilst there is a wealth of literature showing that the composition of follicular fluid surrounding the oocyte before ovulation is sensitive to maternal status such as BMI across different species [93, 94], very few studies have considered how the reproductive tract adapts to maternal nutrition, especially early on during preimplantation. Whilst it is clear that cycle stage and pregnancy status influence reproductive tract fluid composition (summarised in [43]), to date more emphasis has been placed on whether the uterine lining is receptive to implantation or not [95, 96]. Little is known how maternal nutritional status impacts on reproductive tract fluid composition (or histotrophe) which constitutes the environment in which the preimplantation embryo develops [32, 43, 51, 97]. This is an important piece in the puzzle to understand mechanisms underlying the induction of developmental programming, especially when considering the overwhelming evidence derived from ART that in vitro culture conditions can profoundly impact not only on developmental potential but also on offspring health. Reproductive tract fluid composition is regulated via nutrient transporters which, in turn, respond to maternal dietary supplementation and hormonal status [43, 51, 98]. Recent evidence from pregnant mice suggests that uterine fluid amino acid composition quickly responds to maternal diet, but that it does not mirror the changes detected within the maternal circulation [99]. In the human, BMI of women does influence fluid amino acid composition in the non-pregnant uterus, again not reflecting systemic levels in parallel serum samples [100]. This suggests that the reproductive tract may function with a self-regulatory mechanism in controlling or 'buffering' fluid composition permissive for sustained preimplantation embryo development. Less is known about lipid content in reproductive tract fluid and its regulation [51]. Best characterised is follicular fluid lipid composition which displays fatty acid profiles similar to those found systemically in obesity. This, in turn, impacts on fatty acid content of the follicular oocyte and is linked to developmental potential (summarised in [51, 53]).

Expression patterns in the reproductive tract of genes involved in, for example, metabolic and maternal–embryonic communication can be sensitive to maternal obesity. For instance, in the rat uterus, maternal obesity can induce lipid

accumulation and gene expression profiles akin to a pro-inflammatory systemic status, including abnormal expression of genes involved in lipid metabolism [83]. In women, endometrial expression of genes involved in glucose/insulin homeostasis such as Insulin Receptor Substrate 1 (IRS1) and GLUT1 (SLC2A1) is sensitive to BMI and hormonal status [101]. Little detail is known how maternal–embryonic signalling may be affected by obesity, but the involvement of cytokines and growth factors seems plausible [102]. Indeed, in obese cattle, reproductive tract expression of the embryotrophic cytokine Colony Stimulating Factor 2 (CSF2) is reduced whilst Insulin-Like Growth Factor 1 (IGF1) uterine fluid levels are elevated [103, 104].

5.6 Relative Contribution of the Oocyte, Preimplantation Embryo and Reproductive Tract to Programming Through Maternal Obesity

Most of our knowledge of the impact of maternal obesity on developmental programming and offspring health is derived from exposure to maternal obesity from before conception and throughout gestation and lactation. This includes oocyte development and makes it difficult to further narrow down the relative vulnerability to programming at specific developmental stages. Thus, sequence of events and detailed underlying mechanisms remain sketchy. The only way to limit exposure to an obese environment is by restricting it to specific time periods, e.g. periconception only. This usually requires manipulations before implantation takes place, for example: maternal hormone treatment to time ovulation, maximise oocyte recovery and synchronise embryo recipients; in vitro fertilisation; embryo culture; and ET to continue development in a non-obese environment. Although such manipulations can lead to programming in themselves (discussed above), they are invaluable tools to improve our mechanistic understanding and elucidate specific susceptibilities [30]. However, very few studies are available to date, perhaps due to the technical challenges of these manipulations and their practicability. One example shows that exposure to obesity in vivo during oocyte development, fertilisation and either up to blastocyst or two-cell stage before transfer to a non-obese environment is sufficient to lead to fetal growth restriction, developmental abnormalities and altered placental physiology but did not result in compromised offspring metabolic state [24, 105]. Another example demonstrates that when fertilised oocytes from obese mice that had undergone a weight loss strategy were transferred into normal foster mothers the adverse postnatal phenotype observed in offspring from non-weight loss controls was partially alleviated. This suggests the oocyte as one key developmental stage linking maternal status with offspring phenotype [86].

In the next developmental window, the preimplantation embryo is also sensitive to environmental conditions (see above). This is well documented as a consequence

of exposure to compounds found elevated in maternal obesity during in vitro culture and follow-up after ET. For example, short-term culture of murine morulae/blastocysts in the presence of palmitic acid was sufficient to reduce blastocyst cell numbers and alter blastocyst insulin signalling. After transfer, fetal growth restriction and postnatal catch-up growth ensued [106]. Exposure of murine blastocysts to high levels of insulin or IGF1 in vitro increases apoptosis, reduces glucose uptake and implantation and results in fetal growth restriction after transfer. Such detrimental impact could be alleviated by metformin, likely to operate via activation of AMPK involving AKT/mTORC pathways as a rescue mechanism to restore glucose uptake [69, 107]. In a genetic model of maternal obesity in vivo, exposure to metformin during in vitro culture was indeed able to partially reverse adverse blastocyst phenotype [77]. Whilst this sounds promising the long-term effects of such treatment will require some more investigation.

5.7 Current Intervention Strategies

Due to the chronic condition of maternal obesity impacting on oocyte health even before conception, one focus has been on alleviating the impact of exposure of the follicle and oocyte to an obese environment. A number of oocyte defects have been described as a consequence of maternal obesity including spindle abnormalities and meiotic defects, organelle dysfunction (mitochondria and ER), lipid accumulation, reactive oxygen species, epigenetic defects and overall metabolic disturbances [74, 94]. Thus, it is not surprising that maternal diet reversal and weight loss programmes through, for example, bariatric surgery, caloric restriction and/or exercise before conception have become one popular strategy to prevent adverse programming across species and possible transgenerational inheritance [27, 108–111]. However, it remains unknown how intense and over what period of time such strategies need to be continued in order for them to take full effect and remain safe in humans [109]. Various weight loss strategies have had some success in improving ovulation, embryo development and pregnancy rates in subfertile patients as well as alleviating offspring disease risks associated with maternal obesity in rodent models [86, 108, 109, 112]. However, underlying mechanisms and long-term effects on offspring health into later life are less well defined. In addition, timing, duration and intensity of weight loss in relation to conception still remain a subject of debate as there have also been some negative reports [27, 113, 114]. Perhaps the human observational data from the Dutch famine showing that exposure to undernutrition during early gestation increases cardiovascular disease some 60 years later best underpins the call for caution in devising weight loss programmes, especially around conception before the mother is aware of her pregnancy [27, 115]. A better evidence base is needed to improve our mechanistic understanding and animal models where early embryo, fetal and postnatal material is accessible are irreplaceable for this task.

Very few animal models are available to date where reversal of maternal obesity-induced programming has been analysed in relation to early embryonic phenotype and have had mixed success. For example, in mice, normalisation of compromised maternal physiology induced by high-fat diet exposure through a switch onto a non-obesogenic diet for 8 weeks (corresponding to 10–11 menstrual cycles in the human) was insufficient to reverse impaired oocyte quality [116]. However, using a strategy to encourage activity levels voluntarily in mice through environmental enrichment for only 4 weeks has shown more promise in reverting not only impaired maternal physiology but also DNA methylation changes in the oocyte back to normal, improving adverse offspring health consequences. These changes were inherited across at least two generations [86]. Insulin sensitisers such as rosiglitazone which targets PPAR can revert blastocyst phenotype back to that of control embryos [58] similar to the AMPK activator metformin (discussed above) which can alleviate postnatal metabolic phenotype induced by maternal obesity when given throughout pregnancy [117], but long-term consequences into later life have not been followed up. Targeting AMPK activation through manipulating intracellular pathways may also prove a promising strategy to avoid programming by maternal obesity [118]. Compounds such as polyunsaturated fatty acids, resveratrol, curcumin or taurine targeting various signalling pathways (cytokines, transcription factors, enzymes) and metabolic processes (nutrient transporters, metabolic enzymes, glucose/lipid metabolism) associated with mild systemic inflammation or insulin insensitivity as seen in obesity have shown some success in restoring offspring health (reviewed in [119]). However, their safe use during pregnancy requires more investigation. For example, using a non-human primate model of maternal obesity, beneficial as well as cautionary effects on fetal organ development have recently been reported using the insulin sensitiser resveratrol which targets the deacetylase Sirtuin 1 (SIRT1) [120].

5.8 Where to Go Next?

Currently, we have a growing understanding of the consequences of developmental plasticity and the machinery involved in mediating adaptation to prevailing conditions even before implantation, including transgenerational inheritance via epigenetic mechanisms. However, there is a critical gap in knowledge of the upstream mechanisms mediating maternal programming preimplantation. For example, how does the early embryo sense its environment in the first place and how are epigenetic alterations induced? How does the reproductive tract respond to maternal obesity and is it able to give some protection? It is now very clear that metabolic versatility and epigenetic regulation are intricately linked in health and disease states, and even in the preimplantation embryo [14, 94, 121, 122]. Metabolic master regulators and sensing systems functional in early embryos include AMPK (energy levels) and mTORC1 (amino acids, insulin/Pi3k/Akt) signalling pathways [31, 32]. Both have been implicated in embryonic adaptive responses to

environmental cues including maternal obesity (see above) [31]. The role of fatty acids and lipid metabolism, however, has not been examined in much detail in the embryo. Remarkably, the contribution to nutrient sensing of the hexosamine biosynthesis pathway integrating carbohydrate, amino acid, fatty acid and nucleotide metabolism through uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) synthesis has not been well characterised. UDP-GlcNAc is involved in biosynthesis, signalling, and potentially insulin sensitivity through O-linked glycosylation. Since O-linked glycosylation is also critical in histone modifications, this pathway may be another link between environmental stress response and epigenetic changes. The hexosamine pathway is active in early development. Inhibition and knockout studies have shown that this pathway is critical for oocyte maturation and early embryo development and their ability to respond to stress [32]. Moreover, in a perturbed nutrient environment such as hyperglycaemia, this pathway has been implicated in insulin/growth factor resistance in concert with Pi3k/Akt signalling, but a direct link to epigenetic effects in early embryos is still lacking [32].

Other unresolved questions include the quest for critical components inducing developmental plasticity before implantation, their prime targets and the sequence of events. Finding answers will require investigation further upstream of the preimplantation embryo, including the reproductive tract and its relation to the circulation. Whilst we start to understand how development and health of the oocyte are associated with follicular composition in relation to maternal circulation [51, 53, 93, 94, 123], we know very little about the mechanisms regulating oviduct and uterine fluid formation and composition, let alone in relation to maternal nutrition [97, 98]. The latter can only be studied in animal models due to ethical constraints in the human on collecting reproductive tract material whilst an embryo is present. A better understanding of these upstream events will help devising strategies that will ensure the early embryo can be protected safely from maternal malnutrition at a time when the mother is still unaware of her pregnancy.

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Chapter 6
Paternal Obesity and Programming of Offspring Health

Tod Fullston, Helana S. Shehadeh, John E. Schjenken, Nicole O. McPherson, Sarah A. Robertson, Deirdre Zander-Fox, and Michelle Lane

Abstract The physical and nutritional environment experienced by the mother prior to and during conception is imperative to the outcome of pregnancy and offspring health. In addition, there is now mounting evidence that paternal exposures and conditions at the time of conception are also an important determinant of pregnancy outcome and offspring health. Specifically, male obesity is now demonstrated to have detrimental impacts on fertility and fetal development during subsequent pregnancy and can exert programming effects on the phenotype of offspring lasting up to two generations. We summarise the evidence of the effect of environmental exposures on seminal plasma and sperm, focusing on the effects of obesity, and what bearing this has for offspring both in humans and animal models. The current knowledge of what might form the molecular basis of the phenomena of paternal programming of offspring health is also reviewed with consideration given to signals from both seminal plasma and sperm.

T. Fullston (✉) • N.O. McPherson
Discipline of Obstetrics and Gynaecology, Paediatrics and Reproductive Health, School of Medicine, Robinson Research Institute, University of Adelaide, Adelaide, SA 5005, Australia

Freemasons Foundation Centre for Men's Health, The University of Adelaide, Adelaide, SA 5005, Australia
e-mail: tod.fullston@adelaide.edu.au

H.S. Shehadeh • J.E. Schjenken • S.A. Robertson
Discipline of Obstetrics and Gynaecology, Paediatrics and Reproductive Health, School of Medicine, Robinson Research Institute, University of Adelaide, Adelaide, SA 5005, Australia

D. Zander-Fox
Discipline of Obstetrics and Gynaecology, Paediatrics and Reproductive Health, School of Medicine, Robinson Research Institute, University of Adelaide, Adelaide, SA 5005, Australia

Repromed, Dulwich, SA 5065, Australia

M. Lane
Discipline of Obstetrics and Gynaecology, Paediatrics and Reproductive Health, School of Medicine, Robinson Research Institute, University of Adelaide, Adelaide, SA 5005, Australia

Monash IVF Group, Melbourne, VIC 3168, Australia

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Abbreviations

ROS	Reactive oxygen species
8-OHdG	8-hydroxy-2′-deoxyguanosine (oxidatively damaged Guanosine base)
5mC	5-methyl-Cytosine
5hmC	Hydroxymethyl-Cytosine (oxidised form of 5mC)
NOX	NAPDH oxidase
OGG1	8-Oxoguanine glycosylase (enzyme)
BMI	Body mass index
SVX	Seminal vesicle deficient (mouse model)
sncRNA	Small non-coding RNA

6.1 Introduction

For the first time, the current generation of children may have a reduced life expectancy compared to their parents, due largely to an elevated incidence of non-communicable chronic disease. The increase in diseases such as obesity may in part originate in early-life exposures. Overwhelming evidence now demonstrates that peri-conceptional parental exposures to diet, environmental conditions and lifestyle choices alter the integrity of gametes which in turn can have a lifelong impact on the health and disease susceptibility of the offspring. Whilst this evidence initially focused on the mother, often with paternal factors being overlooked, it is now apparent that alterations to sperm that occur as a result of paternal exposures to environmental conditions can also influence embryo and offspring health. In humans, whilst associations exist between male health at conception and offspring health and disease, data demonstrating causality are currently lacking. However, studies in animal models are providing some clues to how paternal health affects offspring, providing evidence that both the molecular structure of the sperm and the composition of seminal plasma are key determinants in the transmission of exposures of the father to his offspring. This chapter will explore how environmental exposures impact the male reproductive tract and how this impacts offspring health.

6.2 Spermatogenesis

The production of a functional spermatozoa capable of successfully fertilising an embryo is a complex process requiring the coordinated activity of thousands of genes, significant remodelling of genetic material and epigenetic marks as well as

substantive modifications to proteins [1]. The production of mature sperm from primordial germ cells is regulated by sex hormones from the hypothalamus, pituitary gland and locally from the testes. The germ cells are enclosed by Sertoli cells (nurse cells) that are bound to each other through tight junctions that form the blood–testis barrier. The blood–testis barrier is selectively permeable, resulting in a specialised environment within the inner compartment of the seminiferous tubules with respect to concentrations of hormones, electrolytes, sugars and amino acids. Testosterone acts on Sertoli cells to regulate sperm differentiation; its concentration within the body is relatively consistent, due to the negative feedback loop that acts on the hypothalamus and pituitary gland to decrease LH production that inhibits the further release of testosterone [2].

Spermatogenesis is comprised of three main stages where type A spermatogonia transition into primary and secondary spermatocytes and then into early round spermatids before being converted to elongated spermatids and eventually spermatozoa. The volume of cytoplasm is vastly reduced during spermiogenesis, whereby excess cytoplasm and organelles are removed from the spermatids by Sertoli cell phagocytosis [3]. Cytoplasmic reduction is vital for the structural conversion of a spermatid into a functional sperm cell and leaves only a small amount of residual cytoplasm. Any residual cytoplasm containing high levels of reactive oxygen species (ROS) may lead to oxidative stress and reduced sperm motility [4, 5]. The remainder of cytoplasmic residue, the cytoplasmic droplet, remains in the elongated spermatid. During epididymal transit, the cytoplasmic droplet migrates from the neck of the sperm to the end of the midpiece; however, the physiological significance of this remains to be fully elucidated [6]. During these final stages of development into the elongated sperm (spermiogenesis), these cells are transcriptionally silent. Consequentially multiple processes that include nuclear condensation, acrosome formation, flagellum formation, cytoplasmic reduction and functional changes that occur in the epididymis must all occur in the absence of transcription/translation [7].

Nuclear remodelling is a key process of spermatogenesis involving the compaction of the sperm nucleus. This nuclear condensation process involves the gradual removal of the chromatin's original histone-based structure and substitution with smaller protamines [8–11]. Histones, which are proteins that DNA is wrapped around [12], are modified via acetylation, methylation and ubiquitination leading to an open and loose chromatin structure [13–15]. Transition proteins then bind strongly to the DNA and are incorporated into the chromatin resulting in the removal of histones. However, it must be noted that a small proportion of histones are retained and not replaced, with up to 15 % retained in human sperm [16, 17]. Finally, transition proteins are replaced by protamines that generate a tightly packaged nucleus [18]. It has been proposed that the specialised protamine-based chromatin structure may be necessary for multiple functions including the generation of a compact and dynamic shape to aid in swimming capacity, the protection of the paternal genome from chemical and physical damage and an involvement in epigenetic signalling [10, 19]. The epigenetic make-up of sperm

is crucial, as it acts to modulate post-fertilisation transcription during embryogenesis [20, 21].

The release of the spermatozoa from the seminiferous tubules occurs via a process of spermiation. It is important to note that whilst structurally intact, the released spermatozoa is not functionally competent. Functionality of the sperm is attained during its transit through several metres of epididymal tubule, which includes the acquisition of motility and the ability for capacitation and oocyte binding/fusion [22, 23]. Remarkably, this acquisition of sperm function occurs in the absence of gene transcription and protein translation. The substantial numbers of proteins that are transferred to sperm by epididymal specific exosomes ('epididymosomes') during this transit are, at least in part, responsible for this acquisition of functionality. Interestingly, both sperm DNA methylation [24] and sperm microRNA content [25, 26] are also modulated throughout epididymal transit. Sperm are then stored in the cauda epididymis for approximately 1 month in humans, or until ejaculation. The entire developmental process that generates mature sperm in men occurs over approximately 72 days, thus allowing for exposure of maturing sperm to environmental influences over an extended period of time [27].

6.3 Seminal Plasma

As well as spermatozoa the seminal fluid contains a plasma fraction, originating from secretions of the male accessory sex glands [28]. The seminal plasma accounts for the vast majority of the ejaculate (~85 % in humans) and contains a complex mixture of bioactive proteins and other agents [28, 29]. In addition to soluble factors, seminal plasma also contains nanovesicles derived from the prostate, called prostasomes [30]. While all accessory sex glands contribute to the composition of seminal plasma, studies in humans have identified that seminal plasma is predominantly made up of fluids from the seminal vesicles and prostate gland [28].

Seminal plasma was traditionally seen as a transport medium to provide sperm to the oocyte at conception. However, we now know that seminal plasma plays a far more complex role. Soluble factors within seminal plasma have been identified to promote the survival of sperm and provide factors to protect sperm from oxidative stress, provide metabolic support, enhance sperm motility and induce capacitation [28, 31]. For example, prostasomes play an important role in the above functions with studies demonstrating that prostasome interactions with sperm influence sperm motility, capacitation as well as acrosome reactions [32–34].

Emerging research in both humans and animal models has provided evidence that seminal plasma has novel roles in exerting influence on the physiology of the female reproductive tract due to the presence of a range of soluble signalling factors including hormones and cytokines, which may act independently of sperm [35–37]. In these studies, seminal plasma has been identified to promote conception, prepare the female reproductive tract for pregnancy and critically promote the

development of a tolerogenic immune environment that is required for the maternal immune system to accommodate the embryo [38–40]. Recent studies in animal models have identified that components of seminal plasma may have an impact on the future phenotype and metabolic health of offspring [41].

6.4 How the Molecular Composition of Sperm Influences the Developmental Program of the Embryo

The chromatin structure of sperm is very different to a somatic cell with a tenfold compaction of DNA being achieved via replacement of histones with smaller cysteine- and arginine-rich basic protamines during the final postmeiotic phase of spermatogenesis [10, 11]. However, several species including the human and mouse retain a small portion of the original histones (H2A, H2B, H3 and H4), which are associated with uncondensed nucleosomal structures. It has been speculated that histone retention in sperm DNA provides a 'histone code' capable of transferring additional epigenetic information to the embryo. This information controls gene expression within the developing embryo, via differential methylation of imprinting control regions, which either up- or downregulate gene transcription [42]. The potential for histone-related epigenetic marks in sperm to carry epigenetic information into the zygote is supported by the non-random location of the retained histone nucleosomes (i.e. at genes of developmental importance) [43] and that post-translational modification of histones and protamines [44] have recently been recognised as paternally derived epigenetic signatures which may contribute to non-genetic transgenerational inheritance.

Noblanc and colleagues found that the regions that retain histones in the sperm DNA were the most prone to oxidative attack, as a result of their relaxed and decondensed chromatin state, with histone-rich and nuclear matrix-attached domains located in the peripheral and basal regions of the sperm nucleus particularly sensitive to this type of damage [45]. Furthermore, although sperm protamines are replaced with maternal histones at fertilisation [46], regions that retained histones in sperm DNA are not replaced by the oocyte post-fertilisation and, therefore, any paternal histone modifications (H3K27me3—repressive; H3K4Me2—active), hold the potential to be inherited into the embryo [43, 47] and alter the transcriptome of the early embryo.

6.5 Impact of Paternal Environmental Exposures on Sperm Function and Molecular Composition

There is substantial evidence that lifestyle and environment can influence both sperm function and molecular make-up. However, traditionally the assessment of sperm has focused on relatively crude measures of spermatogenesis such as count, motility and morphology, with little to no assessment of the molecular composition of the sperm.

6.5.1 Obesity

The most well-characterised lifestyle factor that impacts on sperm function is obesity. The World Health Organisation defines obesity as a body mass index (BMI) of >30 kg/m^2 (weight as a ratio of height). Human and rodent models have demonstrated a correlation between increased BMI/body weight (respectively) and impaired male fertility. Initially human studies focused on the effects that increased BMI had on hormone levels, with obese men reported to have reduced testosterone, increased oestrogen and reduced sex hormone binding globulin (SHBG) concentrations [48–51]. Additional studies have examined the effects of male BMI on conventional sperm parameters (sperm count, motility and morphology) with conflicting conclusions. Some studies support a link between obesity and subfertility including reduced sperm count [52–55], reduced sperm motility [54–56] and increased sperm DNA damage [56, 57], while other studies do not support this link between male obesity and impaired semen parameters [58–60].

A meta-analysis of 21 studies (total of 13,077 men) indicated a negative relationship between BMI and sperm count [61]. A recent systematic review and meta-analysis of 115,158 men concluded that obese men were more likely to experience infertility. Furthermore, these men had a reduced chance of a live birth per cycle of assisted reproduction technology with a 10 % absolute risk increase of pregnancy non-viability and had an increased percentage of sperm with DNA fragmentation and abnormal morphology [62]. There have been some reports on the impact of obesity on sperm DNA integrity with several studies reporting an increase in sperm DNA damage with obesity [56, 57, 60]. Furthermore, increased sperm ROS concentrations have been reported for both human and animal models of obesity, concomitant with increased sperm DNA damage [56, 57, 60].

6.5.2 Smoking

Overall cigarette smoking is known to reduce male fertility, with reductions to count, motility and morphology [63, 64]. But it must be noted that although

reductions in these measures of fertility are consistently reported for smokers, they usually still fall within the normal range, and effects on fertility are instead caused by an increased mutational load and other DNA damage-based mechanisms.

When a male smokes heavily, their seminal plasma shows high levels of oxygen free radicals and their sperm show significantly elevated levels of DNA fragmentation and increased oxidative damage to DNA, as measured by 8-hydroxy-2′-deoxyguanosine (8-OHdG) lesions, compared to the sperm of non-smokers [65]. This DNA fragmentation and damage observed in sperm is presumed to form the basis for increased risks of childhood cancers observed in children born to male smokers [66]. Non-specific oxidative damage to DNA in male germ cells such as deletions, abasic sites and oxidative base change is proposed to increase the mutational load in the embryo [67]. The mutational load then becomes fixed in the fertilised oocyte as a result of abnormal DNA repair at the first cleavage division, demonstrating that genetic damage can be transmitted through the male germ line and this damage may have a major impact on offspring health [67]. Indeed, paternal smoking has been demonstrated to increase the mutation load in children, compared to non-smoking fathers [68].

6.5.3 Age

A recent meta-analysis of 90 studies with a total of 93,839 participants concluded that advanced male age is associated with a decline in semen volume, sperm motility and morphology, but not sperm concentration [69]. In addition, increasing male age has been associated with increased sperm DNA fragmentation, abnormal chromatin packaging and protamine deficiency in both sperm donors and infertile couples [70–72]. It has been demonstrated in a relatively closed Icelandic population that mutations in sperm/offspring increase by approximately two extra mutations per year of paternal age, which estimates a doubling with every 16.5 years of increased age [73]. Furthermore, it was concluded that a father's age is estimated to explain nearly all of the non-random variation in de novo mutation events. Moreover, advanced paternal age alters methylation patterns at imprinted genes and this has been suggested to also increase the risk of neurological disorders found in offspring [74, 75].

This increased DNA damage and epigenetic changes in sperm due to increasing paternal age are implicated as the cause for diminished reproductive outcomes. For example, men >45 years of age are associated with increased miscarriage [76], fetal death [77], pre-eclampsia [78] and low birthweight of live born infants [79]. Advanced paternal age is also associated with an increased susceptibility to complex neurological disorders such as schizophrenia (>55 years old), bipolar disorder (>55 years old) and autism (>50 years old) in their children [74, 80–83]. Advanced paternal age clearly damages the genetic/epigenetic content of sperm and offspring, perhaps reflecting a man's lifetime of acquired environmental/lifestyle exposures that are then transmitted to his offspring.

6.6 Sperm and Seminal Plasma Impact on Pregnancy Progression and Offspring Health

A number of paternal health factors including smoking, advanced age and exposure to industrial chemicals have been associated with increased rates of cancer, mental disorders including autism and congenital abnormalities in subsequent offspring [84–86], which suggests transmission of paternal exposures at or around conception.

Historically parental BMI studies have focused on maternal BMI pre-conception, peri-conception and during pregnancy and lactation and indeed these parameters are imperative to the outcome for the pregnancy and the offspring. There is now mounting evidence that paternal obesity also affects pregnancy outcomes in addition to the reported maternal contributors. Males classified as overweight (BMI 25–30 kg/m^2) or obese (BMI > 30 kg/m^2) currently equates to nearly 75 % of the population of Westernised societies (i.e. the USA, Australia), with co-morbid diseases attributed to obesity becoming increasingly problematic [87, 88]. The rate of obesity alone is ~30 % in these populations and developing nations have some of the most rapid increases in obesity currently [87].

6.6.1 Pregnancy and Embryo Health

Male obesity is associated with decreased pregnancy rates and an increase in pregnancy loss in couples undergoing assisted reproductive technologies [55, 89–93]. Male obesity decreases clinical pregnancy rates, partly explained by perturbed embryo development resulting from poor sperm quality (reduced motility, capacitation, fertilisation, oocyte binding as well as increased intracellular ROS and DNA damage) [55]. These findings were confirmed and extended in rodent models of male obesity which demonstrated delayed zygote cleavage, reduced blastocyst cell numbers, impaired embryo metabolism and reduced implantation as contributing to poorer fertility and pregnancy outcomes [94, 95]. Adverse outcomes have also been replicated by pharmacologically increasing sperm ROS [96, 97]. Zorn and colleagues reported that seminal ROS is a predictor of fertilisation, embryo quality and pregnancy outcomes in conventional IVF patients [98] implicating ROS as a causative agent.

6.6.2 Offspring Health

A case–control study in humans investigated paternal exposures to environmental toxins during the peri-conceptional period and found that there was an increased chance of congenital malformation in children when fathers were exposed to

pesticides, solvents or welding fumes [84]. However, the specific environmental exposures responsible could not be identified due to the diverse range of chemicals potentially involved [84].

Obesity in males is not only associated with subfertility but is also a mediator for paternal programming. Epidemiological studies demonstrate that obese fathers are more likely to father an obese child [99, 100]. However, the extent of the individual contributions of genetic, epigenetic and environment cannot be separated due to the common raising environment shared by both father and child—therefore rodent models have been devised to circumvent this issue. The first rodent model to demonstrate a link between paternal obesity and offspring health was that of Ng and colleagues [101], who fed a HFD to male rats that induced obesity and diabetes. When these males were mated to normal-weight females, their offspring were smaller at birth and female offspring displaying impaired glucose tolerance caused by aberrant insulin secretion and a β-cell defect. The changes to insulin secretion were in part attributed to hypomethylation and downregulation of gene expression in pancreas tissue, suggesting shared responses to programmed systemic factors, or crosstalk between tissues [101]. Subsequent studies have demonstrated that diet-induced paternal obesity in mice resulted in the transgenerational impairment of the metabolic and reproductive health of two resultant generations [102, 103]. In one of these studies, glucose and insulin metabolic defects were not limited to the first-generation offspring, but extended into second-generation offspring, with the most detrimental phenotype in the F_2 generation evident through the F_1 female linage. Interestingly, the molecular composition of the founder sperm was altered, evident as global hypomethylation, altered microRNA content and increased ROS. Therefore, oxidative stress, methylation and microRNA content are possible mechanisms for paternal signals that program offspring health and may initiate the transmission of metabolic syndrome to future generations. The precise mechanism underlying the transmission of paternal programming remains speculative, but it is known that male obesity increases sperm ROS and associated damage, reducing fertilising ability and blastocyst quality as well as compromising offspring health. Furthermore, in other cell types oxidative stress is directly associated with changes to DNA methylation [97, 104, 105], and, although the association in sperm has only been correlative, the significant impact of oxidative stress to sperm, exemplified through rodent models of obesity, implicates ROS as a potential mediator for the observed transgenerational phenotypical changes in the above studies.

6.7 Possible Mechanisms of Paternal Transmission

As evidence mounts that the paternal peri-conception environment can lead to programming of offspring health, investigations have now become directed at finding what changes to the molecular composition of sperm or seminal fluid might enact this programming.

6.7.1 Sperm ROS and Oxidative Damage

Paternal obesity, similarly to paternal smoking, can lead to increased levels of oxidative stress in sperm. The oxidative stress resulting from obesity has been shown to induce DNA damage to sperm [106], leading to delayed embryo development [94, 107]. These studies show that oxidative attack is initiated by ROS and that associations exist between DNA modifications and damage in sperm and impaired offspring health [108]. The classic double-edged sword of redox biology is exemplified by the observation that ROS-dependent signalling has vital roles in normal cellular function at normo-physiological concentrations, but increased amounts can also result in damage to cells, either directly by ROS or by activation of downstream pathways.

There are several different forms of ROS species, namely hydroxyl radical (OH-), superoxide (O2-), singlet oxygen (O-) and hydrogen peroxide (H_2O_2), each with different biological targets with its own spectrum of reactivity. The physiological generation of ROS can occur as a by-product of biological reactions such as those produced by mitochondria, or as a primary function of enzymatic systems such as NADPH oxidase (NOX) [109]. Sperm are highly susceptible to oxidative damage due to the lack of cytoplasmic scavenging enzymes and high levels of polyunsaturated fatty acids found in their plasma membranes [110]. While physiological levels of ROS are necessary for spermatogenesis and post-ejaculation maturation including capacitation and hyperactivation [111], these processes quickly become impaired if the cells enter a state of oxidative stress. Indeed, knockout models of antioxidants exhibit disrupted spermatogenesis [112]. An imbalance in ROS towards oxidative stress in sperm occurs in many male pathologies (male obesity, smoking, ageing, chemical exposure and subfertility), all of which have been shown to increase offspring susceptibility to disease in both animal models and humans [7].

ROS is not limited to within the sperm cells themselves but is increased in the seminal fluid of infertile men as well as with obesity. Seminal fluid ROS concentrations in infertile men correlate positively with sperm DNA damage levels and negatively with pregnancy outcomes [113–115]. Therefore, oxidative stress appears to be a common underlying mechanism for many of the functional defects seen in subfertile sperm including reduced sperm motility, a reduced capacity for fertilisation and substantial losses of DNA integrity (Fig. 6.1) [116–118].

6.7.2 Oxidative Stress and Chromatin Damage

Elevated ROS concentrations impair sperm quality and function [119] and most DNA damage in sperm is oxidatively induced [120, 121]. Sperm chromatin forms a quasi-crystalline structure, i.e. a tight structure that fills every available space, and has very little capacity to respond to DNA damage induced by oxidative attack

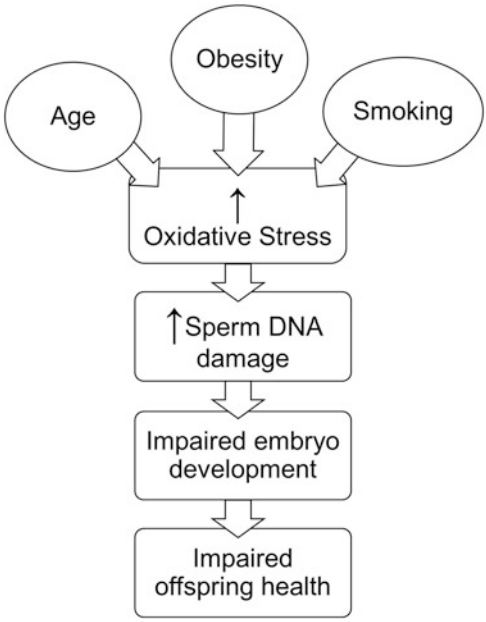

Fig. 6.1 The central oxidative stress hypothesis. A number of paternal environmental exposures that are known to cause defects in both embryo development/quality and offspring health increase oxidative stress in sperm and seminal fluid. Increased sperm DNA damage can result from oxidative attack by free radical species (such as reactive oxygen species; ROS), which impairs embryo development/quality and offspring health

[117]. When oxidative stress occurs, there are two factors that protect DNA from oxidative damage: tight chromatin packaging, facilitated by protamination of DNA, and antioxidants present in the cytoplasm of sperm cells that neutralise free radicals [110, 122, 123]. However, these factors are not effective or sufficient under high oxidative stress load [124]. Although tight chromatin packaging of sperm DNA limits its vulnerability to oxidative attack, not all histones are replaced with protamines. Since approximately 10–15 % of histones are retained in human sperm [11], which is further increased in subfertile men, this suggests that the nuclear condensation process during spermatogenesis may not be in an ideal state, leading to loose and exposed chromatin that is more prone to DNA damage [125]. DNA damage of human sperm appears to be induced oxidatively initially [120], resulting in the formation of oxidative base adducts (e.g. 8-OHdG) that may cause the loss of the affected base leaving an abasic site, which has a strong destabilising effect on the DNA backbone lading to DNA strand breaks [126]. This process establishes a link between oxidative stress and DNA strand breaks [127] and may explain the increase of ROS/oxidative damage and single/double-stranded DNA breaks observed in infertile patients [65, 128–130].

The relationship between oxidative stress and DNA damage in sperm is further consolidated by the demonstration that when sperm were exposed to artificial oxidative stress in vitro (to produce ROS), it induced significant increases in sperm DNA damage, evidenced by modifying constituents of DNA leading to changes in genetic information [131, 132]. This has been reported in the form of deletions, frame shifts, DNA cross-links, DNA strand breaks and chromosomal rearrangements [108, 122, 131, 133, 134]. Increased sperm DNA damage is

associated with reduced fertilisation rates [129, 135], embryo cleavage rates [135–137], impaired blastocyst development rates [138] and reduced clinical pregnancy rates [136, 137, 139, 140]. Furthermore, DNA damage caused by oxidative stress has consequential paternal effects on embryonic growth and development which implicates diminished offspring health [141]. In mice, chemically induced (H_2O_2) sperm DNA damage prior to fertilisation did indeed cause metabolic disturbances in offspring [96]. DNA damage in sperm has been correlated with various offspring pathologies such as miscarriage [142], cancer [66, 86] and neurological defects including autism, spontaneous schizophrenia, bipolar disease and childhood epilepsy [74, 81, 143].

Interestingly, the production of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a result of ROS-induced oxidative DNA damage can also alter methylation patterns of adjacent cytosines. This occurs as a function of increased 8-OHdG abundance that reduces the ability of DNA methyltransferases to bind and add methyl groups to DNA, thus suppressing cytosine methylation by increasing conversion to hydroxymethylation. It could therefore be posited that ROS itself is active in the oxidisation of 5-methyl-Cytosine (5mC) and thereby reinstating a relationship between ROS and epigenetic marks resulting in transgenerational effects [144–146]. Several studies observed that increased ROS impaired spermatogenesis, through cellular and DNA damage that resulted in permanent alterations to DNA methylation, thereby causing epigenetic changes in chromatin organisation associated with an increased risk of disease in offspring [147, 148]. This is supported by an epidemiological study that observed alterations in DNA methylation patterns of newborns born to obese men [149] (Figs. 6.2 and 6.3).

Sperm have a limited capacity to repair oxidative DNA damage since they only possess the first enzyme in the base excision repair pathway, 8-oxoguanine glycosylase (OGG1), and rely on the oocyte to complete this process at fertilisation [150]. It remains a distinct possibility that oxidative damage to sperm at histone-bound DNA regions can be inherited and persist in the embryo. This could act to inhibit paternal pronucleus remodelling and alter the transcriptome of the early embryo.

6.7.3 Seminal Fluid

Recent studies in animal models have raised the prospect that events at conception mediated through seminal plasma contact with female tissues can not only influence the capacity to maintain pregnancy but also may impact the future life course and health of offspring. In mice, in vivo studies utilising mice rendered seminal vesicle deficient by surgical excision of the seminal vesicles (SVX mice) have demonstrated that conception in the absence of seminal plasma not only reduces fecundity but also alters fetal and neonatal outcomes [41]. Evidence for an effect on fetal programming was observed prior to birth, with abnormal blastocyst development and placental hypertrophy seen in pregnant females mated with SVX males

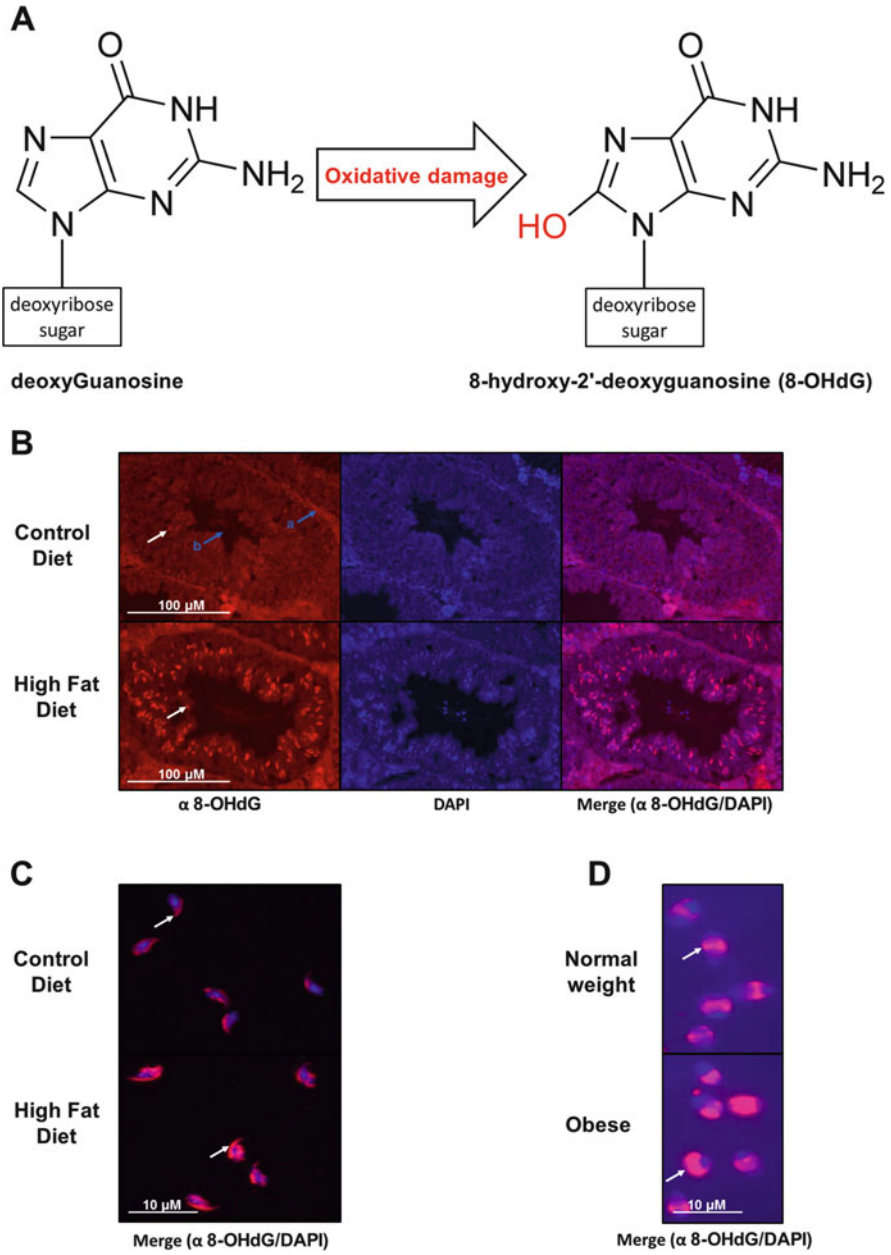

Fig. 6.2 The abundance of oxidative DNA damage (detected by the 8-hydroxy-2′-deoxyguanosine adduct; 8-OHdG) is increased in both a mouse model of obesity (high-fat diet fed) and human obesity, as per the representative images. (**A**) The Guanosine nucleobase is depicted within DNA (attached to the DNA backbone via the deoxyribose sugar) alongside 8-OHdG that is formed following oxidative damage. Immunohistochemical detection using an 8-OHdG antibody (*red*) contrasted against a DNA stain (DAPI—*blue*) in (**B**) a cross section of an individual seminiferous tubule from a mouse testes from mice fed either a control diet or a high-fat diet and (*white arrows* indicate strong positive staining from the heads of spermatids within the

[41]. This placental aberration is commonly observed where placental transport function is compromised and/or where disturbances in fetal growth occur [151]. Further evidence for programming mediated by seminal plasma was evident in the offspring sired by SVX males, where moderate growth impairment could be seen postnatally, followed by growth acceleration and evidence of obesity following puberty, independent of sex [41]. This phenotype was more pronounced in male offspring where a 72 % increase in absolute mass of central adipose tissue was observed. Furthermore, evidence for altered metabolic programming could be seen in adult male offspring with increased metabolic hormones, delayed glucose clearance and increased blood pressure [41]. Development of the altered phenotype was associated with changes observed from the early cleavage stages in embryos sired from SVX males, thus providing a link between altered embryo development and an alteration to the balance of embryotrophic/embryotoxic cytokines in the female tract, demonstrating the importance of seminal plasma's ability to regulate cytokine synthesis in female tissues after conception [41].

A similar phenotype can be observed in the Golden Hamster, where coitus in the absence of seminal plasma leads to abnormal embryo development and transit [152–154] and a reduction in implantation rates and increased embryo death [155] as well as growth impairment during the neonatal period associated with elevated anxiety in offspring [156]. The phenotype in the Golden Hamster offspring has been postulated to result from epigenetic mechanisms, based on the finding that cleavage stage embryos from females not exposed to seminal plasma show reduced acetylation and altered methylation kinetics [155].

In the human, there is no direct evidence linking seminal plasma exposure with the long-term health of offspring. However, similar distorted growth patterns in utero and *post-partum* in humans have been linked with obesity and metabolic disorder later in life [157]. Additionally, clinical observations in assisted reproduction are consistent with the phenotypes observed in mice and hamsters, raising the question of whether, to some extent, altered outcomes after IVF may result from the absence of exposure to seminal fluid at conception. Exposure to semen around the period of embryo transfer during in vitro fertilisation has been shown to improve embryo viability and clinical pregnancy rates [158, 159]. The absence of seminal plasma in the in vitro setting may potentially contribute to the higher rates of implantation failure and reduction in embryo quality which may lead to reduced birth weights and impaired metabolic outcomes, all of which have been observed

Fig. 6.2 (continued) tubule amongst a background of low-intensity basal staining in all other cells; *blue arrows* highlight anatomical features of the seminiferous tubule: (**a**) the outer lamina layer/basement membrane and (**b**) the lumen. (**C**) Mouse sperm from mice fed either a control diet or a high-fat diet (*white arrows* indicate strong positive staining in the heads of extracted epididymal sperm) and (**D**) human sperm from donors that were either of normal weight or obese (*white arrows* indicate strong positive staining in the heads of ejaculated sperm). *Note*: the difference in background colour between mouse (**C**) and human (**D**) sperm staining is due to different slides used, images captured on different microscopes with distinct gain settings

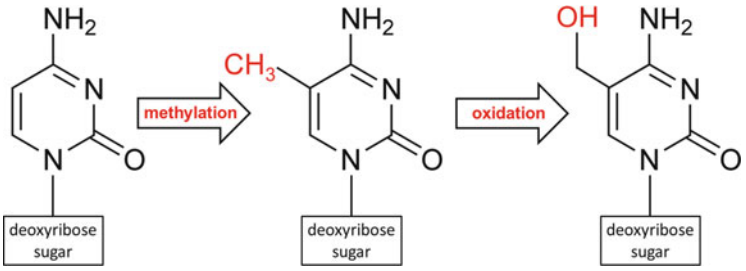

Fig. 6.3 The Cytosine nucleobase is depicted within DNA (attached to the DNA backbone via the deoxyribose sugar) alongside both its methylated form (5mC) and its hydroxymethylated form (5hmC; which is produced as a result of oxidation either by TET enzymes or potentially as a result of direct oxidative damage of the methyl group)

for children born through the early use of IVF [160]. However, these observations require formal investigation to determine the contribution of seminal plasma on perinatal and offspring health outcomes.

6.7.4 Epigenetics

The cardinal example of paternally derived epigenetic influences on offspring phenotype is imprinting. Imprinting disorders can be paternally derived, whereby the paternally inherited imprinted allele is expressed at the expense of the silencing of the maternal allele [161] by mechanisms controlled by methylation and histone modifications [162, 163]. Methyltransferase enzymes transfer methyl groups to DNA bases, reducing transcription and ultimately inhibiting/silencing gene expression, contributing to the regulation of embryonic development, X chromosome inactivation and genomic imprinting [164–166]. Hypomethylation of genes can alter the reprogramming of the male pronucleus in response to environmental exposures such as DNA damaging agents (e.g. chemotherapeutic agents), ultimately leading to the onset of disease in offspring [167–169]. How obesity alters the methylation status of sperm DNA is not understood, but obesity has been demonstrated to modify DNA methylation in somatic cells and lead to hypomethylation of DNA from testes and late elongated spermatids [103, 170].

Acetylation of histones and the incomplete replacement with protamines is an essential process during spermatogenesis. A male mice model of diet-induced obesity demonstrated alterations in acetylation in spermatids and increased DNA damage in sperm, suggesting a protamination impairment [171]. The histones retained in sperm (~10–15 % in human; ~1–5 % in mouse) are capable of carrying epigenetic marks (e.g. acetylation, methylation) that can be transmitted to the oocyte at fertilisation [11] and may modulate embryonic gene expression, which may for part of the programming of embryos generated from obese fathers [172, 173]. Recent reports indicate that spermatogenesis occurs without significant

changes in DNA methylation but rather occurs via atypical chromatin structure that is 'poised' ready for transcription processes from 5mC DNA methylated promoters [174].

The sperm nucleus also contains large populations of RNA, mRNA and small non-coding RNAs (sncRNAs; which includes microRNAs) that contribute to normal fertilisation and subsequent embryo development by active transcriptional and translational regulation [175, 176]. Paternal conditions and exposures have been demonstrated to alter sperm microRNA, which are small endogenous non-coding RNA segments involved in post-transcriptional regulation by binding mRNA targets, usually leading to their degradation or inhibiting translation, consequentially modulating gene expression [177]. Sperm microRNA is essential to the embryo as knockout mice ($Dgcr8^{-/-}$) that lack mature microRNAs produced embryos that arrest during development [178]. Cigarette smoking in men has been found to initiate differential microRNA abundance of 28 sperm microRNAs compared to non-smokers. The differentially abundant microRNAs are involved in pathways vital for normal embryo development including cell proliferation, differentiation and death; however, this study assessed sperm microRNA from only five smokers and five non-smokers making the results inconclusive based on small sample sizes [179]. MicroRNA has also been shown to impact offspring health; e.g. microRNA-124 was injected and artificially overexpressed in fertilised eggs and resulted in frequent twin pregnancies (due to duplication of the inner cell mass in blastocysts) and mouse pups displayed a 30 % increase in size and growth [180]. Further microinjection studies that used a single microRNA into the recently fertilised mouse oocyte also result in altered offspring phenotypes, including loss of pigmentation (microRNA-221/222) [181] and cardiac hypertrophy (microRNA-1) [182]. Interestingly, the sperm sncRNA (including microRNAs) fraction is changed in rodent models by paternal stress, which induces altered behavioural and metabolic phenotype in offspring, and when this sncRNA fraction is extracted from sperm and microinjected into the very early mouse embryo, it was sufficient to recapitulate the offspring phenotype [183]. Diet-induced obese mice have altered expression of 11 testes microRNAs, 4 of which were also altered in sperm [103], although the direct impact of these changes on embryo development is yet to be determined. Overall, these studies demonstrate that sperm microRNA content is sensitive to environmental exposures and can potentially result in altered embryo development, induce multiple pregnancies and ultimately impact offspring health.

Sperm RNAs have been shown to be localised to histone retained regions and potentiated genes, implicating sperm RNA in chromatin packaging and genomic imprinting [175]. The role of sperm-borne RNAs in embryogenesis is unclear, but the delivery of sperm RNAs to the oocyte at fertilisation has been reported necessary in early zygotic and embryonic development [184, 185]. Although sperm contain a vast array of RNA, the mRNA fraction comprises a small fraction of this, and as such very few studies have investigated the impact of obesity on mRNA in sperm. Despite this, mouse models of diet-induced obesity and diabetes have shown differences in mRNA levels of several genes within testes compared with lean controls [103, 186]. Overall, these studies indicate that RNA is delivered

to the oocyte and is necessary for embryo development and may modulate offspring phenotype.

6.8 Summary

Paternal conditions and environmental exposures appear to influence sperm and seminal fluid by creating genetic alterations, epigenetic marks and extracellular signals, in turn affecting offspring phenotype. The culmination of these signals must persist through—or be applied during—two rounds of epigenetic reprogramming within the embryo. Epigenetic reprogramming is a vital process that ensures embryo totipotency and the removal of epigenetic mutations to prevent the transmission of disease to offspring [187, 188]. During embryo development, the epigenome of the pre-implantation embryo is incompletely reprogrammed and DNA methylation is re-established, with the exception of imprinted genes, whereby one of the parental alleles is methylated and becomes silenced in the embryo [189]. The fetus' own primordial germ cells undergo a second round of epigenetic reprogramming and epigenetic marks are reacquired in a sex-, cell- and tissue-specific manner, which potentially leads to epigenetic inheritance in offspring [188, 189]. There are many suggested mechanisms involved in paternal transmission and the most widely recognised (but are not limited to) include methylation changes of imprinted genes maintained into the embryo, oxidative damage to DNA, modifications of retained histones in sperm (acetylation and methylation) transmitted from sperm to embryo [67, 173], alterations to sperm sncRNA (including microRNAs) content that modifies embryonic gene expression [103, 180, 190] and seminal fluid composition influencing the expression of genes in the female reproductive tract before implantation [41]. All of these mechanisms are posited to ultimately impact on the molecular constitution of the developing embryo and induce pathologies in subsequent offspring. Although these candidate mechanisms shed light on the potential pathways involved in the paternal transmission of disease to offspring, more investigations are required to examine the consequences of the molecular alterations during spermatogenesis and how this impacts reprogramming during embryo development and leads to the onset of disease in offspring. Furthermore, relatively simple lifestyle interventions aimed at improving dietary intake and/or physical activity have demonstrated promising results for both improvements to sperm quality and offspring health [95, 191]. These interventions represent a potential circuit breaker for the transgenerational transmission of obesity and promise to improve overall health and fertility.

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Chapter 7
The Impact of Maternal Obesity and Weight Loss During the Periconceptional Period on Offspring Metabolism

L.M. Nicholas and I.C. McMillen

Abstract The current global obesity epidemic has resulted in more women entering pregnancy with a body mass index in the overweight and obese range. It has been shown that offspring of obese women are at increased risk of obesity and type 2 diabetes in childhood and adult life, thus giving rise to an 'intergenerational cycle' of metabolic dysfunction. Importantly, studies in recent years have highlighted that the oocyte and/or early pre-implantation embryo is particularly vulnerable to the effects of maternal obesity resulting in long-lasting endocrine and metabolic effects for the offspring. Investigations into the molecular mechanisms underlying the programming of obesity and insulin resistance in liver, muscle and adipose tissue have highlighted the role of epigenetic changes within these tissues, which are recruited within the developing embryo and/or fetus. The periconceptional period is also an important period for intervention where dietary intervention in overweight/obese women is relatively more feasible. While dieting before pregnancy may have metabolic benefits for the offspring, there are however also metabolic and endocrine costs for the offspring. Thus, we need a better evidence base for the development of dietary interventions in obese women before pregnancy and around the time of conception to maximise the metabolic benefits and minimise the metabolic costs for the next generation.

Keywords Maternal obesity • Periconceptional period • Metabolism

Overweight and obesity affect 39 % and 13 %, respectively, of adults worldwide [1]. Consequently, more women in the developed world are entering pregnancy

L.M. Nicholas (✉)
Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5000, Australia
e-mail: ln305@medschl.cam.ac.uk

I.C. McMillen
Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5000, Australia

The Chancellery, University of Newcastle, Callaghan, NSW 2308, Australia

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either overweight or obese [2–4]. Obese women are more insulin resistant than their normal-weight counterparts, both before and during pregnancy [5], and this is associated with an increased risk of developing gestational diabetes mellitus (GDM) and of giving birth to a large baby with increased fat mass [5–8]. Indeed, as of 2013, 17 % of all live births were associated with hyperglycaemia in pregnancy [9]. Importantly, exposure to either maternal obesity or to impaired glucose tolerance during pregnancy is also associated with an increased risk of obesity and features of insulin resistance in childhood, adolescence and adult life [10–12]. Globally, 42 million preschool children were overweight in 2013 [13]. This suggests that exposure to maternal obesity may result in an 'intergenerational cycle' of obesity and insulin resistance [3, 14, 15]. There has, therefore, been significant interest in understanding the type of dietary and lifestyle interventions such as increased physical activity both before and during pregnancy, which may lead to optimal outcomes for the mother and her offspring [15, 16]. This chapter will summarise the epidemiological, clinical as well as experimental studies that have highlighted the relationship between maternal obesity with or without pre-existing GDM and type 2 diabetes mellitus (T2DM) and the later onset of obesity and insulin resistance in the offspring in childhood and adult life. Furthermore, some of the potential benefits and risks of maternal dietary restriction and weight loss will also be highlighted.

This chapter will also highlight some of the molecular mechanisms underpinning the programming of obesity, insulin resistance and T2DM specifically in insulin-sensitive tissues and what is known about the epigenetic mechanisms that are recruited within the developing embryo in the face of maternal obesity or dietary restriction and weight loss, which result in the programming of these metabolic pathways.

7.1 Maternal Obesity and Its Association with Short- and Longer-Term Pregnancy Outcomes

Maternal overnutrition and overweight or obese status has a significant impact on the health of the offspring in later life. Data from more than 200 countries between 1980 and 2008 highlight that there is a steady increase in the prevalence of obesity in every region of the world, including most countries of low and middle incomes, with the steepest rises in higher-income countries [17]. Not surprisingly, this global obesity 'epidemic' includes women of reproductive age with more women entering pregnancy with a BMI in the overweight (i.e. a BMI $\geq$ 25 kg/m^2) or obese (i.e. a BMI $\geq$ 30 kg/m^2) range [3]. The prevalence of obesity in women aged between 20 and 39 years is now around 15–28 % in women in the USA, UK and Australia [2–4, 18, 19]. In the USA, La Coursiere and colleagues found an increase in the proportion of women entering pregnancy both overweight and obese between 1991 and 2001 [20]. At present, more than 60 % of all pregnancies in the USA are in women who are either overweight or obese at conception [21, 22]. Similarly, in

Australia, the prevalence of maternal overweight and obesity was 34 % in a population giving birth between 1998 and 2002 and 43 % in a population measured at their first antenatal visit between 2001 and 2005 [23, 24]. Furthermore, the incidence of childhood obesity, which strongly predicts adult obesity [25], is also increasing; an estimated 22 million children aged under 5 years are estimated to be overweight or at risk of becoming overweight worldwide [26] and 1 in 10 children (155 million) aged between 5 and 17 years are overweight [27].

Obesity imposes a number of serious risks during pregnancy including increased rates of twinning and miscarriage in early pregnancy, and maternal obesity has also led to increased rates of hypertension, pre-eclampsia and venous thromboembolism [28].

7.1.1 Maternal Obesity and the Developmental Programming of Obesity and Insulin Resistance: Evidence from Human Studies

In addition to the clinical risks conferred by obesity to the pregnant mother, maternal obesity also has longer-term consequences for the offspring including increased adiposity [5–8]. The underlying mechanisms that result in obesity in the offspring of overweight or obese women most likely are a result of a dysregulation of glucose, insulin and lipid metabolism in these offspring [29, 30]. Women who enter into pregnancy obese are more insulin resistant than their lean and overweight counterparts, particularly before pregnancy and in early gestation [5], resulting in an increased risk of developing insulin resistance and GDM [31]. Exposure of the developing fetus to maternal hyperglycaemia results in excess fetal growth; while maternal glucose freely crosses the placental barrier, there is no trans-placental transfer of maternal insulin [32]. The fetal pancreas responds to the increased glucose supply from the mother by synthesising and secreting insulin to maintain its own glucose homeostasis. Insulin acts as a fetal growth hormone promoting growth and adiposity [32]. Obese women are, therefore, at a greater risk of giving birth to a larger, heavier and fatter baby [5–8]. This has been demonstrated in various epidemiological and clinical studies, which have shown that overweight/obese women tended to have infants with an increased risk of macrosomia; defined as birth weight $\geq$4 kg [33–36].

Furthermore, these offspring of obese and/or diabetic mothers are not only heavier at birth but remain so throughout childhood and adult life. According to a recent systematic review, an increase in pre-pregnancy overweight/obesity in women increases the risk of having a large for gestational age (LGA) baby and these babies are subsequently at an increased risk of being overweight/obese in later life [37]. It has also been shown that the offspring of Pima Indian women with pre-existing GDM and T2DM were larger for gestational age at birth and, at every age, were heavier than the offspring of prediabetic or non-diabetic women [7, 38,

39] and that children of women with diabetes during pregnancy were on average 30 % heavier than expected for their height at 8 years of age [40]. Boney and colleagues also reported that obesity in children at 11 years of age was a strong predictor of insulin resistance [10]. Furthermore, children of obese mothers that were born LGA were at twice the risk of developing the metabolic syndrome accompanying childhood obesity at this age [10]. The impact of maternal obesity is still present in her offspring in adult life. Parsons and colleagues carried out a longitudinal study of the 1958 British birth cohort to determine the influence of birth weight on BMI at different stages of later life. BMI of the participants was measured at ages 7, 11, 16, 23 and 33 years, and they concluded that maternal weight or her BMI largely explained the association between a high birth weight and a high adult BMI of the offspring [41].

A recent longitudinal study of 421 mother–daughter pairs [42] has highlighted the concern that the increase in obesity in women entering pregnancy will, in turn, lead to propagation of an 'intergenerational' cycle of obesity and insulin resistance [3, 15]. Kubo et al. found that girls who were exposed to maternal GDM and hyperglycaemia in utero were at a higher risk of increased adiposity and that this risk increases if the mother was overweight/obese [42]. Moreover, they found that the risk of obesity was highest among offspring of mothers with GDM and pregravid obesity [42].

7.1.2 Maternal Obesity and the Developmental Programming of Obesity and Insulin Resistance: Evidence from Animal Studies

As most women who are obese at conception remain obese through their pregnancy, it is difficult to determine the separate or interdependent contributions of maternal pre-pregnancy BMI, gestational weight gain and glycaemic control on the metabolic outcomes for the offspring in human studies. Experimental studies in animals are, therefore, key to address these questions. Indeed, studies in both small and large animals have also provided evidence for the association between maternal obesity and subsequent programming of obesity and insulin resistance in the offspring. Studies in rodents have involved maternal consumption of either a high-fat only [43–45] or high-fat, high-sugar junk food diet, which is reflective of an obesogenic Western diet in humans [46, 47] from before pregnancy and during gestation. In some studies, the period of overnutrition was also extended to encompass lactation [45–47]. Despite the differences in diet composition, length of exposure to maternal overnutrition and whether offspring were weaned onto a standard chow or high-fat diet, these studies all found that a common outcome was increased adiposity in the offspring [43–48]. Furthermore, it has also been shown that exposure of the offspring to a high-fat diet resulted in increased adiposity specifically in the visceral fat depot [49, 50]. Visceral fat accumulation

has been shown to be associated with the development of insulin resistance [51, 52]. Studies have also documented that maternal overnutrition in rodents is associated with insulin resistance in the offspring [46, 47, 49] due to altered expression of key components of the insulin signalling pathway [53] as well as poor glucose tolerance [43, 46, 47, 49], associated with a combination of β-cell dysfunction and insulin resistance [44, 49] in the offspring. Furthermore, in some cases, maternal obesity during pregnancy and lactation also resulted in obesity-induced non-alcoholic fatty liver disease (NAFLD) in the offspring, which is characterised by evidence of steatosis, liver injury, raised inflammatory cytokines and the beginning of fibrogenesis [54].

Similar observations linking maternal obesity and the development of increased adiposity and insulin resistance in the offspring have also been made in a primate model of maternal overnutrition [55]. One of the earliest studies conducted in the baboon showed that overnutrition in the pre-weaning period permanently increased offspring adiposity as a consequence of fat cell hypertrophy [56]. Sheep have also been used as a large animal model to study the impact of maternal overnutrition/obesity on the developmental programming of obesity and insulin resistance in the fetus and in the offspring. The sheep fetus is similar to the human fetus in its dependence on glucose as a major source of energy [57–59]. Moreover, both sheep and humans are precocial species, exhibit the same newborn-to-maternal weight ratios and have the same temporal pattern of fetal organ development throughout pregnancy [58, 60]. Importantly, in both sheep and humans, the appetite regulatory network develops in the hypothalamus before birth, in contrast to rodents, in which this neural network develops after birth [61]. There have been a series of studies on the effects of maternal overnutrition/obesity in the sheep from 60 days before conception and throughout pregnancy on the fetal and postnatal lamb. Similar to findings in rodent studies, maternal obesity in sheep leads to increased adiposity in the offspring in late gestation [62], which persists after birth [63]. These changes were attributed to increased gene expression and protein abundance of fatty acid (FA) and glucose transporters (GLUTs) as well as increased expression of enzymes mediating FA biosynthesis in fat depots of the offspring [62]. Maternal obesity also resulted in decreased pancreatic weight and β-cell number in the fetus in late gestation as well as in a reduction in circulating plasma insulin concentration at term [64]. Moreover, these offspring displayed upregulation of inflammatory signalling pathways in skeletal muscle, which promotes adipogenesis and downregulates myogenesis [65, 66]. This along with the increased adiposity would result in an increased risk of developing insulin resistance and T2DM in the postnatal animal. Indeed, when the offspring of obese ewes were subjected to ad libitum feeding at maturity, they showed increased appetite, growth rates and adiposity and decreased glucose tolerance and insulin sensitivity [67].

Late gestation has also been identified as a period during which exposure to maternal obesity can result in programming of obesity in the offspring. Exposure to maternal overnutrition during the last month of pregnancy resulted in an increase in fetal glucose and insulin concentrations and an upregulation of key adipogenic, lipogenic and adipokine genes, including peroxisome proliferator-activated

receptor (PPAR) γ, leptin and adiponectin in perirenal adipose tissue of the sheep fetus in late gestation [68] as well as increased fasting plasma glucose concentrations and a higher relative subcutaneous fat mass in the first month of life in the postnatal lamb [69]. Furthermore, leptin expression was higher in both the perirenal and subcutaneous fat depots in the postnatal lamb of the overnourished ewe [70]. It is clear, therefore, that maternal overnutrition in late gestation results in an initial upregulation of adipogenic and lipogenic genes in the perirenal fat in fetal life followed by an upregulation in leptin expression in the perirenal and subcutaneous fat depots and the emergence of leptin resistance in the hypothalamic network which regulates appetite in postnatal life. In contrast to its effects on programming of the fat–brain axis, exposure to maternal obesity in late gestation had less impact on hepatic glucose metabolism in the offspring. Maternal overnutrition during the last month of gestation resulted in decreased hepatic expression of the mitochondrial (PEPCK-M) isoform of phospho*enol*pyruvate carboxykinase both before birth and in postnatal life. There was, however, no impact on the cytosolic isoform of PEPCK (PEPCK-C), glucose-6-phosphatase, PPARγ co-activator (PGC)-1, PPARα and 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) in the postnatal lamb [71].

7.2 The 'Intergenerational Cycle' of Obesity and Insulin Resistance

Although there is variation between studies in the length of the feeding regimes and types of dietary challenges, studies in both small and large animals show that exposure to maternal overnutrition/obesity consistently results in a similar phenotype in the offspring including a high body fat mass and in most cases impaired insulin signalling in the liver, muscle and adipose tissue [15, 72]. Together, these findings implicate a possible 'intergenerational cycle' of obesity and insulin resistance (Fig. 7.1). In a recent study by Graus-Nunes et al., a maternal high-fat diet consumed before and throughout pregnancy and lactation resulted in impaired whole-body metabolism as well as altered development of the pancreas in the F1 and F2 offspring [73]. The maternal contribution to the intergenerational transmission of obesity and insulin resistance appears to be mediated, at least in part, by the transgenerational accumulation of epigenetic modifications [74]. This has led to a significant interest in the type of dietary and lifestyle interventions that could be imposed before and during pregnancy that could lead to optimal metabolic outcomes for the mother and the offspring [15, 75] (Fig. 7.1).

Fig. 7.1 The intergenerational cycle of obesity and insulin resistance. Is there a role for pre-pregnancy weight loss to break this cycle?

7.3 Molecular Mechanisms Underlying the Programming of Insulin Resistance and T2DM in Insulin-Sensitive Tissues

Defects in insulin signalling itself are among the earliest indications that an individual is predisposed to the development of insulin resistance and subsequently T2DM [76, 77]. To date, however, the underlying molecular mechanisms which result in resistance to the actions of insulin including transcriptional and post-transcriptional dysregulation of genes involved in insulin signalling as well as post-translational modification(s) and degradation of their corresponding protein (s) are poorly understood [78, 79]. Furthermore, although it has been shown that maternal obesity is associated with an increased risk of obesity and insulin resistance in the offspring, the genetic and/or epigenetic modifications within insulin-sensitive tissues such as liver and skeletal muscle which contribute to the insulin-resistant phenotype remain unknown.

7.3.1 Metabolic Role of the Liver

In postnatal life, both in human and in sheep, the liver plays an essential role in carbohydrate metabolism by maintaining plasma glucose concentrations within a very narrow range over both short and long periods of time. This is partly achieved by the actions of insulin, which regulates hepatic glucose output by suppressing gluconeogenesis and glycogenolysis [80–82]. Insulin acts through a complex, highly integrated network that controls several processes downstream of the insulin receptor (IR) [77, 83]. The IR itself is found enriched in caveolae, which are flask-shaped invaginations of the plasma membrane. Structural components of caveolae are made up by the caveolin (Cav) gene family, namely Cav-1 and Cav-2, which are usually co-expressed in adipose tissue and the liver, and Cav-3, which is expressed

in skeletal, cardiac and smooth muscle [84]. Localisation of the IR within caveolae serves to ensure metabolic signalling specificity downstream of it and Cav-1 also stabilises the receptor against proteasomal degradation [84].

In the presence of insulin, IR phosphorylates IRS proteins that are linked to the activation of the PI3K–Akt pathway, which is responsible for most of the metabolic actions of insulin [77]. PI3K acts to catalyse the formation of the lipid second messenger PIP_3, which allows the localisation and activation of PDK1 and the subsequent activation of Akt. PDK1 is also critical for activating $aPKC_\zeta$, which in the liver leads to an increase in lipid synthesis [77]. In contrast, activation of Akt results in a reduction in phosphorylation of the transcription factor FoxO1, which together with the transcriptional co-activator PGC1α plays an important role in regulating the expression of both PEPCK-C and G6Pase [80–82, 85]. PEPCK-C is mainly considered to be the rate-limiting enzyme in gluconeogenesis whereas hydrolysis of glucose-6-phosphate by G6Pase is the 'final common pathway' and is rate determining for the release of glucose into the circulation by gluconeogenesis and glycogenolysis [86].

The liver is also the most important site for the removal of FFA from circulating blood plasma [87, 88]. It coordinates the synthesis of FAs and the esterification of FAs to produce TGs and their subsequent packaging into VLDLs for export to adipose tissue [81, 87, 89]. The liver also regulates the rate of FA oxidation and ketogensis [81, 87, 89] and is therefore able to handle large amounts of fat without accumulating triacylglycerol and causing peripheral lipotoxicity [89].

7.3.2 Maternal Obesity Programs Molecular Changes in Hepatic Metabolic Processes in the Offspring

Shankar and colleagues found that at 21 days after birth, the offspring of rats that were overnourished from 3 weeks before until conception had increased phosphorylation of the IR and Akt, two key molecules of the insulin signalling network [90]. Furthermore, in another rodent model of metabolic programming by maternal obesity, female mice were fed a highly palatable diet high in sugar and fats for 6 weeks prior to pregnancy and throughout gestation and lactation [91]. Offspring of obese dams, which were weaned onto a control chow diet, had decreased hepatic IRS-1 abundance at 3 months of age. Moreover, hepatic phosphorylation of IRS-1 at Ser 307, which results in inhibition of insulin signalling, was also increased in these offspring [91].

In addition to defects in the insulin signalling network, increased hepatic fat deposition is also a very common feature of obesity and insulin resistance [92, 93] and NAFLD has been shown to be initiated by insulin resistance [94]. Most studies investigating the effects of maternal obesity on hepatic metabolism have examined perturbations in lipid metabolism. For example, studies investigating the impact of either a high-fat, high-sugar or high-fat only diet from before and during pregnancy

and lactation on the offspring found that these mice developed a condition with marked similarity to NAFLD including increased liver TGs and hepatic fibrogenesis [54]. These offspring also had changes in gene expression, which indicated upregulation of lipogenesis, oxidative stress and inflammation [95]. Furthermore, rodent offspring exposed to maternal obesity also show impaired FA oxidation possibly due to a decrease in hepatic mitochondrial function [96]. Finally, a study by McCurdy and colleagues investigating the effect of a chronic high-fat diet on the development of fetal metabolic systems reported that maternal high-fat feeding triggered development of fatty liver and hepatic oxidative stress in the fetus, which persisted in postnatal life [97].

7.3.3 Metabolic Role of Skeletal Muscle

Skeletal muscle is the major site of postprandial glucose clearance from the circulation and accounts for up to 75 % of insulin-dependent glucose uptake [98]. The rate-limiting step for glucose clearance is the transport of glucose across the plasma membrane by facilitated diffusion of glucose through a family of specific GLUTs. GLUT-4 is the predominant glucose transporter in postnatal life, and in the postprandial state, binding of insulin to its receptor on myocytes leads to translocation of GLUT-4 to the plasma membrane, thereby permitting glucose entry into the cell [99, 100]. In the presence of insulin, IR phosphorylates IRS proteins, which then act as docking proteins for the activation of PI3K. PI3K acts to catalyse the formation of the lipid second messenger PIP_3, which allows the localisation and activation of PDK1 and the subsequent activation of Akt and aPKC through phosphorylation of the Thr 308 and Thr 410 sites, respectively [100]. The positive actions of PI3K can be negatively regulated by phospholipid phosphatases, e.g. PTEN, which dephosphorylate and inactivate PIP_3 [77].

Activation of Akt acts to phosphorylate and inhibit AS160, which is involved in the regulation of glucose uptake through the redistribution of GLUT-4 from intracellular vesicles to the plasma membrane [101, 102]. This ultimately leads to an increase in glucose transport into the cell [101, 102]. Similarly, aPKCs have also been shown to play a role in insulin-stimulated glucose uptake and GLUT-4 translocation in adipocytes and muscle [99]. In skeletal muscle, glucose is utilised to generate energy via glycolysis and is also converted to glycogen for storage [103]. Akt is also involved in the regulation of glycogen synthesis through the actions of the serine/threonine kinase GSK3 [77, 104, 105]. GSK3 consists of two highly homologous isoforms, GSK3α and GSK3β, and acts to phosphorylate and inactivate GS [104, 105]. In resting cells, GSK3 activity is high, but on stimulation, GSK3 is inactivated through phosphorylation; GSK3α is phosphorylated at Ser 21 and GSK3β at the equivalent residue, Ser 9 [104, 105]. There is evidence from both human and animal studies that defects in these downstream components of the insulin signalling pathway are present in the insulin-resistant state [100, 104].

7.3.4 Maternal Obesity Programs Molecular Changes in Metabolic Processes in Skeletal Muscle of the Offspring

Although there is a wealth of evidence showing the association between maternal obesity and the subsequent insulin-resistant phenotype in the offspring, there is a paucity of studies that have been carried out to try and determine the molecular basis of this relationship. One study by Shelley and colleagues observed that 3-month-old mice that were exposed to maternal obesity before and during gestation and lactation had decreased abundance of IRS-1. IRS-1 is required for the activation of PI3K, which is needed for the phosphorylation and activation of Akt and subsequent glucose uptake. These mice also had decreased abundance of the catalytic subunit of PI3K, p110β, as well as decreased phosphorylated Akt [53].

Studies in sheep have produced similar results although different molecules in the insulin signalling cascade appear to be affected. A study by Yan and colleagues where ewes were overnourished from 60 days before conception and throughout pregnancy reported that there were defects in insulin signalling in skeletal muscle of the adult offspring at the receptor level, which is in contrast to rodent studies that showed impaired post-receptor signalling [53, 106]. Similar to rodents, however, these lambs also had increased phosphorylated IRS-1 abundance and decreased phosphorylated Akt abundance at 22 months of age [106].

7.3.5 Metabolic Role of Adipose Tissue

Most energy reserves are stored in adipocytes as triacylglycerol (TAG), which arises from two major processes: uptake of free fatty acids (FFA) from plasma or de novo lipogenesis from non-lipid precursors such as glucose [107]. Insulin plays a key role in the latter as it stimulates glucose uptake into adipose tissue. Glucose is transported into adipocytes by facilitated diffusion through a family of specific GLUTs. GLUT-4 is redistributed from intracellular vesicles to the plasma membrane in response to insulin as it is in skeletal muscle via activation of the IR [99, 100]. Furthermore, insulin also plays an important role in uptake, esterification and storage of FFA in adipocytes [108]. Insulin stimulates the activity of lipoprotein lipase LPL, which generates FFA for TAG synthesis by enabling the release of FFA from lipoproteins and is the important first step in TAG synthesis [107]. Insulin also suppresses the activity of hormone-sensitive lipase (HSL), which is expressed in white and brown adipose tissue and is a principal regulator of FFA release from adipose tissue [107].

Adipose tissue also secretes a number of adipokines, e.g. leptin and adiponectin, which play a key role in the development of insulin resistance associated with increased adiposity [109]. For example, obese patients with insulin resistance/T2D have reduced adiponectin [110]. Adiponectin also regulates hepatic glucose

production through its actions on gluconeogenic genes [111]. Another adipokine, leptin, acts as a circulating signal of fat mass [112]. Indeed, there is a direct relationship between cord blood concentrations of leptin at delivery and birth weight or neonatal adiposity both in normal pregnancies [113, 114] and in pregnancies complicated by maternal diabetes [115].

7.3.6 *Maternal Obesity Programs Molecular Changes in Metabolic Processes in Adipose Tissue of the Offspring*

Borengasser and colleagues have investigated the early effects of programming that occur prior to the emergence of adiposity and weight gain in 21-day-old offspring of dams that were overfed from before pregnancy [21, 96]. They found that these offspring had increased expression of adipogenic, lipogenic and adipokine (leptin and adiponectin) genes in white adipose tissue (WAT) [21]. Furthermore, the increased abundance of adipogenic proteins PPARγ, CCAAT-enhancer-binding protein (C/EBP)-α and C/EBP-β resulted ultimately in increased adipocyte differentiation, which was present in the offspring of obese dams at 21 days of age and persisted at 100 days of age [21]. In addition to these changes, insulin signalling was also upregulated in WAT of the offspring. This was characterised by increased protein abundance of the IR, GSK3α/β and increased GLUT-4 gene expression [21]. Phosphorylation of Akt following acute insulin stimulation was also approximately 1.9-fold greater in these offspring [21].

Interestingly, these findings associated with insulin signalling are in contrast to those of Fernandez-Twinn et al. in their study to dissect out the effects of maternal diet-induced obesity on offspring insulin resistance that were independent of the increased adiposity [72]. Using a mouse model of maternal diet-induced obesity with a diet rich in fat and simple sugars representative of a Western human diet, they found that the young mice of the obese dams displayed impaired adipose tissue insulin signalling [72]. These mice had reduced IR, IRS-1, the regulatory and catalytic subunit of PI3K, Akt1 and Akt2 protein abundance in WAT [72]. These findings suggest that although exposure to maternal obesity contributes to an obese phenotype, the adipose tissue of these offspring develops resistance to the actions of insulin even before the appearance of increased adiposity. What is also interesting is that, taken together, both these studies highlight the fact that adipose tissue is exquisitely sensitive to programming by maternal obesity and that the differences in timing, duration and type of maternal over-feeding can produce contrasting changes prior to the onset of obesity in the offspring. Exposure to pre-pregnancy obesity appears to program increased insulin signalling in WAT of the offspring, whereas when the period of exposure to maternal obesity is extended to encompass both gestation and lactation, there appears to be a switch to decreased insulin signalling.

7.4 Exposure to Maternal Obesity During Gestation and the Epigenetic Origins of Obesity

A number of recent studies in rodents have investigated the impact of a maternal high-fat diet throughout gestation on epigenetic changes in the adipose tissue and liver of the offspring [74, 116, 117]. Transcriptomic changes in the study by Borengasser et al. outlined in the previous section have been shown to be associated with alterations in DNA methylation of CpG sites and CpG island shores, which are proximal to developmentally important genes including C/EBP-β [21]. Changes in adiponectin and leptin expression in adipose tissue of offspring exposed to maternal high-fat diet were found to be due to alterations in both acetylation and methylation of histone H3K9 within the adiponectin promoter and changes in methylation of histone H4K20 within the leptin promoter [116]. The offspring of high-fat fed dams also showed increased hepatic expression of the cytosolic isoform of the gluconeogenic gene, phospho*enol*pyruvate carboxykinase, which was attributed to histone modifications associated with transcriptional activation [117]. Importantly, the effects of maternal high-fat feeding appear to be transgenerational. The F_2 offspring derived from both grand-maternal and maternal obesity appeared to be extremely susceptible to developing obesity due to the transgenerational accumulation of epigenetic modifications including histone methylation, which then contribute to an increase in lipogenesis [74].

Recent evidence has also identified microRNAs (miRNAs), a class of small (~22 nt), non-coding RNAs, as important regulators of insulin signalling through its role in post-transcriptional gene regulation either by cleavage or translational repression of their specific target mRNAs [79, 118–120]. In their study described above, Fernandez-Twinn and colleagues showed that the decreased IRS-1 protein abundance in WAT of offspring exposed to maternal diet-induced obesity before and during pregnancy and lactation was linked to increased expression of miR-126 [72]. He and colleagues have also shown in a rat model of T2DM that expression of all members of the miR-29 family (miR-29a, miR-29b and miR-29c) was upregulated in liver, adipose tissue and skeletal muscle of Goto-Kakizaki rats [118]. The authors then mimicked insulin resistance in 3 T3-L1 adipocytes in order to determine the molecular mechanisms involved in the regulation of insulin signalling by the mir-29 family. They found that there was an increase in expression of miR-29a and miR-29b as well as a parallel decrease in the abundance of insulin signalling molecules, in particular, Ser 473 phospho-Akt, which resulted in a subsequent decrease in glucose uptake [118]. The mir-103/mir-107 family have also emerged as regulators of hepatic insulin signalling in two models of insulin-resistant, obese mice. Specifically, Trajkovski and colleagues have shown that miR-103/miR-107 were among the five most upregulated miRNAs in the livers of insulin-resistant obese mice [120]. Cav-1 carries a seed match to miR-103/miR-107 in its 3'UTR and is, therefore, its direct target. Consequently, Cav-1 expression was decreased after overexpression of miR-107 in the liver of mice.

Finally, Jordan and colleagues have shown that expression of hepatic miR-143 was increased in db/db mice as well as mice exposed to a high-fat diet. They also showed in a transgenic mouse model of miR-143 overexpression that these mice had impaired glucose metabolism through the induction of insulin resistance as shown by a decreased in phosphorylation of Akt [79].

7.5 The Periconceptional Period Is a Critical Window for the Development of Postnatal Obesity

Currently, it appears that for obese women, pre-pregnancy BMI rather than gestational weight gain is associated with an increased risk of pre-eclampsia, GDM and the delivery of a macrosomic infant [121]. It has also been reported that siblings born to women who had undergone bariatric surgery for the treatment of severe obesity had a lower BMI and obesity risk than their siblings who were born prior to maternal surgery and weight loss [122]. Moreover, previous studies have shown that even in women who are ovulating regularly, increased BMI correlates with reduced conception rates [34, 123], suggesting that obesity affects critical periconceptional events [124]. The very earliest stages of embryo growth are primarily controlled by the quality of the oocyte, also known as oocyte developmental competence. Clinically, oocyte quality has been directly assessed in only three studies with the most recent and larger study finding that although there was no difference in the number of follicles aspirated, the number of mature oocytes was significantly reduced in morbidly obese women [125].

An experimental study in mice by Minge and colleagues, which investigated the impact of maternal obesity on oocyte and early embryo development, showed that when cultured in vitro oocytes from obese mice exhibited slower development to the four- to eight-cell stage and through to the blastocyst stage. These blastocysts also have reduced level of mitochondrial DNA and these changes were still present in the fetus at 14.5 days even though the blastocysts were transferred to normal-weight surrogates [126]. Furthermore, they also showed that these negative effects of maternal obesity can be improved by treatment with insulin sensitisers administered around the time of conception. These results indicate that maternal obesity as well as her peripheral insulin sensitivity during the periconceptional period is an important determinant of the developmental outcomes of the offspring [127]. This indicates that the periconceptional period, which includes some or all of the following early developmental stages: oocyte maturation and follicular development, i.e. pre-pregnancy events, conception and embryo/blastocyst growth up until implantation [15], is therefore a critical window for the development of postnatal obesity in the offspring.

7.5.1 An ovine Model of Maternal Obesity in the Periconceptional Period and Programming of Later Obesity In the Offspring

In order to determine whether exposure to maternal obesity in the periconceptional period alone has any specific impact on adiposity and metabolic function in the offspring, we have developed an embryo transfer model in sheep [128–132]. In this model, non-pregnant donor ewes were either overnourished or normally nourished for at least 4 months before conception. One week after conception, single embryos were transferred from obese or normal weight 'donor' ewes to non-obese 'recipient' ewes, which were maintained on a control diet for the remainder of pregnancy [128–132]. Thus, exposure to a high nutrient environment encompassed oocyte maturation, follicular development, conception and growth of the early pre-implantation embryo. This model is unique in that exposure of the offspring to maternal obesity is confined strictly to the periconceptional period.

Donor ewes that were overnourished during the periconceptional period were heavier than ewes on the control level of nutrition at 4 weeks before conception [131], and these ewes also had an obese phenotype at conception [131] as determined by their body condition score [133]. Furthermore, similar to obese humans [134], donor ewes that were overnourished also had increased plasma insulin but not plasma glucose concentrations [131].

7.5.2 Impact of Exposure to Obesity in the Periconceptional Period on Fat Mass in the Offspring

There is a sex-specific effect of exposure to maternal obesity during the periconceptional period on the body fat mass of lambs at 4 months of age [131]. Female but not male lambs, conceived in obese ewes, had an increased total fat mass. Specifically, the greatest impact of maternal periconceptional obesity appeared to be on the visceral fat depots, i.e. the perirenal and omental fat depots, in these female lambs [131]. Interestingly, the weights of these depots were also higher in female compared to male lambs [131]. There was also a significant relationship between total fat mass of female lambs at 4 months of age and the weight of donor ewes at conception [131].

Furthermore, an investigation of the expression of genes that regulate the differentiation and the development of adipose tissue as well as the storage of lipids in the perirenal, omental and subcutaneous fat depots of these lambs found that the increased adiposity in female lambs was not due to changes in expression of the adipogenic, lipogenic and adipokine genes PPARγ, glyceraldehyde 3-phosphate dehydrogenase, lipoprotein lipase, leptin and adiponectin [131]. Further studies on the role of the insulin signalling and other key metabolic pathways which regulate lipogenesis within these fat depots are required.

7.5.3 *Impact of Exposure to Obesity in the Periconceptional Period on Insulin Signalling in the Liver and Muscle in the Offspring*

Follow-up studies have also been carried out to determine whether exposure to maternal obesity during the periconceptional period may result in the molecular features of insulin resistance in the offspring either as a consequence of the impact of increased adiposity on insulin-sensitive tissues such as the liver and muscle [135, 136] or as a consequence of the programming of specific changes in the abundance of insulin signalling molecules in these tissues of metabolic importance [137–139].

While exposure to maternal obesity around the time of conception resulted in an increase in body fat mass in female lambs, we found that there were programmed changes in gene expression and protein abundance of key insulin signalling molecules in the liver and to a more limited extent in skeletal muscle of both male and female lambs conceived in obese ewes [128, 129]. There was a decreased hepatic abundance of the insulin receptor as well as phosphorylated Akt (Ser 473) and FoxO1 (Thr 24) in the young offspring of obese ewes. Interestingly, however, there was a paradoxical effect on the expression of key factors which regulate hepatic gluconeogenesis; expression of 11βHSD1, PEPCK-C and PEPCK-M was decreased in lambs exposed to maternal obesity [129]. These contrasting changes suggest that there are distinct mechanisms involved, which are programmed by maternal obesity during the periconceptional period and which impact hepatic insulin signalling and gluconeogenic factors separately. Findings in skeletal muscle of the offspring, however, showed that exposure to maternal obesity during the periconceptional period did not appear to impact directly on the early components of the insulin signalling pathway. Instead, this exposure resulted in specific changes in the abundance of molecules downstream of Akt [128]. Taken together, these results suggest that exposure to maternal obesity during the periconceptional period acts directly, independently of increased adiposity, to program changes within the insulin signalling pathway in the liver and skeletal muscle.

7.5.4 *Impact of Exposure to Obesity in the Periconceptional Period on Hepatic Fatty Acid Metabolism in the Offspring*

In addition to defects in the insulin signalling network, increased hepatic fat deposition is also a common feature of obesity and insulin resistance [92, 93]. Indeed, maternal obesity both before and throughout pregnancy has been shown to result in alterations in hepatic FA oxidation and lipogenesis in the offspring [54, 97]. In addition to changes in the abundance of hepatic insulin

signalling molecules, we also found that exposure to maternal obesity during the periconceptional period resulted in downregulation of hepatic PGC1-α and PPARα and also resulted in a compensatory increase in the abundance of AMP-activated protein kinase (AMPK) α1 and α2, which may initially limit the impact of these changes on intra-hepatic FA oxidation in the young offspring [130]. Furthermore, hepatic expression of sterol regulatory element-binding protein 1, a key lipogenic gene, was also increased in these lambs. It is possible that with ageing and/or exposure to a high-caloric diet, these offspring may be susceptible to hepatic lipid accumulation and steatosis [130].

7.5.5 Exposure to Maternal Obesity During the Periconceptional Period and the Epigenetic Origins of Obesity

During the early stages of development, the differentiation and development of different cell types is regulated by epigenetic mechanisms, which play a role in modulating chromatin architecture [140]. Furthermore, epigenetic regulation plays a key role in conferring phenotype plasticity, which allows organisms to adapt their gene expression and function in response to the environment [141]. Each cell type, therefore, has its own epigenetic signature which reflects genotype, developmental history and environmental influences. This is ultimately reflected in the phenotype of the cell and organism [142]. Early embryogenesis in mammals is a critical period for the establishment of the epigenome [143]; during the period between conception and implantation, there is de-methylation of the genome followed by a wave of re-methylation shortly after implantation [142]. This period, therefore, represents a critical window in development during which the embryo is vulnerable to environmental and/or nutritional cues that disrupt the establishment of epigenetic marks such as DNA methylation, histone modification and miRNAs [144]. Importantly, although the genome of an individual is largely stable, the epigenome has the potential to be reversibly modified by exposure to a range of nutritional and environmental factors [145]. Parental nutrition has been shown to permanently influence metabolism of the offspring through epigenetic mechanisms and these changes also appear to be stable and transgenerational [146].

Using the embryo transfer model described above [128–132], we found that exposure of the oocyte/early embryo to maternal obesity resulted in upregulation of hepatic expression of miR-29b, miR-103 and miR-107 [129]. Expression of these miRNAs has been shown to be related to decreased insulin signalling in adipocytes [118] and liver [120] and is increased in murine models of obesity and T2DM [79, 118, 120].

Since defects in insulin signalling are among the earliest indicators that an individual is predisposed to the development of insulin resistance and T2DM [77], our results suggest that miRNAs may be potential epigenetic regulators that

are sensitive to programming by the metabolic and nutritional environment associated with maternal obesity specifically during the periconceptional period. MiRNAs could, therefore, play a key role in the transduction of the metabolic consequences of maternal obesity from the mother to the offspring.

7.6 Weighing Up the Benefits and Costs of Maternal Dietary and Lifestyle Interventions in Obese Mothers for Their Offspring

Clinical and experimental studies have provided clear evidence that exposure to a maternal obesogenic environment from before pregnancy and around the time of conception has long-lasting metabolic consequences for the offspring. There has been a growing focus, therefore, on nutritional health of women in the periconceptional period and on what weight loss interventions can be safely introduced in overweight/obese women seeking to become pregnant [15]. There is, however, now more than ever a need for a better understanding of both the benefits and the possible negative effects of maternal dietary and lifestyle interventions in obese mothers for their offspring.

7.6.1 The Benefits of Maternal Dietary Restriction and Weight Loss: Breaking the 'Intergenerational Cycle' of Obesity and Insulin Resistance

Previous studies have suggested that childhood obesity may be prevented by normalising body composition and nutrition and improving the general health of young women of childbearing age before becoming pregnant, thereby preventing the prevalence of the 'intergenerational cycle of obesity' and the serious co-morbidities associated with obesity [147]. Other studies have focused on limiting gestational weight gain through both physical activity and the reduction of dietary intake [148–150] on the premise that a healthy, active pregnancy may help to minimise the cycle [151]. Although most studies showed favourable weight-related outcomes indicating that interventions can help pregnant and postpartum women manage their weight, knowledge gaps still remain regarding the benefits and potential harm associated with dietary and lifestyle interventions for overweight and obese pregnant women and their offspring [148–151].

Furthermore, as traditional 'diet and exercise' approaches do not often achieve robust and sustained weight loss [152], surgical approaches have also emerged as an effective albeit complex strategy to promote durable weight loss, improve insulin resistance and improve or reverse T2DM especially in morbidly obese women [152, 153]. A number of studies have found a decreased prevalence of obesity in

the offspring of mothers who underwent maternal surgical weight loss prior to pregnancy [154, 155]. Furthermore, a recent study by Guénard and colleagues found that the improved cardiometabolic risk profiles of offspring born after maternal weight loss surgery were associated with epigenetic changes including differential methylation patterns of glucoregulatory genes [156].

In contrast to human studies, there have been relatively few experimental animal studies, which have investigated whether maternal dietary restriction and/or exercise are able to reverse the outcomes associated with maternal obesity. We have shown in the sheep that dietary restriction and weight loss experienced by the mother in the periconceptional period alone is able to ablate the effects of a high maternal pre-pregnancy weight on offspring adiposity [131]. Furthermore, in a study where the nutritional intake of female rats that were previously on a high-fat diet was 'restricted' to normal chow from 1 month prior to conception and throughout pregnancy and lactation, dietary 'restriction' was able to normalise fat mass, serum triglycerides, leptin and insulin levels in 3-week-old male offspring [157]. Interestingly, these changes occurred even though these dams that were exposed to dietary 'restriction' remained heavier than their control counterparts at conception [157], indicating that in this instance, it is the metabolic response to dietary restriction rather than a lower maternal body weight, which confers metabolic benefits in the offspring. Studies investigating the impact of exercise from before and/or throughout pregnancy in rodents have found that exercise is able to ablate the increase in plasma leptin and triglycerides caused by exposure to maternal obesity [158]. Expression of key genes involved in glucose and lipid metabolism as well as markers of inflammation was also reduced to control levels in skeletal muscle and adipose tissue of offspring from dams that underwent voluntary exercise during pregnancy [159].

7.6.2 The Metabolic and Endocrine Costs of Maternal Dietary Restriction and Weight Loss

While maternal dieting before pregnancy has metabolic benefits, there are also potential metabolic and endocrine costs for the offspring. Studies of people born at the time of the Dutch famine in 1944–1945 have shown that exposure to undernutrition during both early and mid-pregnancy in a population that was previously well nourished was associated with a reduction in glucose tolerance and increased insulin concentration at age 50 and 58 [160]. Furthermore, experimental evidence in sheep has shown that nutritional restriction imposed in ewes with a normal body across both the periconceptional and early gestation periods (from 60 days before until 30 days after conception) has an adverse impact on the glucose–insulin axis of the offspring in postnatal life [161]. This impaired glucose tolerance also persists in the adult offspring [162]. It is not known, however, whether a similar period of

dietary restriction and weight loss imposed on obese ewes will also have consequences for the metabolic health of the offspring.

We have also shown that in most instances dietary restriction and weight loss in the periconceptional period were unable to ablate the effects of maternal obesity on the abundance of insulin signalling proteins in both the liver [129] and skeletal muscle [128] or on signalling molecules involved in hepatic lipid metabolism in the postnatal lamb [130]. Instead, exposure to dietary restriction in either normal-weight or obese ewes resulted in a downregulation of a different subset of insulin signalling proteins within both liver and skeletal muscle compared to offspring exposed to maternal obesity. Furthermore, the reduced abundance of some insulin signalling molecules were conserved both in liver and skeletal muscle of offspring exposed to maternal dietary restriction during the periconceptional period [128, 129]. This suggests that the mechanism involved is one that is able to impact tissues that arise from different cell lineages as the liver and skeletal muscle arise from the endoderm and mesoderm, respectively [163]. This leads us to ask the question of whether the oocyte/early embryo is most sensitive to perturbations during the period encompassing 1 month before and 1 week after conception. Moreover, since the specification of different cell types is regulated by epigenetic mechanisms such as histone or DNA modification, which can modulate chromatin architecture [140], it is possible that changes in the abundance of insulin signalling molecules in liver and skeletal muscle of the offspring are due to the recruitment of epigenetic mechanisms within the developing embryo. Indeed, studies which have investigated the impact of maternal undernutrition during the periconceptional period in sheep have found that changes in the abundance of insulin signalling molecules in liver and skeletal muscle were inversely associated with changes in expression of specific miRNAs in these metabolic organs [164, 165].

It has also been shown in sheep that moderate dietary restriction imposed during the periconceptional period results in an increase in fetal arterial blood pressure and in an earlier activation of the fetal pre-partum cortisol surge [166, 167]. Furthermore, dietary restriction in both normal weight and obese ewes during the periconceptional period has also been shown to result in an enhanced cortisol response to stress in female lambs at 3–4 months of age [132]. Investigation into the possible mechanism(s) underlying this observation found that both male and female lambs from normal-weight ewes that were exposed to dietary restriction during the periconceptional period had a loss of DNA methylation within the differentially methylated region (DMR) of *H19/IGF2* in the adrenal gland [132]. Moreover, these lambs had increased activation of the downstream components of the intra-adrenal renin–angiotensin system through an increase in the abundance of angiotensin-converting enzyme and angiotensin type 1 receptor in the adrenal cortex [168]. Interestingly, Heijmans and colleagues have shown that individuals whose mother was exposed to the Dutch Hunger Winter famine during the periconceptional period had lower methylation of the *IGF2* DMR measured in their blood six decades later [169].

7.7 Summary and Conclusions

There is, therefore, solid evidence that exposure to maternal obesity or to impaired glucose tolerance in utero programs an increased risk of obesity and features of insulin resistance in the offspring, thus potentially fuelling an 'intergenerational cycle' of obesity and insulin resistance. Importantly, it appears that the oocyte and/or early pre-implantation embryo is particularly vulnerable to the effects of maternal obesity, resulting in long-lasting endocrine and metabolic effects for the offspring. Furthermore, the impact of maternal obesity on different insulin-sensitive tissues may be programmed independently rather than as a result of the indirect effects of increased adiposity. Investigations into the molecular mechanisms underlying the programming of obesity and insulin resistance in liver, muscle and adipose tissue have highlighted the role of epigenetic changes within these tissues, which are recruited within the developing embryo and/or fetus (Fig. 7.2).

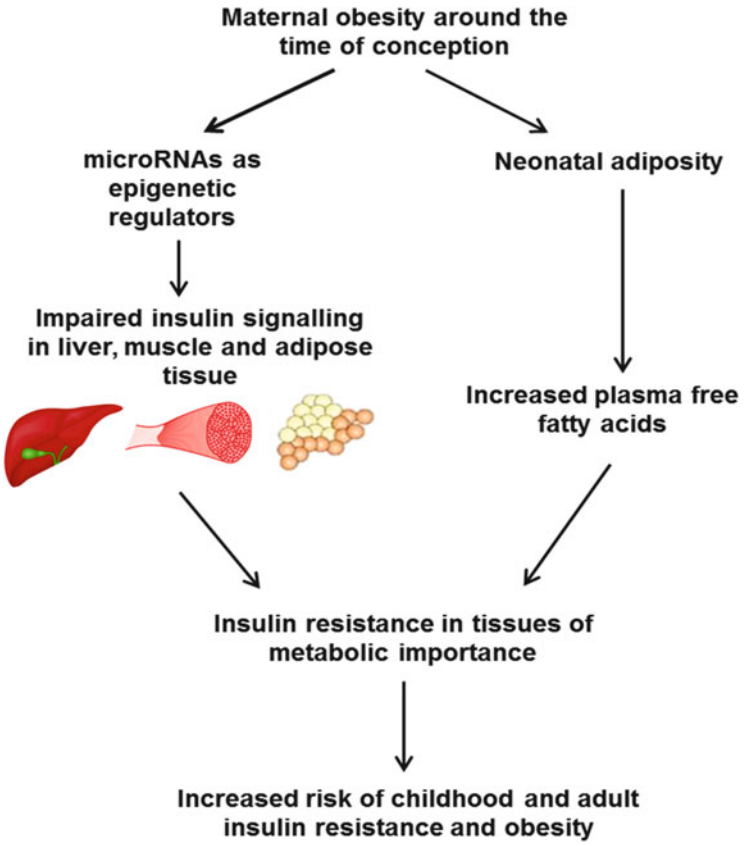

Fig. 7.2 The impact of maternal obesity during the periconceptional on different insulin-sensitive tissues in the offspring may be programmed independently rather than as a result of the indirect effects of increased adiposity. Epigenetic changes within these tissues may be the conduit through which a life of metabolic vulnerability is programmed in tissues of metabolic importance

Finally, it is clear that weight loss achieved through dietary restriction in overweight/obese women prior to and around the time of conception may not be the optimal intervention to break the 'intergenerational cycle' of obesity and insulin resistance. Furthermore, the longer-term effects of maternal exercise in overweight/obese mothers during the pre-conception period on the offspring remain to be determined. These findings highlight the need for a better evidence base for the development of dietary interventions in obese women before pregnancy which maximise the metabolic benefits and minimise the metabolic costs for the offspring.

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Chapter 8
Mechanisms Linking Maternal Obesity to Offspring Metabolic Health

Laura Dearden and Susan E. Ozanne

Abstract A wealth of animal and human studies demonstrate that perinatal exposure to maternal obesity results in predisposition of offspring to develop metabolic diseases later in life. This process is a contributing factor to the exponential rise in obesity rates. Metabolic disease in offspring exposed to maternal obesity is associated with disruption of a number of organ systems including the heart, liver, and endocrine pancreas as well as the central nervous system (CNS). These disruptions are mediated through structural and gene regulatory changes, and although the precise molecular mechanisms underpinning these modifications remain uncharacterized, they are likely to involve alterations to offspring epigenetic marks. This chapter summarizes our current knowledge of how maternal obesity programs offspring metabolic health and explores the mechanisms that could mediate these effects.

Keywords Maternal obesity • Developmental programming • Glucose homeostasis • Central nervous system • Cardiovascular system • Aging • Epigenetics • Metabolic hormone

8.1 Introduction

In recent decades, worldwide obesity levels have increased exponentially. Obesity is no longer just a health problem but represents an astronomical financial burden for society. It has been estimated that over the next 20 years, obesity-related costs will account for around 16 % of health spending in developed countries [1], and it was revealed recently that the cost of treating diabetes in England already accounts for 10 % of all prescribing costs [2]. While several genetic polymorphisms linked to obesity have been discovered [3], these are few, only account for small increases in body weight, and explain less than 5 % of the heritability of the condition. In recent

L. Dearden (✉) • S.E. Ozanne
Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Box 289, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK
e-mail: ld454@medschl.cam.ac.uk; seo10@cam.ac.uk

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years, the importance of the early-life environment in shaping later life disease risk—including susceptibility to develop obesity—has been established.

An interaction between the early-life environment and later life metabolic disease risk was first proposed in the seminal papers by Hales and Barker, who reported an association with low birth weight (as a proxy for restricted fetal growth) and cardiometabolic disease in adulthood [4, 5]. Further studies examining individuals who were in utero during the Dutch Hunger Winter, a famine in the Netherlands during the Second World War, confirmed the association between in utero undernutrition and the development of metabolic disease [6]. As well as the detrimental effects of exposure to undernutrition in utero, there is now a wealth of evidence that demonstrates early-life exposure to overnutrition—for instance, in cases of maternal obesity—is also associated with increased metabolic disease. Comparative studies of siblings born before and after the mother underwent gastric bypass surgery have revealed that the children born after the mother had lost weight had great improvements in insulin sensitivity, reduced adiposity, and reduced blood pressure compared to their siblings [7].

8.1.1 Birth Weight and Overnutrition During the First Weeks of Postnatal Life

Since the initial observations by Hales and Barker, it has been confirmed by numerous other studies that individuals born small for gestation age (SGA) show an increased incidence of metabolic disease later in life. Interestingly, recent studies have demonstrated a U-shaped relationship between birth weight and adolescent adiposity [8], showing that being born large for gestational age (LGA) is also associated with metabolic disease. Rapid postnatal catch-up growth after SGA birth appears to exaggerate the effect of suboptimal growth in utero on risk of metabolic and cardiovascular diseases later in life [9]. In addition, there is evidence that accelerated early postnatal growth, independent of growth in utero, is associated with increased obesity [10]. However, these associations are dependent on the socioeconomic environment that the child grows up in [11], and so results from cohorts in different countries must be interpreted independently.

8.1.2 The Use of Animal Models in the Field of Developmental Programming

While it is primarily desirable to examine results from human cohorts in relation to any health issue, for ethical and practical reasons this is often not possible. The early age of sexual maturity and shorter gestation periods of rodents have made mouse and rat models extremely popular within the developmental field.

Furthermore, important and highly translatable research has been conducted in nonhuman primate (NHP) models as well as other large animal models such as sheep. Within the developmental programming field, NHP, ovine, and rodent models of maternal obesity have produced phenotypes in offspring remarkably similar to human observations.

The use of animal models has enabled researchers to address questions that it simply wouldn't be possible to investigate in human subjects. For example, the question of whether maternal diet or maternal adiposity is more important in determining offspring outcomes cannot be conclusively answered in human studies due to the inaccuracy of food intake questionnaires and shared dietary habits within a family household. In contrast, animal models allow researchers to strictly control both maternal and offspring diet, as well as the genotype of the mother and offspring. Furthermore, animal models give the option to examine at a molecular level organs such as the brain that require a terminal end point and for obvious reasons are not possible in humans. Some recent progress has been made in identifying biomarkers in human blood that could be indicative of exposure to an adverse early-life event (see Sect. 8.3.1.4). However, we are a long way from these biomarkers being effectively used in human health care and diagnostics, and therefore extensive research in this field is still required.

Genetically modified rodents are invaluable in elucidating the molecular mechanisms underpinning phenotypes in offspring that have been exposed to an adverse perinatal environment. For example, Vogt et al. have recently utilized genetically modified mice which lack the insulin receptor specifically in pro-opiomelanocortin (POMC) neurons in the hypothalamus, to demonstrate that insulin signaling mediates the disruption in these neuronal projections in offspring exposed to maternal overnutrition [12]. The use of other genetically modified rodent models allowing cell-specific deletion or overexpression of specific proteins will undoubtedly further our understanding of the cellular events mediating the detrimental effects of exposure to maternal obesity.

8.2 Alterations to Organ Structure and Function

8.2.1 Cardiovascular System

Cardiovascular dysfunction and metabolic disease are intrinsically linked. Cardiovascular disease is one of the most serious comorbidities associated with obesity and causes a significant social and financial burden. Evidence from the Helsinki birth cohort has demonstrated significant associations between both gestational weight gain (GWG) and offspring birth weight with enlarged ventricular mass, as well as an association between maternal obesity and offspring cardiovascular disease [13, 14]. Causative associations between maternal nutrition and offspring cardiovascular function have been demonstrated in animal models showing striking

evidence of cardiac structural and functional changes independent of offspring body weight.

8.2.1.1 Cardiac and Renal Structure

In rodent models, maternal obesity is associated with cardiac hypertrophy and increased left ventricular thickness in offspring [15–17]. Furthermore, sheep fetal offspring exposed to maternal obesity and/or overnutrition display increased left ventricular thickness, cardiac hypertrophy, and increased heart weight, all of which are indicative of reduced cardiac function [18–20].

In humans, there is a U-shaped relationship between birth weight and chronic kidney disease [21, 22], suggesting an interaction between the early-life nutritional environment and kidney function. Supporting this theory, recent studies have also demonstrated a positive association between formula feeding of babies and kidney mass in individuals as adults [23, 24]. Similarly, it has been shown in a rodent model of neonatal overnutrition that offspring display morphological changes in the kidney indicative of reduced renal function [25, 26].

8.2.1.2 Hypertension

It has recently been demonstrated that hyperleptinemia is instrumental in mediating the development of obesity-associated hypertension [27]. This is of particular concern as leptin is elevated in mothers in obese pregnancies, and maternal and fetal leptin levels are directly correlated [28, 29]. A shared phenotype in experimental models of both maternal hyperleptinemia and maternal obesity is offspring hypertension [30, 31]. Furthermore, offspring hyperleptinemia during the perinatal period (induced by exogenous administration of leptin) results in the development of hypertension [32, 33]. There is emerging evidence that the development of hypertension in offspring is due to increased sympathetic tone [16, 34, 35]. Recent results from Samuelsson et al. suggest that increased sympathetic tone is due to altered melanocortin signaling in the central nervous system (CNS) of offspring [36]; given the other reports of developmental programming of the hypothalamus (see Sect. 8.2.4.1), this is certainly an important avenue for further investigation.

8.2.2 Liver and Endocrine Pancreas

The liver is essential to maintaining energy homeostasis due to its vital roles in maintaining both glucose and lipid homeostasis. A common offspring phenotype reported in rodent, sheep, and NHP models of maternal obesity is ectopic fat storage in the liver, resulting in nonalcoholic fatty liver disease [37–39]. This negatively

impacts on hepatic function, and therefore glucose homeostasis, resulting in insulin resistance in offspring [40].

Another vital organ in maintaining glucose homeostasis is the endocrine pancreas. Situated within the endocrine pancreas, β-cells are the only cells in the body that can produce insulin. These highly important β-cells can be damaged by chronic hyperglycemia, resulting in less endogenous insulin production and the need for exogenous insulin (as in cases of poorly controlled T2DM). In NHP, both maternal obesity and overnutrition result in impaired vascularization of offspring pancreas and increased markers of pancreatic inflammation and insulin resistance in peripheral tissues [41, 42]. Rodent and sheep models of offspring exposure to maternal obesity have also reported signs of altered pancreatic structure such as altered β-cell number and volume [43, 44]. Recent evidence from a rodent model of maternal obesity showed that impaired pancreatic development and function is stably transmitted to later generations [45].

There is also evidence that in addition to altered structure of the pancreas and liver, exposure to maternal obesity can alter the innervation of these organs by the CNS. In both NHP and rodents, it has been shown that in offspring exposed to maternal overnutrition, there is decreased central innervation of the liver and pancreas, respectively [12, 46].

8.2.3 Adipose Tissue

The amount, type, and distribution of adipose tissue has a substantial impact on metabolic and long-term health independent of body weight [47]. It is now accepted that adipose tissue plays a pivotal role in maintaining whole-body insulin sensitivity by engaging in insulin-dependent glucose uptake and influencing the sensitivity of other tissues to insulin by releasing free fatty acids and adipokines such as leptin and adiponectin.

Fetal sheep exposed to maternal obesity have increased peri-renal brown adipose tissue mass [48]. Adult offspring from the same model display increased adiposity and altered expression of adipose nutrient transporters [49]. Rodent models have consistently reported increased adiposity in the offspring of obese mothers, often due to adipocyte hypertrophy [31]. Exposure to maternal overnutrition during the perinatal period can also alter the distribution of fat between the various peripheral depots. Volpato et al. have reported an increase in epididymal and inguinal fat stores at the expense of subcutaneous adipose depots [50]. This is significant as the distribution of fat depots has an influence on metabolic health independent of total adiposity levels.

8.2.4 CNS

Increased weight gain in offspring exposed to maternal obesity is often preceded by hyperphagia, implicating altered central regulation of energy homeostasis as an underlying cause of metabolic phenotypes. Central control of energy homeostasis can be broadly divided into two areas: homeostatic control of energy homeostasis originating in the hypothalamus and reward-related feeding and behavior orchestrated through the mesolimbic pathways.

8.2.4.1 Homeostatic Feeding Pathways

Over the past two decades, the importance of the hypothalamus within the brain in regulating whole-body energy homeostasis has become increasingly clear. Neurons expressing the orexigenic Neuropeptide Y (NPY) and anorexigenic POMC situated within the arcuate nucleus (ARC) of the hypothalamus are instrumental in sensing changing nutrient status in the rest of the body. These NPY and POMC neurons project to other regions of the hypothalamus including the paraventricular nucleus (PVH) and the brain stem in order to mediate downstream physiological effects to maintain energy homeostasis.

Pioneering work by the Bouret laboratory and others has shown that development of the hypothalamus is plastic and sensitive to metabolic signals in the perinatal period [51]. Evolutionarily, the requirement for metabolic hormones in hypothalamic development enables the hypothalamus to develop in line with the nutritional state of the ex utero environment. However, it also leaves hypothalamic development extremely vulnerable to disruption in instances where metabolic and fetal hormone levels are altered, for example, as a consequence of maternal obesity.

Rodent studies have demonstrated that the offspring of obese mothers display a reduced number of axonal projections between the ARC and PVH [52], as well as a reduction of projections between the ARC and dorso-medial and lateral hypothalamus [12]. This programming of ARC projections occurs even when offspring exposure to maternal obesity is limited to the suckling period, which corresponds with the reported timing of development of these projections. This suggests that the disrupted circuitry reflects a disruption of axonal projections, rather than a cellular defect. These changes are thought to be mediated through altered neuronal insulin and leptin signaling, highlighting the importance of both maternal and fetal metabolic hormone levels during the perinatal period (see Sect. 8.5.1).

8.2.4.2 Reward-Related Feeding

Maternal obesity can also influence offspring feeding behavior and alter dietary preferences. In rodents, maternal obesity has been reported to increase the preference for fatty and sugary food in offspring, leading to obesity [53–55]. This is

particularly relevant when considering the increased availability of highly palatable fat and sugar-rich foods in modern society. The offspring of obese mothers also display increased frequency of feeding episodes and a longer duration of feeding during a given episode [56]. Interestingly, it has also been reported that the offspring of obese mothers display alterations to reward systems in the brain that could explain the frequently reported hyperphagia. Several studies have reported programming of the mesolimbic reward system in offspring, resulting in altered activation in response to diverse stimuli including feeding, and reduced anticipatory responses for food rewards [54, 57, 58].

8.3 Changes to Gene Expression

Changes in the transcriptome and/or proteome of all major organ systems have been reported in the offspring of obese mothers, across a range of species. These include alterations in peripheral organs including heart [59], adipose tissue [60], kidneys [61], and the liver [62]. In the CNS, changes in the expression of genes involved in both energy homeostasis and reward-related feeding have been demonstrated in the hypothalamus and mesolimbic pathways, respectively [63–65].

8.3.1 Epigenetics

The stable nature of phenotypes throughout the lifetime of the exposed offspring, and the recently reported intergenerational transmission of programming effects, suggests permanent changes in gene expression in the exposed individuals. Epigenetic regulation represents a stable but modifiable level of genomic regulation; the term epigenetics literally means “on top of genetics” and refers to a system of processes that induce heritable changes in gene expression without altering the genomic sequence. In utero regulation of epigenetic machinery has recently received a lot of interest as a potential mechanism for causing permanent, heritable changes to gene expression.

There is emerging evidence from human cohorts of the importance of changes to the epigenome. In a recent study of siblings born before and after maternal gastric bypass surgery, significant differences in the methylation of glucoregulatory genes were observed in blood samples [7]. In a different study, maternal glycemic level was shown to contribute to the methylation state of a specific site near the leptin gene, which was in turn associated with cord blood leptin levels [66]. A recent report from the ALSPAC team identified four loci at which offspring methylation state is correlated with maternal GWG [67]; however, this association failed to validate in larger cohorts leading to doubt over the strength of the association [68].

It is worth noting that the (in)heritability of the epigenome can be context dependent (i.e., altered epigenetic markers that are permanent and inheritable) or

germ line dependent (i.e., altered epigenetic markers in offspring gametes that will produce the next generation). Therefore, epigenetic markers must be identified in the F2 and subsequent generations in order to identify truly heritable changes to the epigenome. Additionally, changes to epigenetic marks of functionally relevant genes must be present prior to the development of a phenotype in order to prove causality. This is why animal models of maternal programming are particularly important, as they allow access to vital organs during early life and before the development of metabolic phenotypes.

8.3.1.1 Histone Modifications

The DNA in cells is stored as chromatin. The basic unit of chromatin is a nucleosome, which consists of roughly 147 bp of DNA wrapped around a core histone octamer made up of two copies each of the H2A, H2B, H3, and H4 proteins. This organization leaves the N-terminal tails of histones accessible to modifications including methylation, acetylation, and phosphorylation [69]. Histone acetylation is associated with an active euchromatin state, whereas histone methylation can confer activation or inactivation of associated chromatin, depending on which component of the histone octamer and which particular lysine of that protein is modified [69].

The Histone Acetyl Transferase (HAT) family performs histone acetylation, associated with transcription, whereas the Histone De-acetylase (HDAC) family of proteins performs histone de-acetylation that is inhibitory to transcription. In an NHP model, fetal offspring exposed to maternal overnutrition display reduced HDAC activity, which is associated with hyperacetylation at H3K14 in the liver [70]. Unfortunately, due to the difficulty of analyzing the histone code (which is more technically challenging compared to analyzing for example DNA methylation state), data on histone modifications resulting from exposure to maternal obesity are sparse. However, sheep offspring exposed to IUGR display increased H3K9Ac and decreased H3K27Me3 modifications associated with the POMC promoter. These changes are observed specifically in the hypothalamus, although they are not associated with a corresponding change in *Pomc* mRNA [71]. Although these histone modifications occur in response to offspring exposure to maternal undernutrition—rather than obesity—they demonstrate the dynamic nature of the histone code in relation to the early-life environment.

8.3.1.2 DNA Methylation

DNA methylation is an essential component of normal genomic regulation. Methylation at the 5′ position of a Cytosine base within a CpG dinucleotide is a stable epigenetic mark that can be transferred between generations during mitosis. CpG dinucleotides are randomly distributed throughout the genome, but are particularly frequent near the 5′ promoter regions of genes. Areas with a high frequency of CpG

dinucleotides are termed CpG islands. Whereas CpG dinucleotides are usually methylated, Cytosine residues within CpG dinucleotides in CpG islands are usually un-methylated. A high percentage of CpG methylation is associated with transcriptional silencing of nearby genes, whereas CpG island hypomethylation is associated with transcriptional activation. This is in part due to the fact that the attachment of methyl groups can directly inhibit the interaction between DNA and transcriptional machinery, for example, by attaching to cytosine residues within a transcriptional response element and thus repressing transcription [72, 73]. Furthermore, promoter methylation can also cause recruitment of other proteins (for example, Methyl Binding Domain proteins), which facilitate binding of histone-modifying complexes that subsequently alter chromatin activation state as discussed above [74].

Within normal genomic regulation, DNA methylation is particularly important for the silencing of imprinted genes and the X chromosome during development. The regulation of imprinted genes is also subject to programming by the early-life environment, as demonstrated in a rodent model of hyperglycemia in which reduced expression of the imprinted genes *Igf1* and *H19* in pancreatic islets is attributed to hypermethylation of the promoter regions [75].

DNA methylation patterns are established during the early stages of development, and this time is therefore a critical period during which methylation patterns are vulnerable to change. During the preimplantation stage, the embryonic genome is subjected to widespread demethylation, and then de novo methylation occurs at specific regions to generate a pattern of methylation that is inherited by daughter cells [76]. As the DNA methylation pattern is essentially maintained throughout life, changes to the activity of methyl transferases during these critical periods of development can cause lasting changes to gene regulation.

Tissue-specific expression and relative levels of several hormones—including insulin, leptin, and adiponectin—are regulated by the methylation state of promoter regions, making these genes susceptible to altered expression and abundance. Furthermore, Masuyama et al. have recently demonstrated that the methylation state of the leptin and adiponectin genes can be inherited [77]. Neonatal overnutrition causes hypermethylation of the POMC promoter in the hypothalamus specifically at CpG dinucleotides within a transcription factor binding site, resulting in a lack of *Pomc* mRNA regulation in response to leptin or insulin [78]. Similarly, offspring exposed to maternal obesity in utero display hypermethylation of a region upstream of the POMC gene, which corresponds with decreased *Pomc* expression and increased body weight [79].

8.3.1.3 MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNAs (between 22 and 25 nucleotides in length) that are able to post-transcriptionally modify gene expression. miRNAs bind to the 3′ untranslated region of mRNA transcripts and therein either interact with the DICER complex to target the bound transcript for degradation or inhibit translation of the transcript by physically inhibiting the binding of translational

machinery. Interestingly, miRNA expression can be regulated both dependently and independently of the host gene in which they reside, making their expression highly dynamic.

To date, only a few observations of altered miRNA expression in tissues of offspring exposed to maternal obesity have been published. For example, mir133 is increased in the heart of offspring in a murine model [16], and NHP exposure to maternal obesity results in increased expression of miRNAs associated with cardiovascular disease [80]. Furthermore, in a sheep model of maternal obesity the fetuses displayed altered levels of mir-29b, -103, and -107 in the liver [62]. The only current evidence for miRNA activity mediating maternal obesity-induced changes in gene expression comes from a mouse model in which the levels of mir126 are elevated in the epididymal fat of offspring [81]. As mir126 is a regulator of *Irs1*, this increased mir126 activity could explain the decreased expression of *Irs1* that is also observed in these offspring. Importantly, the effects of maternal obesity on mir126 and *Irs1* expression are cell autonomous and are maintained in vitro when pre-adipocytes are differentiated in culture [81].

8.3.1.4 Epigenetic Markers in Blood for Human Diagnostics

A current challenge for studies of human epigenetic marks is that they are limited to easily accessible biological samples, most commonly blood. Researchers therefore need to identify epigenetic marks that are uniform throughout the whole organism, despite the fact that they may only confer a functional role in specific (inaccessible) tissues. Metastable epialleles are regions of the genome at which DNA methylation is established stochastically in the early embryo and then maintained in differentiated tissues. Focusing on metastable epialleles allows researchers to work around limitations in sample collection from human subjects. Recently, Dominguez-Salas and colleagues have shown persistent changes in DNA methylation in offspring born to mothers either during the rainy season or the dry season in the Gambia [82]. Pregnancies during these two seasons vary greatly in maternal nutrient intake, making this an interesting model of changes to maternal diet. Candidate methylation analysis of blood and hair samples (selected as mesodermal and ectodermal tissues, respectively) from the children of these mothers demonstrated increased methylation of six metastable alleles in individuals who were born in the rainy season.

A recent study by Sharp et al. of the ALSPAC cohort has provided the first evidence that the influence of maternal obesity on offspring metabolic health may be mediated via altered DNA methylation. This study is the first to examine DNA methylation levels at three time points: in neonatal cord blood and later in peripheral blood at 7.5 and 17 years of age. The authors identified several CpG sites that were differentially methylated in blood of offspring exposed to maternal obesity and associated with offspring adiposity. Replication of these results in larger cohorts will identify molecular pathways underpinning maternal programming of

offspring health and potentially lead to the identification of novel epigenetic markers that can be used as a stable indicator of exposure to an early-life insult.

8.4 Aging

As humans undergo the natural aging process, they display increased body weight, a shift in adipose distribution, and deteriorating function of organs such as the heart, kidney, and reproductive system. Many of these natural aging processes recapitulate phenotypes observed in offspring exposed to adverse perinatal nutritional environments. Indeed, a study by Reynolds et al. has shown that the children of obese mothers have a decreased life expectancy due primarily to cardiac dysfunction [83]. This has led researchers to consider whether accelerated aging is one of the primary molecular mechanisms underpinning the changes in health after exposure to an adverse early-life environment. Indeed, macronutrient restriction and undernutrition causes accelerated cellular aging in offspring pancreatic islets, and markers of accelerated aging in the liver [84, 85].

Telomeres are guanine-rich nucleotide sequences present at the ends of chromosomes that prevent chromosomal deterioration. An essential part of the aging process in telomerase-negative somatic cells is telomere shortening that occurs after each cell division. More recently, telomeres have also been shown to shorten in response to oxidative stress [86, 87]. When telomeres become critically short in length, they undergo a conformational change which results in them representing double-stranded breaks, causing the cell to enter growth arrest and senesce or become apoptotic [88]. Differences in telomere length have been implicated in developmental programming in response to maternal nutritional state. Low birth weight offspring of protein-restricted mothers cross-fostered to control dams to enable rapid recuperation during the postnatal period have reduced longevity accompanied by accelerated telomere shortening in several tissues [85, 89, 90]. While there is no evidence yet of accelerated telomere shortening in response to maternal overnutrition, the shared commonality in cellular responses to both ends of the nutritional spectrum predicts that this process is also likely to occur with exposure to maternal obesity.

8.5 Maternal Factors

In order to develop effective intervention strategies and healthcare guidelines, it is necessary to elucidate the mechanisms by which the maternal nutritional and endocrine state is transmitted to and/or sensed by offspring during early life. Put simply, what is the "programming factor" that we need to target in order to prevent the early-life programming of metabolic disease risk? Traditionally, research has focused on maternal hormone and nutrient levels, particularly those that are able to

cross or modulate function of the placenta. However, emerging evidence highlights a role for novel mechanisms by which the maternal environment influences offspring early development. For example, recent research has shown that maternal stress during pregnancy alters the vaginal microbiome, and exposure to this altered microbiome programs development of offspring brain and gut [91].

8.5.1 Metabolic Hormones

A host of metabolic hormones are altered in the obese mother during pregnancy and consequently in the developing fetus. Elevated levels of many of these hormones have been implicated in mediating the effects of the perinatal environment on offspring development (Fig. 8.1).

Maternal leptin levels are elevated in obese pregnancies, and although there is some debate, it is generally accepted that leptin can cross the placenta. As discussed earlier, high leptin levels are implicated in the development of hypertension in both obese individuals and offspring exposed to maternal obesity [27, 34]. Furthermore, the correct regulation of leptin levels in the perinatal period is essential for correct

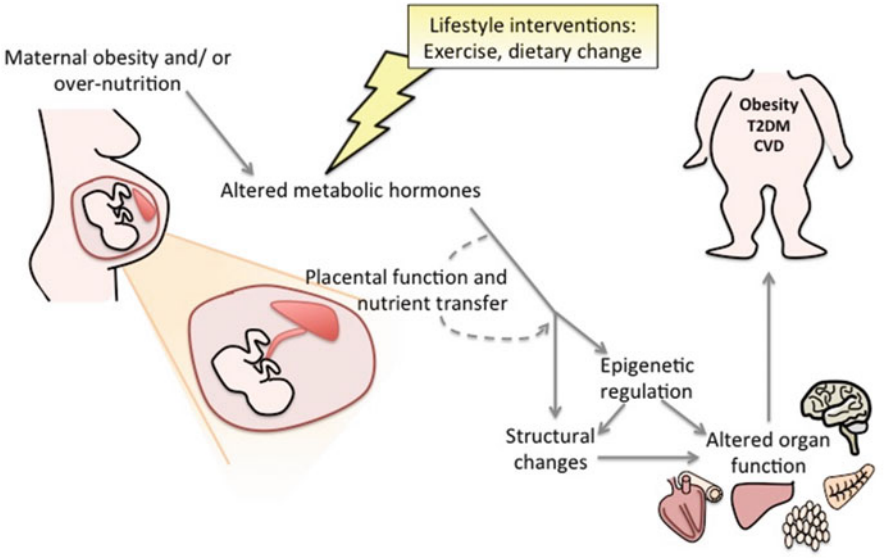

Fig. 8.1 Maternal obesity and/or overnutrition is associated with altered levels of metabolic hormones. These hormones are able to modulate placental function and nutrient transfer, and some can also act on the fetus directly to modulate epigenetic machinery and cause structural changes in organs. These processes ultimately result in a change in organ function and the development of obesity later in life. Lifestyle interventions that increase the mother's metabolic fitness are a promising intervention to inhibit the effects of maternal obesity on offspring metabolic health

development of the hypothalamus, and thus altered leptin levels during this period can have long-term detrimental effects on the ability to maintain energy homeostasis [51]. It is of note, however, that in a rodent model of leptin deficiency, maternal protein restriction is still associated with adverse metabolic outcomes in offspring [92], and therefore leptin cannot be solely responsible for all offspring phenotypes observed in response to an adverse nutritional environment.

Hyperglycemia and insulin resistance, leading also to elevated circulating insulin levels, usually accompany maternal obesity. While insulin can only cross the placenta in limited amounts, maternal hyperinsulinemia is usually accompanied by hyperglycemia, which in turn can induce elevated levels of both fetal insulin and glucose. Increased fetal glucose levels cause increased insulin and IGF signaling and subsequently fetal growth [93, 94]. Furthermore, the activity of members of the DNMT family has been shown to be altered by changing glucose concentrations [95]. As demethylation followed by de novo methylation is an essential process during early embryogenesis, changes to the activity of the epigenetic machinery caused by the in utero nutritional state would cause significant long-term effects on offspring epigenetic regulation. Like leptin, insulin also has a significant role in hypothalamic development [12, 96], and fetal hyperinsulinemia as a response to maternal hyperglycemia will therefore have significant effects on the development of hypothalamic energy homeostasis pathways.

Maternal obesity can alter the macronutrient and hormonal composition of milk, and this is therefore a likely contributing factor to changes in offspring development during the early postnatal period. In particular, elevated leptin content in milk has been reported in both obese human and rodent mothers [38, 97]. Furthermore, cross-fostering of control offspring to a GDM dam during the lactation period has been shown to cause perturbations to the development of hypothalamic energy balance circuitry, suggesting consumption of milk from a diabetic mother can cause long-term changes to body weight and food intake in offspring [98].

8.5.2 The Placenta

An obvious place to look for maternal influence on offspring development is at the maternal–fetal interface, or more specifically at the placenta and at the hormones and nutrients that are able to act on or pass through the placental barrier to the developing fetus. The placenta acts as an important nutrient sensor during pregnancy and can respond to external stimuli to dynamically regulate the transfer of nutrients, including glucose, to the developing fetus [99]. As glucose is the primary fuel source for the developing fetus, the placenta plays an important role in ensuring that fetal circulating glucose levels are maintained within a physiological window to avoid excess or impaired fetal growth.

It is becoming increasingly apparent that nutrient transfer across the placenta can be altered as a consequence of the metabolic state of the mother during pregnancy.

Fetal glucose uptake via the placenta is dependent on the metabolic status of the mother [100]. Numerous murine models of maternal obesity and gestational hyperglycemia have demonstrated increased placental nutrient transfer to the fetus in utero, resulting in increased birth weight [101, 102]. There is also considerable evidence from human studies that maternal hyperglycemia and GDM can alter placental function [103]. NHP models of obesity have shown significant damage to the placenta caused by maternal overnutrition [104]. These changes are independent of maternal obesity but are exacerbated in pregnancies complicated by maternal obesity and insulin resistance. The general clinical accessibility of the placenta as a whole tissue after birth makes it possible to investigate structural and functional changes that occur during obese human pregnancies, and this active area of future research will undoubtedly increase our understanding of the placental origins of developmental programming.

8.6 Interaction Between Genes and the Environment

It is now largely accepted that the polygenic nature of obesity means that susceptibility to develop cardiometabolic diseases is due to a complex interaction between genetic susceptibility and environmental exposures during early life. Perhaps most convincing is a recent study by Rosenquist et al., which demonstrates that the well-studied Fat mass and Obesity-associated (FTO) polymorphism only has a significant association with BMI in individuals born after 1942 [105]. Furthermore, in the Dutch Hunger Winter cohort, there is a significant interaction between a polymorphism in the Peroxisome Proliferator-Activated Receptor γ2 (PPARγ2) gene and famine exposure on glucose and insulin metabolism; the mutant allele is associated with impaired glucose tolerance and T2DM as an adult only if offspring were also exposed to the famine specifically during mid-gestation [106].

The Avon Longitudinal Study of Parents and Children study has also revealed complex gene and environment interactions. The insulin gene variable number of tandem repeats (INS VNTR) is associated with adult obesity and T2DM, and the ALSPAC study revealed that there is an interaction between INS VNTR genotype and postnatal weight gain in relation to adolescent BMI [107]. Other studies have revealed interactions between genetic risk alleles and diet [108, 109] in determining childhood adiposity. However, these studies have focused on offspring diet, rather than maternal diet. Therefore, although there is evidence that the environment and genetic factors interact, this needs to be further explored in both human and animal models where the maternal nutritional state is taken into account.

8.7 Paternal Influences

The predominantly maternal influence that has been noted in most studies of metabolic disease transmission suggests the in utero environment has an effect independent of genetic heritability. However, although the maternal environment undoubtedly exerts a strong influence on fetal development, the paternal environment can in theory exert an independent effect on fetal development through gamete transmission.

There are conflicting reports on the influence of the father's metabolic state during early life and at conception on offspring metabolic disease. Offspring of two overweight parents have an increased risk of childhood obesity compared to offspring with just one obese parent [110, 111]. However, when examining the individual influence of maternal and paternal obesity, the mother–child association is consistently stronger than father–child in relation to offspring BMI [111]. It is therefore important to remember that sex-specific inheritance of X chromosome linked genes and mitochondrial DNA from the mother must also be considered as these confer increased maternal influence in heritability. Interestingly, a recent study in China has suggested that the effects of paternal BMI on fetal growth are sex specific; a positive association between paternal BMI and intrauterine growth was reported in male but not female offspring [112]. A transgenerational link has also been proposed between the paternal grandfathers nutrition during adolescence and incidence of obesity and cardiovascular disease in later generations [113, 114].

Although these few studies suggest that paternal metabolic state around conception may be associated with offspring metabolic disease risk, there is a lack of compelling evidence in humans that this is due to true programming of offspring metabolic regulation, rather than a shared family lifestyle and genetic inheritance. In animal models, however, there is evidence that combined parental obesity has a greater detrimental effect on oocyte implantation and early fetal development than maternal obesity alone [115]. In drosophila, paternal consumption of a high sucrose diet is sufficient to program alterations to fat storage in subsequent generations of offspring [116]. Furthermore, the daughters of obese male mice display disrupted pancreatic function and transcriptional changes in adipose tissue [117, 118]. Given the exponentially increased incidence of obesity in both men and women of reproductive age, it is imperative that the precise influence of paternal metabolic state on offspring metabolic health is defined in order to inform health guidelines.

8.8 Intervention Studies

The use of intervention studies (particularly in animal models) gives us an unrivaled insight into the mechanisms mediating changes in the nutritional environment on offspring health. In the first instance, simple lifestyle or behavioral changes are of primary preference as they are more likely to be adopted by mothers than

pharmaceutical regimes that may have side effects (Fig. 8.1). Also, historic cases such as the devastating effects of thalidomide use during pregnancy have made many people wary of taking medications during pregnancy.

A behavioral intervention model that is being trialed simultaneously in both humans and animal models is the encouragement of maternal exercise during obese pregnancies. Exercise is extremely effective at improving insulin sensitivity and therefore glucose homeostasis, even independently of decreased adiposity [119, 120], and is therefore a viable option for improving the mothers "metabolic fitness" (i.e., glucose and insulin sensitivity) independent of her weight. The UK Pregnancies Better Eating and Activity Trial (UBPEAT) recruited a large cohort of obese pregnant women and encouraged them to partake in a mild exercise regimen alongside weekly meetings with health trainers. The initial results from the UPBEAT trial have shown that although the behavioral intervention was not adequate to reduce the incidence of LGA births, mothers in the intervention group had decreased GWG and skin fold thickness [121]. As both GWG and maternal adiposity have been associated with metabolic parameters in adolescent offspring, close follow-up of this cohort will establish whether the improvement of these maternal parameters is also beneficial to offspring. Indeed, in rodent models both maternal exercise during pregnancy and offspring exercise during the early postnatal period are sufficient to augment the detrimental effects of maternal obesity on offspring metabolic health [122, 123].

Studies conducted in NHPs have suggested that control of maternal diet during pregnancy (even if the mother remains obese) is extremely effective in ameliorating offspring phenotypes. These studies have utilized naturally occurring diet-resistant females who remain lean despite consumption of a HFD to demonstrate that exposure to maternal overnutrition alone (without maternal obesity) causes changes in offspring liver function [37, 63]. Furthermore, switching the diet of NHP obese females immediately prior to pregnancy reverses the alterations observed in offspring hypothalamic feeding pathways—despite the mothers remaining obese—suggesting that this phenotype is mediated by maternal diet alone [63]. These studies therefore suggest that changing the maternal diet to a healthier diet before pregnancy is sufficient to ameliorate the transmission of detrimental phenotypes to offspring. In a human study of dietary intervention, gestational diabetic mothers following a strict calorie-controlled diet have a reduced incidence of LGA births and less birth complications compared to those on a diet of their own choice [124]. Unfortunately, however, it is not clear from this study whether the beneficial effects on offspring are as a result of the mother's dietary change alone, or from the beneficial effect of a healthier maternal diet on diabetes management.

8.9 Conclusions

Extensive evidence from animal models and human studies demonstrates that early-life exposure to maternal obesity increases offspring susceptibility to develop metabolic disease later in life. While the molecular mechanisms remain largely uncharacterized, evidence suggests that altered levels of metabolic hormones in both the mother and fetus cause significant changes to organ development, which may be caused by changes in gene regulation due to altered activity of epigenetic machinery. Disrupted organ development can cause decreased function later in life, resulting in the inability to maintain metabolic homeostasis and the development of obesity. The mother's metabolic health can be improved by lifestyle interventions such as dietary changes and exercise, and therefore represents a tractable target for intervention. Future research using intervention studies conducted in parallel in human and animal models will help elucidate the precise molecular mechanisms mediating the detrimental effects of maternal obesity on offspring metabolic health.

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Chapter 9
The Effect of Maternal Overnutrition on Reward and Anxiety in Offspring

Aya Sasaki, Suzanne Erb, and Patrick O. McGowan

Abstract Obesity has reached epidemic levels in developed countries. Maternal overnutrition has been linked to a number of poor health outcomes in offspring, including metabolic, cardiovascular and mental disorders, some of which do not become apparent until later in life. In particular, maternal overnutrition is linked to increased risk for hedonic and stress dysfunctions. Previous studies in animal models indicate that maternal overnutrition, typically using a diet high in fat, impacts the function of the mesolimbic pathway, leading to attenuated function of the reward system and decreased dopamine-related behaviour. Also maternal overnutrition affects the function of the hypothalamic–pituitary–adrenal axis, leading to activated stress system and increased anxiety-like behaviour. This chapter focuses on what is known about the effects of maternal intake of high-fat diet on the reward and stress systems in offspring brain and behaviour. We discuss the likely role of epigenetic regulation of these pathways in the long-term changes in brain function associated with the perinatal environment.

Keywords High-fat diet • Overnutrition • Maternal • Dopamine • Glucocorticoid • Stress • Diet-induced obesity • Anxiety • Reward • Epigenetics

A. Sasaki • S. Erb (✉)
Department of Cell and Systems Biology, University of Toronto, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4

Department of Psychology, University of Toronto, Scarborough, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4
e-mail: erb@utsc.utoronto.ca

P.O. McGowan (✉)
Department of Cell and Systems Biology, University of Toronto, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4

Department of Psychology, University of Toronto, Scarborough, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4

Department of Biological Sciences; Department of Physiology; Center for Environmental Epigenetics and Development, University of Toronto, Scarborough, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4
e-mail: patrick.mcgowan@utoronto.ca

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Abbreviations

D1R	Dopamine receptor D1
D2R	Dopamine receptor D2
DAT	Dopamine transporter
GR	Glucocorticoid receptor
HPA	Hypothalamic–pituitary–adrenal
MR	Mineralocorticoid receptor
NAC	Nucleus accumbens
PFC	Prefrontal cortex
TH	Tyrosine hydroxylase
VTA	Ventral tegmental area

9.1 Introduction

Disorders associated with lifestyle choices, such as the overconsumption of energy-rich foods, have reached epidemic levels in developed countries. Type 2 diabetes was once a disease primarily in adults. Increasingly, however, it is presenting in adolescents and even in children, as the incidence of obesity increases in these populations. In fact, childhood obesity has doubled in children and tripled in adolescents in the past 30 years [1], and, accordingly, one in every three American children born in 2000 is likely to be diagnosed with diabetes in their lifetime. In addition to being at higher risk for developing diabetes, obese youth are at greater risk for cardiovascular disease [2] and many other diseases, including psychiatric disorders later in life [3].

Globally, consumption of energy-dense foods high in fat has increased dramatically in the past 30 years, as has the average serving size. Compounding the problem, the higher caloric intake is tending to be accompanied by generally lower rather than higher levels of physical activity, corresponding to generally more sedentary lifestyles. In fact, the USA is ranked 1st in the world for percent of overweight individuals, with more than half of the American population being overweight. Worldwide, 2.3 billion people were overweight in 2010, and this number is predicted to increase.

Overnutrition is common among pregnant women [4], and it is clear that obesity propagates across generations. Thus, maternal obesity may have health consequences not only for the mother but also for her offspring. Postnatal lifestyle is the most immediate cause of obesity. However, in humans, evidence of the influence of maternal diet is found in the association between birth weight and adult obesity and metabolic disease. Likewise, animal studies have shown that maternal nutrition history predicts obesity in adult offspring, independent of postnatal diet [5].

It has been suggested that the transgenerational impact of maternal obesity occurs via metabolic programming [6]. During early critical periods in

development, the organism has the ability to adapt to the environment, and these adaptations are reflected in permanent changes in metabolic processes. The critical developmental time window for this programming is during gestation and lactation, a time when offspring are fed by their mothers and when offspring metabolism and risk for future obesity is particularly sensitive to maternal diet. Indeed, shifts in metabolic programming as a consequence of maternal diet during this period are considered to have at least contributed to the epidemic rise in obesity. Although it is likely that the long-term effects of changes in metabolic programming involve interactions with multiple neural systems (e.g. those related to the rewarding properties of food), the biological mechanisms mediating these long-term effects are largely unknown.

Eating behaviours are regulated by peripheral and central processes that directly or indirectly affect the brain's reward pathways. Palatable or high-fat diet activates dopaminergic pathways within the mesolimbic reward system, implicated in natural reward processes and drug addiction [7, 8]. As we discuss below, maternal high-fat diet alters dopaminergic gene regulation, dopaminergic transmission in the reward pathway and the locomotor-activating effects of amphetamine [9–11]. Moreover, activation of dopaminergic systems interferes with hormones such as leptin that regulate satiety, thereby promoting consumption of palatable food. Maternal overnutrition also affects anxiety behaviour and stress physiology in offspring [12, 13]. These effects of maternal diet are significant in that the motivational processes mediating responses to rewards and stressors are intimately related. For example, drug addiction is considered as a chronic cycle of reward-directed behaviour followed by withdrawal-induced negative affect [14]. It is possible that similar such cycle may operate in the context of natural reward-related behaviours, such as high consumption of palatable food [15].

In this chapter, we will discuss how maternal diet during and after gestation affects behaviour in offspring; furthermore, we will link behavioural outcome to neural mechanism and to the presentation of altered reward- and stress-related phenotype. To date, the hypothalamus, which regulates the homeostasis of energy intake, has provided the main focus for studies aimed at examining the influence of maternal overnutrition on brain function in offspring (see [16, 17]). More recently, however, this work has extended to include a consideration of the extrahypothalamic systems, including midbrain and cortical dopamine systems, and stress-related systems of the hypothalamus and limbic forebrain. It is these systems that will provide the focus in this chapter.

9.2 Studies of the Effects of Maternal Diet on Offspring: Caveats to Consider

Before proceeding with a discussion of the effects of maternal diet on behavioural and neural phenotype in offspring, caveats pertaining to work in the area more generally should be briefly addressed. First, it should be cautioned that studies of maternal overnutrition tend to be variable on a number of critical parameters. The majority of work in the area involves a maternal diet manipulation given during and/or after pregnancy, and most studies use diets that are high in fat. However, studies differ in the proportion and quality of fat (saturated, unsaturated or transfats) in the diet, the carbohydrate content, the use of 'cafeteria' diets in some cases and the timing of exposure to the diet manipulation (e.g., before, during and/or after pregnancy). Here, the focus will be on diets high in saturated fat, the most common fat used to drive overnutrition. Thus, we use the term 'overnutrition' interchangeably with 'high-fat diet', unless otherwise specified.

Second, it is worth noting that although the focus here is parental obesity and offspring behavioural phenotype, the majority of studies examining the behavioural effects of overnutrition are performed using diet-induced obesity models, where the diet is fed continuously in adulthood only. In these diet-induced obesity studies, therefore, it is not always clear whether the effects of the diet result from current diet, diet history or a combination. In the context of this chapter, the diet-induced obesity studies will serve to illustrate instances where the effects of high-fat diet in development diverge from those of chronic high-fat diet in adulthood, as well as where common brain mechanisms appear to be altered by high-fat diet exposure.

9.3 Effects of Maternal Overnutrition on the Offspring Dopamine System

Dopamine circuitry is associated with neural reward mechanisms that can serve to alter animals' preference for energy-dense palatable foods. The regulation of food intake by the central nervous system involves homeostatic mechanisms in the hypothalamus and interactions with the mesolimbic dopamine pathway mediating reward and motivation [18]. Both natural reinforcers (e.g. palatable food such as high-fat diet) and drug reinforcers act on the mesolimbic pathway, which originates in the ventral tegmental area (VTA) and provides dense dopamine innervation of the nucleus accumbens (NAC) and prefrontal cortex (PFC). Activation of this pathway by both natural rewards and drugs of abuse results in increased dopaminergic transmission within the NAC.

In rodents, the development of dopaminergic neurons is not fully established until the second and third weeks of postnatal life, a time period that overlaps with maternal lactation [19]. Thus, it has been considered that the maternal nutritional

environment may alter the function of the mesolimbic pathway to alter behavioural and neural responses in offspring, including overconsumption of a high-fat diet.

9.3.1 Reward-Directed Feeding Behaviour and Psychostimulant-Induced Locomotor Activity

In rodent models, maternal overnutrition increases the preference for palatable foods in offspring. For example, maternal consumption of a palatable high-fat diet 3 months prior to conception, and during gestation and lactation, increases preference for fat and sugar intake in the offspring [9]. Human studies are in support of these findings, indicating that maternal dietary content predicts adiposity in childhood [20] and that child fat intake is associated with prenatal rather than postnatal maternal fat intake [21].

Appetite regulation is largely mediated by hypothalamic regions involved in appetite control and by peripheral factors such as leptin, insulin and ghrelin that regulate energy balance [22]. Offspring exposed to maternal high-fat diet appear to have an increased hunger for fat-rich food that overrides satiety signals that usually maintain the balance between energy intake and expenditure in the body. Clearly, however, feeding is about more than the regulation of energy intake and expenditure; it produces a pleasure state that involves the activation of reward pathways in the brain.

Recent studies have suggested that pre- and postnatal (perinatal) exposure to a diet high in fat increases the preference for high-fat diet and the drive to consume palatable foods in adulthood. Likewise, maternal consumption of a palatable diet increases the preference and consumption of food that is high in fat and sugar, when compared to a micronutrient-balanced control diet [9, 23] or food rich in proteins [10, 24]. Importantly, the increased preference for palatable food by maternal overnutrition appears not to be due to increased appetite per se. When animals are given a control diet instead, they do not show increased appetite (i.e. increased consumption) for the control food [12, 24, 25]. These studies suggest that maternal effects on offspring dietary preferences involve changes in the salience of particular food-related stimuli (i.e. palatable diet), rather than merely an increase in energy intake. As was mentioned above, studies in humans likewise show that specific dietary preferences for fats are associated with maternal food intake during pregnancy [20, 21].

9.3.2 Dopamine-Related Neural Gene Expression

Although little work has been done to explore the neurobiological basis of the effects of maternal diet on food preferences in offspring, there are data consistent

with the idea that dopamine is involved. Indeed, maternal overnutrition has been shown to alter the expression of multiple dopamine-related genes in the mesolimbic pathway of adult offspring, including tyrosine hydroxylase (TH), dopamine receptors D1 and D2 (D1R, D2R) and dopamine transporter (DAT) [10, 11, 26]. However, the direction of expression of these changes and the specific dopaminergic genes exhibiting changes is variable between studies, possibly owing to differences related to the specific diet administered. Of note, increased DAT expression in the NAC is associated with DNA hypomethylation, suggesting that the change in gene expression is transmitted via an epigenetic modification [9].

As discussed, both natural and drug reinforcers alter the function of the dopaminergic system. Thus, an interesting question is whether maternal overnutrition alters the sensitivity of offspring to the locomotor-activating effects of psychostimulants. Indeed, the activational effects of drugs such as amphetamine, cocaine and morphine are mediated via dopamine transmission in the NAC [27]. In one study that addressed this question, it was found that maternal overnutrition was associated with attenuated amphetamine-induced locomotion and attenuated expression of amphetamine-induced sensitization [11]. Moreover, the attenuated effect of amphetamine on locomotor activity corresponded to blunted dopamine transmission in the NAC [26].

9.3.3 Models of Diet-Induced Obesity

As mentioned, models of diet-induced obesity involve exposing rodents to a high-fat diet for a long period of time in adulthood. Overall, the results of these studies are in agreement with observations relating to the effect of maternal overnutrition in offspring. Thus, rodents consuming a high-fat diet exhibit increased motivation to work for sucrose pellets [28], attenuated amphetamine-induced locomotor sensitization [29] and decreased dopamine turnover in the mesolimbic system (NAC) [30]. Also similar to the effects of maternal overnutrition, diet-induced obesity leads to changes in dopamine-related gene expression in the mesolimbic pathway, including reduced expression of TH, D1R and DAT in the NAC [29, 31]. Altogether, these studies consistently point to associations between consumption of high-fat diet and blunted reward function at both behavioural and neural levels.

One neurochemical of relevance to this discussion is leptin. Leptin is an adipose-derived hormone that acts on hypothalamic leptin receptors to regulate energy balance. Specifically, leptin regulates appetite by signalling when an individual has had enough to eat [32]. However, whereas increased leptin levels generally suppress feeding behaviour, a failure to do so is commonly found in cases of obesity, including obesity in pregnancy [33]. This so-called leptin resistance is also reflected in the results of studies utilizing maternal high-fat diet and diet-induced models of obesity [34–36].

Of note, leptin is known to regulate dopaminergic state, via actions at leptin receptors in the VTA. For example, infusion of leptin into the VTA reduces the

firing rate of dopamine neurons, while blocking the leptin receptors reverses this effect [37]. Additionally, conditional leptin receptor knockdown by siRNA in the VTA leads to an overall increase in feeding, as well as a preference for high-fat diet, as measured by the amount of food consumed after switching from standard to high-fat diet [37].

In accordance with its effects on dopaminergic transmission in VTA, leptin modulates the induction of locomotor sensitization to amphetamine. In one study, the sensitizing effect of amphetamine on locomotor activity was prevented in ob/ob mice lacking the genes coding for leptin [38]. Conversely, systemic treatment with leptin for 2 weeks resulted in the induction of amphetamine sensitization in the knockouts and an enhancement of this effect in wild-type mice [38].

Although maternal overnutrition is known to result in the expression of chronically high levels of leptin in offspring, these offspring exhibit reduced sensitization to the locomotor-activating effects of amphetamine [11]. This result would seem at odds with what might be expected based on the outcome of the work with ob/ob mice. It is possible, however, that differences in the developmental context of leptin exposure in these two models may induce changes in leptin levels within specific neural circuitries. Also, in the context of developmental exposure to high-fat diet, the mechanism for high leptin levels in offspring appears partly due to the increased number of new neurons expressing a number of orexigenic peptides, galanin, encephalin and dynorphin, in the hypothalamus that are known to interact with anorexigenic peptides such as leptin [23]. These data suggest changes in brain structure with exposure of offspring to maternal high-fat diet that are not observed with genetic deletion of leptin.

9.4 Effects of Maternal Overnutrition on the Offspring Stress Response System

In humans, exposure to maternal overnutrition and high-fat diet during development increases the risk in offspring of developing anxiety disorders and depression [39]. A number of lines of evidence suggest that the increased in risk may be driven, at least in part, by disruption in the development of neural pathways regulating responses to stress [40, 41].

9.4.1 Anxiety Behaviour and Stress Physiology

Animal studies have shown that exposure to maternal overnutrition impacts the expression of anxiety-like behaviour across the lifespan. For example, in a rat model, maternal overnutrition increased anxiety-like behaviour in adult offspring, as measured in the Open Field and Elevated Plus Maze tasks [12, 42, 43]. These

results are similar to primate studies showing that developmental exposure to maternal overnutrition increases novelty-induced anxiety in adult offspring [40, 44]. Finally, in human studies, results generally point to a positive association between the co-occurrence of childhood obesity and anxiety disorders [45].

The expression of anxiety-like behaviours is known to be sensitive to changes in the function of the hypothalamic–pituitary–adrenal (HPA) axis, which mediates the endocrine response to stress in part through negative feedback inhibition of corticosterone release. Likewise, maternal overnutrition influences the function of the HPA axis of offspring in a long-term manner. For example, maternal overnutrition is associated with lower levels of circulating corticosterone in male and female rats [12] and mice [46], and female rats exposed to maternal overnutrition exhibit prolonged elevation in corticosterone after physical restraint stress [12]. Likewise, neonatal rats exposed to maternal overnutrition exhibit an elevated corticosterone response to ether stress, suggesting that the programming of the HPA axis by maternal high-fat diet occurs in early postnatal life [47].

9.4.2 Stress-Related Neural Gene Expression

HPA function can be altered by changes in the expression of mineralocorticoid (MR) and glucocorticoid receptors (GR) within limbic brain areas, including the amygdala and hippocampus; these receptor populations differentially regulate basal and stress-activated levels of corticosterone in circulation [48, 49]. Of note, we recently reported that MR and GR transcripts are elevated in the amygdala of offspring whose mothers were fed a high-fat diet during pregnancy and lactation [12]. These data are in agreement with other studies of maternal stress manipulations, showing that increased GR in the amygdala enhances the corticosterone-mediated response to stress [50]. They are also in agreement with a study showing that the offspring of nonhuman primates fed with high-fat diet exhibit increased hypothalamic expression of proopiomelanocortin transcript, a gene that affects HPA function by altering levels of adrenocorticotropin-releasing hormone and, in turn, cortisol release [51].

9.4.3 Stress-Related Responses in Models of Diet-Induced Obesity

The behavioural effects of chronic exposure to high-fat diet in adult rats are similar to those of the offspring of mothers fed a high-fat diet. For example, after 10–12 weeks of consuming a high-fat diet, adult rats exhibited a relative increase in behavioural anxiety on the elevated plus maze, open field and light dark task [52, 53]. Moreover, these elevated anxiety-like behaviours are associated with an

altered HPA axis response, consisting of elevated corticosterone levels after restraint stress. These results agree with several other studies showing that chronic consumption of a high-fat diet in adulthood generally leads to elevated circulating levels of glucocorticoids and an enhanced corticosterone response to stress [54–56], but see [57]. Finally, chronic consumption of a high-fat diet exacerbates the effects of stress by impairing the negative feedback inhibition of the corticosterone response to psychosocial stress [53, 58].

Chronic exposure to high-fat diet in adulthood also leads to alterations in stress-related gene expression within stress-related neural circuitry that is similar, but not identical, to those of offspring exposed to maternal overnutrition. For example, animals consuming high-fat diet show reduced transcript in the hippocampus of both MR and GR, when compared to animals consuming standard house chow [52]. The offspring of animals exposed to maternal overnutrition, on the other hand, exhibit increased expression of the GR transcript within the amygdala. Given the opposing roles of the hippocampus and amygdala in the regulation of the HPA axis, however, both findings are consistent with the idea that the stress system is heightened in response to both dietary manipulations and that both manipulations lead to enhanced behavioural anxiety.

9.5 Relationship Between Food and Drug Addiction

Based on the relationship between maternal overnutrition and responses to psychostimulants in offspring, it is of interest that drug addiction, like disorders involving food consumption, has been linked to dysregulation within the primary brain pathways regulating reward and stress. Characterized by compulsion to seek drugs, drug addiction consists of a chronic cycle of drug intoxication followed by withdrawal and relapse [14]. This cycle corresponds to powerful positive reinforcement (drug intoxication) and, over time, the emergence of a negative emotional state (anxiety) after withdrawal. It has been argued that this negative emotional state may, at least in the short term, perpetuate drug seeking [14]. It has also been argued that drug addiction is characterized by a shift in the motivational processes mediating ongoing consumption from positive reinforcement induced by the drug to negative reinforcement resulting from the relief of negative affect upon resuming drug taking [14]. And it has recently been proposed that a similar transition may occur in the case of disordered eating leading to obesity [15]. Although the motivational and corresponding neural mechanisms involved in drug addiction are perhaps better understood than those involved in obesity, it has been suggested that compulsive drug and food consumption may be regulated by common neural and molecular mechanisms, including those related to dysregulated dopaminergic and stress-related function [59].

9.6 Conclusion and Prospective: The Potential Role of Epigenetic Mechanisms

In this chapter, we have highlighted research concerning reward- and stress-related effects that may help explain the rapid rise in metabolic dysfunction in offspring as a result of maternal diet. This question is particularly relevant since up to 30 % of human pregnancies in developed countries are now complicated by factors owing to maternal obesity [60]. Identifying the mechanisms through which maternal overnutrition results in altered reward and stress pathways later in life will enable the understanding of risk factors for disorders characterized by dysregulated hedonic and negative emotional states.

Epigenetic mechanisms, which modify gene function in the absence of a change in gene sequence, have been proposed to program gene expression as a function of early-life experience [12]. Long-term changes in gene regulation can occur via epigenetic modifications of DNA and chromatin structure. In contrast to chromatin modifications, which may be transient and are tightly coupled to gene expression, DNA methylation is a relatively stable modification that, in regulatory elements, typically leads to persistent repression of gene expression [61]. For example, levels of maternal behaviour received within the first week of life are associated with offspring HPA function and levels of DNA methylation in stress-related genes [62, 63]. Recently, a number of studies of candidate genes have indicated that maternal overnutrition alters levels of DNA methylation in gene promoters in offspring [9, 64–68].

With the support of technological advances in high-throughput DNA sequencing, it is now possible to extend this work from a consideration of candidate genes to candidate pathways. Many of the ways in which environmental exposures alter epigenetic mechanisms in offspring remain unknown. Improved methods for genome-wide detection of epigenetic alterations, however, have greatly advanced complex disease research by providing the means to identify mechanisms leading to stable changes in cellular function [69]. How these changes might distribute across dopaminergic and HPA-related gene networks is one related topic of active research.

In future research, it will be of interest to identify how epigenetic marks indicating enhanced or repressed transcriptional potential may relate to dysfunction in reward and stress pathways across a variety of conditions. Because epigenetic marks are potentially reversible, identifying the manner in which they are altered in the dopaminergic and HPA-related pathways of offspring whose mothers who were fed a high-fat diet will offer insight into mechanisms leading to stable disease states; this work may also inform novel routes to pharmacological intervention. Altogether, such studies will illuminate our understanding of risk factors for disorders characterized by dysregulated emotional processing.

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Chapter 10
The Implications of Maternal Obesity on Offspring Physiology and Behavior in the Nonhuman Primate

Elinor L. Sullivan and Paul Kievit

Abstract Exposure to maternal obesity and high-fat diet (HFD) consumption during perinatal development impacts numerous aspects of offspring physiology and behavior. Epidemiologic studies indicate that maternal obesity is associated with increased risk for metabolic, mental health, and neurodevelopmental disorders. As factors such as a shared environment and genetics could contribute to this association, animal studies are critical. The use of nonhuman primates is particularly important as they have a similar developmental timeline, physiology, and behavior as humans. Evidence from animal models supports the findings from human studies and indicates that maternal obesity induced by HFD consumption impairs the development of many organ systems including the brain, pancreas, liver, and cardiovascular system. These studies suggest that offspring are predisposed to obesity due to hyperphagia, increased preference for fat and sugar, and reductions in energy expenditure. Rodent and nonhuman primate offspring exposed to maternal HFD consumption exhibit increased anxiety, impairments in social behavior, and decreased cognitive performance. These observed behavioral changes are though to be due to alterations in the development of neural circuitry critical in behavioral regulation such as the serotonin, dopamine, and melanocortin systems and increased activity of the hypothalamic–pituitary axis. Mechanisms for these developmental changes include alternations in maternal behavior due to HFD consumption and the increased levels of inflammatory factors, nutrients and hormones that are associated with maternal obesity. Given the high levels of maternal

E.L. Sullivan, PhD (✉)
Department of Biology, University of Portland, 5000 N. Willamette Blvd, Portland, OR 97203, USA

Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, USA

Division of Diabetes, Obesity and Metabolism, Oregon National Primate Research Center, Beaverton, OR, USA
e-mail: sullivae@up.edu

P. Kievit
Division of Diabetes, Obesity and Metabolism, Oregon National Primate Research Center, Beaverton, OR, USA

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obesity and HFD consumption in developed nations, we postulate that future generations are at increased risk for obesity and metabolic, neurodevelopmental, and mental health disorders.

Keywords Maternal obesity • High-fat diet • Pregnancy • Energy balance • Energy expenditure • Food preference • Programming • Nonhuman primate • Anxiety • Autism • ADHD

10.1 Introduction to Maternal Obesity

Perinatal exposure to maternal obesity, impaired metabolic state, and high-fat diet (HFD) consumption is commonplace in developed nations. Currently, a third of women of childbearing age in the USA are obese and two-thirds are overweight [1]. Obesity during gestation is associated with adverse outcomes for both the mother and child such as gestational diabetes [2, 3], preeclampsia [4, 5], high blood pressure [6], placental dysfunction [7, 8], prematurity [9, 10], and infants born either large or small for gestational age [11]. Given the high prevalence of maternal obesity worldwide, it is critical to investigate the long-term effects of exposure to maternal obesity on the developing offspring. A HFD is commonly used to induce maternal obesity in animal models, and in humans a HFD typically accompanies maternal obesity. This chapter will discuss the effects of both maternal obesity and HFD consumption and will assume, except where noted, that maternal HFD consumption results in obesity. This chapter will also examine the impact of exposure to maternal obesity and HFD consumption during perinatal development on the physiology, behavior, neural development, and HPA axis of the offspring with a special focus on evidence from nonhuman primate (NHP) models.

10.2 Translational Potential of NHP Studies to Human Health

The development of research models of disease in animals has progressed our understanding of human diseases tremendously. From the basic biology of organ function, to the intricate communications between organ systems in endocrinology, to the study of the pathophysiology of debilitating diseases such as cancer, cardiovascular disease, and neurodegenerative diseases, animal models have helped us to devise methods and treatments to improve human health. In the field of developmental programming, rodent models fed a HFD have predominantly been used to study the effect of the maternal obesity on fetal development. These studies have been extensive and in depth, providing us with an understanding of how maternal diet can affect cardiovascular disease, glucose metabolism, and many other diseases. However, there are several limitations to solely using rodents when comparing the outcomes to human development.

The use of NHPs is particularly important in the examination of the impact of maternal obesity and HFD on offspring brain development, behavior, and physiology as these animals have a comparable developmental timeline, physiology, and behavior as humans. Nonhuman primates have a similar developmental ontogeny of the brain as humans with the majority of brain development occurring prenatally. This is an area of divergence between rodent and human development as much of the neural circuitry critical in regulating physiology and behavior occurs postnatally in rodents. For example, the melanocortinergic system, an important regulator of energy balance, develops rather late in development, occurring during the third week after birth in rodents [12, 13] and during the third trimester in humans and NHPs [14, 15]. The similar gestational and developmental timeline of NHPs and humans ensures that the developing offspring has similar exposure to the disrupted hormones, elevated circulating lipids, and nutrients associated with maternal obesity. Nonhuman primates also have similar placental structure and function allowing for the developing fetus to be similarly impacted by the excess nutrients and lipids transported through and the inflammatory factors secreted by the placenta. Another example of diverging physiology is the pathophysiology of obesity and the development of type 2 diabetes mellitus (T2DM). Rodents are often resistant to diet-induced obesity and generally do not develop diabetes [16], while NHPs develop the full spectrum of metabolic disease as observed in humans, including age or diet-induced obesity, hypertension, hyperlipidemia, insulin resistance, and central adiposity [17–20]. Nonhuman primates are also ideal for studies examining behavior as they have complex social and mental health-related behaviors allowing the behavior tests to be similar to those used in clinical assessment of human behavior. The NHP model of maternal obesity developed by our group also allows for the investigation of the relative impact of exposure to maternal metabolic phenotype (obesity and insulin resistance) versus HFD during pregnancy on the development of offspring physiology and behavior. In this NHP model, two-thirds of adult females become obese and insulin resistant when consuming the HFD, while one-third remain lean and insulin sensitive. This is important as human studies demonstrate a link between maternal obesity and the risk of offspring obesity [21] and mental health disorders [22–29]; however, these studies do not have the ability to separate diet effects from maternal metabolic phenotype effects. Considering the prevalence of obesity and wide consumption of a HFD worldwide, use of the NHP model to understand the impact of exposure to maternal obesity and HFD consumption is critical as it allows the direct translation of research findings to humans.

10.3 Adult Obese State

10.3.1 The Metabolic State during Maternal Obesity

Pregnancy requires metabolic, physiological, anatomical, and mental exertion from the mother. From early events like implantation to the increased requirements of nutrients necessary to feed the developing fetus, all these events are coordinated to prepare both mother and fetus for the labor, delivery, and feeding of the newborn child. This metabolically taxing state necessitates changes in maternal metabolism (glucose, insulin, leptin, lipids). For instance, pregnancy results in resistance to the action of the hormone insulin resulting in increased circulating glucose and lipids and therefore making higher levels available to the fetus [30–32]. Maternal hyperlipidemia is also present in pregnancy, manifesting as temporary rises in circulating triglycerides and cholesterol that provide a source of lipids for the developing fetus [33]. Obesity, a state already accompanied by increased levels of circulating triglycerides and insulin resistance, therefore exacerbates these rises in insulin [34] and lipids [35] during the pregnancy of an obese mother.

In addition to the dysregulation of hormones such as insulin, maternal leptin resistance is also affected by maternal obesity. In normal physiology, pregnancy is associated with a state of leptin resistance [36, 37], where food intake increases even though circulating leptin levels are also increasing. The mechanisms for the increased levels of leptin and leptin resistance remain unclear, but it is very well known that leptin can have effects on brain development [38]. In addition, dysregulation of leptin has been implicated in the development of mental health disorders. Since maternal obesity already results in a state of hyperleptinemia, exposing the fetus in the early stages of development to these higher levels of circulating leptin could have significant effects for the offspring and obese mother. In addition to the dysregulation of insulin, triglyceride, and leptin levels, maternal obesity also predisposes the mother to many other complications, such as gestational diabetes, preeclampsia, and longer hospital stays [39].

10.3.2 Maternal Obesity in Humans is Associated with Inflammation

The obese state is associated with low-grade chronic inflammation. Adipocytes secrete inflammatory factors including c reactive protein, interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α [40, 41]. The levels of these circulating inflammatory factors are proportional to adipose tissue mass. Inflammatory cytokines are also elevated in many organs in obese individuals including the brain [40] and placenta [42, 43]. Elevated levels of these inflammatory factors increase the risk for many metabolic diseases including cardiovascular disease, heart disease, insulin resistance, type II diabetes mellitus, and hypertension [40]. The increased

inflammatory cytokines associated with obesity during gestation are believed to cause dysfunction in the endothelium [44] and placenta [45]. Maternal obesity during gestation exposes the developing fetus to an elevated level of inflammatory factors, which are postulated to impair the development of several organ systems including the brain.

10.3.3 Maternal Obesity Is Associated with Placental Dysfunction

Many of the pregnancy complications associated with maternal obesity [46] that impact the developing fetus are postulated to be associated with placental dysfunction. Evidence from animal models indicates that maternal obesity induced by consumption of a HFD causes placental inflammation decreasing functionality of the placenta. In sheep, maternal obesity impacts the placenta by increasing the activation of inflammatory cytokines and downstream signaling factors [47], reducing uterine blood flow, and causing a 33 % reduction in the mass [48]. A reduction in placental mass was also observed in rodents fed a HFD [49]. Similarly, in our NHP model, we report an increase in inflammatory cytokines in the placenta from adult female macaques consuming the HFD and an elevation in the levels of cytokines in the fetal compartment [50]. In macaques, maternal HFD consumption is also associated with a 35–50 % decrease in blood flow through the uterine artery to the placenta [50]. The obese state further exacerbates the placental dysfunction resulting in a higher rate of stillbirths, due to increased placental infarctions and reduced blood flow to the fetus [50]. Thus, evidence from animal models consistently indicates that maternal HFD consumption impairs placental function leading to pregnancy complications. Moreover, the elevated levels of inflammatory cytokines secreted by the placenta likely initiate the generation of cytokines by the fetus [51, 52], further increasing the inflammation that the fetus is exposed to during development. Increased levels of inflammatory cytokines modulate growth factors critical for fetal development [53] and impact the development of neural pathways critical in regulating behavior and physiology.

10.3.4 Maternal Metabolic State and Nutrition Impact Maternal Behavior

Mounting evidence supports an important and persistent role for parental care particularly during the early postnatal period on offspring behavior and physiology. A wide body of literature in rodents indicates that naturally occurring individual differences in maternal care during early development will program the behavior and response of offspring to stress [54, 55]. For example, rat offspring exposed to

decreased maternal attention and grooming exhibit increased anxiety-like behavior as adults [55, 56], and offspring from attentive mothers are less anxious and display improved regulation of stress [56, 57]. Offspring social behavior is also impacted by maternal behavior with male rats exposed to higher levels of maternal licking and grooming displaying less aggression toward their peers [55]. However, the impact of maternal diet on maternal behavior has been largely unstudied. Three studies indicate that maternal HFD increases nursing behavior [58–60]. The effects of increased nursing during the perinatal period on offspring behavior have not been directly assessed. However, overfeeding via experimental reduction of litter size results in offspring that are hyperphagic and heavier due to impairments in critical energy balance regulatory circuitry in the hypothalamus [61]. Two studies demonstrate an impact of maternal HFD consumption on maternal grooming [58, 59]. However, one study reports a decrease in grooming behaviors [58], while the other reports an increase in the grooming of pups [59].

Maternal behavior also plays an important role in programming offspring behavior in NHPs [62–65]. For example, infant rhesus macaques exposed to maternal rejection are at increased risk for later developing anxiety [62]. Interestingly, Japanese macaques exposed to early maternal rejection exhibit increased independence in social situations and decreased stress response as infants [64]. The offspring's behavioral outcome appears to be dependent on the developmental age when it is exposed to the maternal separation or rejection. Rhesus macaques that experienced maternal separation at 1 week of age demonstrated elevation in self-comfort behaviors such as thumb sucking, while maternal separation at 1 month of age resulted in offspring seeking increased social comfort [65]. The impact of maternal HFD on maternal behavior has not been previously examined in NHPs. For the past 5 years, we have characterized maternal infant interaction in control and HFD-consuming adult females. We observed an association between maternal HFD consumption and an increase in nursing behavior during the early postnatal period and a decrease in grooming behavior (Sullivan et al., in preparation), which is consistent with the findings in rodent models.

In humans, mental health disorder such as postpartum depression are well documented to influence maternal behavior towards her infant and increase the risk of offspring developing mental health disorders as adults [66]. Perinatal exposure to postpartum depression is associated with violent and internalizing behavior [66]. Daughters of mothers suffering from major depression are at increased risk of developing mental health disorders in adolescence [67]. As a HFD has been shown to increase the symptoms of postpartum depression, maternal diet may impact offspring behavior by modulating maternal mental health [68]. Preliminary evidence also indicates that mothers classified as obese interact differently with their infant offspring than mothers classified as normal weight. Obese mothers spent less time interacting and feeding their infants; however, these infants still had an increased overall caloric intake due to increased consumption of "complementary" foods (cereal, fruit pudding, apple sauce, etc.) [69]. Another study confirmed these findings by reporting that women who entered pregnancy in the obese state introduced complementary foods earlier than women whose pre-pregnancy weight was

classified as normal [70]. Together these studies provide evidence of the interdependence of maternal behavior with maternal diet and metabolic state, which may each impact offspring behavior. Future studies need to parse out the contributions of maternal behavioral differences versus maternal diet on offspring behavior. It is critical that future nutritional studies identify the optimal dietary composition to be consumed during gestation and lactation to benefit both maternal and infant behavior and decrease the infant's risk of developing neurodevelopmental and psychiatric disorders.

10.4 The Impact of Maternal Obesity on Offspring Physiology

10.4.1 Energy Balance Regulation

Human studies consistently demonstrate that maternal obesity is associated with increased risk of the child developing obesity and metabolic disorders [21]. The impact of maternal obesity on offspring risk of obesity appears to be independent of co-occurring metabolic disorders such as diabetes mellitus, as women with obesity and normal blood glucose regulation still have children who are heavier and have increased adipose tissue mass [71]. Even though evidence from human studies implicates exposure to maternal obesity and HFD in programming offspring obesity, numerous environmental and genetic factors could also contribute to the association. It is very challenging to accurately measure the diet of pregnant women and is potentially unethical to manipulate the diet until we gain a further understanding of the optimal diet during gestation. It is also very difficult to accurately measure energy expenditure and energy intake in children. Thus, animal models of maternal obesity and HFD consumption are critically important to directly examine mechanism, identify critical periods of development, and develop potential therapeutic interventions.

Using an NHP model of HFD-induced maternal obesity, our group documented an increase in body weight, adiposity, and leptin levels in juvenile offspring exposed to maternal obesity and HFD consumption [72]. In this model, we note that both maternal HFD and obesity play a role in programming an offspring's body weight as juvenile offspring from control mothers that spontaneously develop obesity were heavier than offspring from lean control mothers [73]. Rat pups exposed to maternal HFD consumption during gestation and lactation are heavier and have increased adiposity and hyperglycemia as compared to pups exposed to a control diet [74]. Mouse offspring of diet-induced maternal obesity exhibit increased food intake and decreased locomotor activity resulting in increased adipose tissue mass [75]. Together these studies provide consistent evidence that in animal models, exposure to maternal HFD consumption programs offspring to be at an increased risk of obesity.

Rodent studies consistently find that exposure to maternal HFD consumption during perinatal development programs hyperphagia [75–77]. Exposure to maternal HFD consumption has been well documented to impact the development of neural circuitry in the hypothalamus critical in food intake regulation [78, 79] including the melanocortinergic system (discussed in detail in Sect. 10.6.1). Rat offspring exposed to maternal HFD consumption during early development exhibit an increase in the expression of the orexigenic peptides galanin, encephalin, and dynorphin in the paraventricular nucleus of the hypothalamus (PVH) and melanin-concentrating hormone and orexin in the lateral hypothalamus [76]. Gestational exposure to maternal HFD also triggers the growth of neuronal and neuroepithelial cells of the third ventricle and stimulates their migration to the hypothalamus producing a greater percentage of neurons expressing orexigenic peptides [76]. Lastly, HFD exposure reduces offspring's sensitivity to leptin's anorectic action [77]. Rodent studies provide evidence that maternal HFD consumption during fetal development disrupts the development of critical neural circuitry in the hypothalamus resulting in hyperphagia.

In contrast to the numerous studies that have investigated the effect of maternal HFD and obesity on offspring food intake, very few studies have examined the impact on energy expenditure. In the NHP model, we note that HFD consumption results in a compensatory increase in physical activity; however, this increase appears to be independent of maternal diet [80]. In rodent models, physical activity has been assessed in a few studies. However, the findings to date are inconsistent potentially due to the use of different measurement techniques and experimental designs. Dark cycle locomotor activity measured via telemetry was found to be reduced in mouse offspring exposed to maternal HFD consumption during gestation and lactation [75]. In another murine model, male offspring exposed to maternal HFD consumption during gestation were hyperactive during the open field test [81]. However, this increase in activity is likely to indicate anxiety as it was observed in a novel environment. A rat study examined locomotor activity during the day by placing rats in a box that detected activity via animal's movement across electromagnetic fields and found that offspring exposed to a diet high in saturated fat (coconut oil) during gestation and lactation did not exhibit a difference in activity as compared to animals exposed to the control diet [82]. However, in the same study rat offspring exposed to a diet high in unsaturated fat (sunflower oil) during perinatal development exhibited an increase in locomotor activity [82]. Thus, the type of fat in the diet impacts the directionality of the change in physical activity due to perinatal dietary programming. The primary component of energy expenditure is metabolic rate. However, the effects of maternal HFD consumption and obesity on metabolic rate have only been examined in one study. The examination of perinatal programming by maternal HFD and obesity on offspring metabolic rate is an important future direction of the field. In addition, future studies should examine the impact of perinatal HFD exposure on metabolic

adaptation to different states of energy balance such as dieting, fasting, and chronic consumption of a HFD.

10.4.2 Food Preference

Mounting evidence indicates that perinatal nutrition and maternal metabolic state impact children's food preference and feeding behavior. An increased preference for high-fat food in children aged 3–5 years was related to increased body fat of the child, as measured by skinfold thickness, and increased parental weight [83]. In addition, children with parents of normal weight consumed a reduced percentage of calories from fat than children with parents who were overweight [84]. However, environmental factors such as familiarity with high-fat foods and genetic factors can also contribute to difference in food preference; thus, the impact of programming by maternal obesity and HFD consumption remains uncertain. Animal studies are critical in elucidating the role of programming by HFD and obesity versus shared environmental factors on offspring food preference.

In NHPs, exposure to maternal obesity and HFD consumption during perinatal development programmed an increased preference for fat and sugar in offspring [73]. This finding was confirmed by rodent studies that also document an increased preference for fat and sugar in offspring exposed to maternal HFD consumption. For instance, exposure to a junk food diet during gestation or lactation programmed an increased preference for fat, sugar, and salt in adult rat offspring [77, 85, 86]. Interestingly, the type of fat that the offspring is exposed to during the perinatal period impacts the offspring's preference for fat, with offspring exposed to diet high in saturated fat displaying a preference for fat, whereas offspring exposed to a diet high in polyunsaturated fatty acids do not [86]. As discussed in Sect. 10.6.3, evidence from rodent [82] and NHP [73] studies indicates that exposure to maternal HFD consumption impacts the development of the dopamine system which likely contributes to the observed differences in food preference. Evidence from human, NHP, and rodent studies consistently report an increased preference for fat and sugary food in offspring exposed to maternal obesity and HFD consumption. It will be important for future studies to determine the role that the type of fat plays in programming food preference, as this will guide nutritional studies focused on determining the optimal perinatal diet.

10.4.3 Pancreas

The relationship between glucose homeostasis and maternal diet was originally discovered in a cohort of men born in Hertfordshire, UK, in whom it was observed that there was a relationship between birth weight and glucose intolerance at a later age [87]. This relationship was underscored by findings from studies of people who

were in gestation during the Dutch Hunger Winter [88, 89], where a severe famine restricted nutrients during a very sensitive time of development, which ultimately resulted in the metabolic changes later in life. For instance, by performing glucose tolerance testing in men and women from the Dutch Famine Birth Cohort, de Rooij et al. demonstrated that people exposed mid-gestation to severe nutrient restriction had a dysfunction in insulin secretion [88]. Contrary to famine, the global epidemic of obesity has been paralleled by a global increase in diabetes. In the USA, the number of people diagnosed with diabetes has quadrupled in the last 30 years, and currently almost 10 % of the population has this disease [90]. Of people with diabetes, 90–95 % of the cases are T2DM. The pathophysiology of T2DM is a complex interplay between genetics, epigenetics, and environment. Recent research in several models, including human, are focusing on the role of maternal obesity in the development of diabetes and the central role the pancreas plays in this.

There are several important differences in the rodent versus the primate in regard to the pancreas. For instance, the timing of development occurs during different windows of gestational age [91], the intra-islet cytostructure is different, as well as the innervation of the islets [92–94]. To obtain a better understanding of the effect of maternal obesity on glucose homeostasis, research will need to investigate the changes in the pancreas of NHPs. Using a NHP model of maternal obesity, our group has demonstrated that maternal HFD leads to dysfunctional development of the fetus [50, 72, 95, 96]. Indeed, as was observed in the other tissues such as liver and placenta, maternal obesity resulted in dysregulation of the islet composition, demonstrating that a HFD fed during the gestational period results in a decrease in α-cells, thus increasing the β- to α-cell ratio [97] in 1-year-old animals. This work postulates that the decrease in the number α-cells is a compensatory response to the increased production of glucose by the liver in these animals. Although this question has not been directly addressed, there is a possibility that the paracrine action of α-cells could affect insulin secretion. Future work should focus on determining which components of the diet are driving the changes in α-cell development. Subsequent work in this model investigated the vascularization and innervation of the islet. Pound et al. demonstrated that offspring from obese NHP mothers have decreased innervation and vascularization in the third trimester of development and that this reduction in vascularization persists at least 1 year postnatally [98].

10.4.4 Cardiovascular System

Cardiovascular disease is particularly affected by the increasing rates of obesity, hypertension, and diabetes [99, 100]. The original work by Dr. David Barker in humans clearly demonstrated that birth weight is correlated with subsequent cardiovascular disease, highlighting the fact that the heart and other players in the cardiovascular system are affected by the fetal environment [101–103]. The importance of maternal diet in cardiovascular programming was underscored by rodent

studies that showed that a maternal low protein diet induced hypertension in offspring [104]. Further studies in both rodent and human models confirmed and expanded upon these studies, showing that other maternal insults can have dramatic effects on the cardiovascular system, including maternal obesity [105–108]. The breadth of research studying the effects of maternal obesity has used the rodent as an experimental model because of the short life span, lower cost, and availability of genetic models. To date, only a handful of studies have utilized the NHP model to study the impact of maternal obesity and all have focused on the early indicators of vascular dysfunction.

Recent work using a baboon model demonstrated that feeding newborn baboons a HFD for the first 16 weeks of life resulted in long-lasting changes in adipose development independent of body weight, although these preweaning diets did not necessarily increase atherosclerosis at 5 years of age [109, 110]. New work is now demonstrating that it is the combination of maternal diet and postweaning diet that is detrimental to the development of cardiovascular disease. Early changes in gene expression and the expression of microRNA have been described in a model of maternal obesity in the baboon [111] where the mothers were fed a high fat/high fructose diet. In these fetuses, investigated during the third trimester, there was already evidence of myocardial fibrosis. On the molecular level, there was differential expression of several of the cardiac microRNAs, perhaps a sign of maternal programming. Our work using the earlier described model of maternal obesity in the Japanese macaque demonstrated that both a maternal and postnatal HFD exacerbate the development of vascular and endothelial function [96], resulting in increased intimal thickness in the abdominal aorta and a decrease in the vasodilation capacity in offspring of obese mothers. Interestingly, some of the negative effects of maternal HFD on offspring cardiovascular function were partially ameliorated when offspring were weaned onto a healthy diet. This suggests that an early dietary intervention may be effective in mitigating cardiovascular dysfunction programmed by maternal obesity and HFD consumption.

10.4.5 Liver

Maternal obesity and/or gestational diabetes is a major contributor to the increase in nonalcoholic fatty liver disease (NAFLD) in obese children [112, 113] and neonatal infants [114, 115]. Research in rodent and other animal models are now demonstrating that this excessive hepatic lipid storage is occurring during fetal development [116, 117]. Maternal obesity results in elevated glucose, insulin, and fatty acid levels during development of the fetus, and this presents an issue early on in development when the fetus has not developed subcutaneous fat storage. Although the liver requires lipids for normal functioning during development, excess lipids can be cytotoxic. Excess levels of intracellular lipids can cause a variety of cellular damage, including the production of reactive oxygen species. This finding has been demonstrated in many different animal models, including the NHP. McCurdy

et al. demonstrated that during fetal development, offspring from obese monkeys consuming a HFD had threefold higher levels of triglyceride in the liver. This resulted in early signs of liver toxicity as evident by increased levels of oxidative stress at the cellular level [72]. Similar results have been observed with studies in mouse models [118, 119], demonstrating that early exposure of the fetus to maternal HFD consumption can lay the foundation for future NAFLD. Subsequent studies in the NHP showed that fetal exposure to a HFD resulted in persistent changes, even if the postnatal diet was switched to low fat [120]. This phenotype could be the result of extensive epigenetic programming in the liver. Studies in rodents, humans, and NHPs have identified several epigenetic changes in response to exposure to a HFD either during adulthood [121] or fetal development [122, 123], the contribution of these changes to programming of NAFLD is a topic of future research. In addition, research by Grant et al. showed that hepatic innervation and hepatocyte apoptosis is different as well, providing evidence that many pathways in the NHP liver are affected by maternal diet [95, 124]. An interesting observation from the study by McCurdy et al. was the inclusion of animals that remained lean on the HFD. When studying offspring from these non-obese mothers, it appeared that similar dysfunction was noted in the liver, suggesting that the majority of the liver damage can be contributed to maternal diet, independent of maternal obesity. More importantly, a reversal of the HFD to regular chow during the pregnancy of obese mothers partially reversed the liver damage [72]. Although additional work needs to be done, these findings could support clinical dietary interventions during pregnancy as a first step in combating early NAFLD.

10.5 Maternal HFD Consumption Programs Offspring Behavior

10.5.1 Exposure to Maternal Obesity Increases the Risk of Mental Health Disorders

Evidence from epidemiologic studies indicates that exposure to an unhealthy diet and maternal obesity during early development increase the risk of the child developing mental health and neurodevelopmental disorders including attention deficit hyperactivity disorder (ADHD) [25, 125] and autism spectrum disorder (ASD) [23]. Children from mothers who were obese during pregnancy were more likely to have difficulties in emotional regulation [26] and increased risk of depression and withdrawal [126]. Importantly the high prevalence of obesity in women of childbearing years in recent decades is postulated to contribute to the concurrent increase in the rates of ASD [27] and ADHD [127–129] in the USA. Exposure to maternal obesity during gestation was reported to double the risk of a child developing ADHD symptoms [125]. Also, children with ADHD are twice as likely

to have a mother who was obese [25]. Risk of ASD and developmental delays in children aged two to five were also shown to be increased by perinatal exposure to maternal obesity [23].

Metabolic disorders associated with obesity may also be associated with increased risk of offspring developing neurodevelopmental and mental health disorders. The number of studies examining the impact of maternal metabolic disorders on offspring's risk of neurodevelopmental and mental disorders is limited, focusing primarily on diabetes. Children exposed to diabetes during gestation display greater rates of ADHD symptoms [130]. Gestational diabetes is also associated with increasing the offspring's risk for anxiety, depression, and social problems [131]. Exposure to maternal diabetes is associated with greater risk of ASD and developmental delays in young children [23]. Hypertension and pre-eclampsia during gestation were also associated with increased ASD risk [132–134]. Together this evidence suggests an important link between exposure to maternal obesity and associated metabolic disorders and offspring mental health and risk for behavioral disorders. However, the relative contribution of the prenatal versus shared postnatal environment remains unclear, as does the contribution of each metabolic disorder. Also, common genetic factors could underlie both obesity and mental health disorders. To more fully examine these questions, well-controlled animal experiments are needed. Substantial evidence from animal models demonstrates that maternal consumption of a HFD during the perinatal period impacts various aspects of offspring behavior.

10.5.2 Maternal HFD Impacts Offspring Anxiety

Exposure to maternal HFD during gestation is associated with heightened anxiety in both NHP [135] and rodent offspring [136]. Male rodent offspring whose mothers consumed a diet with a high content of saturated or trans fat during the perinatal period displayed increased anxiety in adulthood. Interestingly, a difference in anxiety behavior was not evident in female offspring indicating gender differences in maternal diet programming of offspring behavior [136]. However, female offspring from both diet groups had a higher level of anxiety than male offspring; thus, it is possible that a ceiling effect prevented the increase anxiety in HFD female offspring to be detected. In this model, the investigators postulate that maternal intake of a HFD increases offspring exposure to inflammatory factors that directly impact brain development [136]. In an NHP model of HFD-induced maternal obesity, our group has demonstrated an increase in anxiety in female, but not male Japanese macaque offspring [135, 137]. The increase in anxiety in female offspring was associated with a suppression of central serotonin synthesis in offspring from HFD mothers [135, 137]. Importantly, this increase in anxiety in female macaque's offspring is consistent with the evidence in humans that reports a marked gender dimorphism in anxiety prevalence. In humans, females reported to have an increase in anxiety susceptibility and a more profound link between anxiety

and obesity [138]. There is evidence in rodent models that the developmental time period in which offspring are exposed to the HFD impacts the outcome on offspring's behavior. With offspring exposed to a HFD during gestation exhibiting increased anxiety [136], while those exposed to the diet solely during lactation do not. In the NHP model of maternal HFD consumption, the developmental timing of HFD exposure has not yet been examined as mothers consume the HFD during both gestation and lactation.

Human studies support the findings from animal studies and contribute to the evidence that maternal obesity increases the risk of offspring anxiety. Children from mothers who were obese during pregnancy were more likely to have difficulties regulating emotions such as sadness and fear [26] and were reported to have an increased risk for internalizing problems including depression and withdrawal [126]. Maternal obesity is associated with pregnancy complications such as infants being born small or large for gestational age [11, 139, 140], which increases the likelihood of offspring developing anxiety and depression as adolescents [141]. Also, as noted above offspring exposed to maternal obesity are at a much greater risk of becoming obese themselves as children and adults. Childhood obesity is associated with higher rates of internalizing behaviors such as anxiety and depression and social problems [131]. Measures of obesity during infancy (high birth weight and top 10 % ponderal index) were found to be positively associated with adult depression [142]. Moreover, obesity in adulthood is well documented to be associated with anxiety and depression [143].

10.5.3 Maternal HFD Programming of Social Behaviors

Social interaction and the development and maintenance of social networks are critical for the survival of most species as they allow for procreation, procurement of food and resources, and protection from predators. Recent evidence indicates that maternal diet and metabolic state during the perinatal period may impact offspring social behavior. The first evidence that maternal diet impacted offspring social behavior came from a study by Raygada et al. in which investigators found that maternal consumption of diet high in polyunsaturated fatty acids led to increased aggression in female offspring in three different strains of mice [144]. This increase in aggression was postulated to be due to an upregulation of protein kinase C (PKC) activity in the hypothalamus. To date, very few studies have examined the impact of maternal HFD on offspring social behavior. Kang et al. found that female offspring from HFD mothers exhibited social impairments using a social interaction test [81]. Interestingly, the deficits in social behavior were not found in male offspring and a dietary intervention during the lactation period was found to reduce the social deficits in HFD female offspring. In this study, increased inflammatory cytokines and microglial activity were also observed in female HFD offspring and were postulated to underlie the deficits in social behavior. These findings from rodent studies are consistent with the results in our NHP

model in which we observe a decrease in social interactions in HFD offspring when exposed to a novel peer and in their normal social housing (Sullivan et al. in preparation). These findings from animal models support evidence in human studies that indicate that disorders such as ASD, which are characterized by impairments in social behavior, occur at higher rates in offspring from obese mothers [23].

10.5.4 The Impact of Maternal HFD Consumption on Learning and Memory

Epidemiologic studies have recently linked obesity and consumption of a HFD in adulthood with cognitive impairment [145], Alzheimer's disease [146], and dementia [146]. A high intake of saturated fat during midlife was associated with decreased cognitive function and memory and an increased risk of cognitive impairments [145], while a high intake of polyunsaturated fats and fish was associated with improved memory and cognitive function [145]. Rodent studies support these findings by providing consistent evidence that consumption of a HFD accompanied by obesity impairs spatial learning and memory [147–155]. To date, the impact of consumption of a HFD and obesity during adulthood on cognition have not been examined in NHPs. It will be important for future studies to pursue this as NHPs provide an important link between the mechanistic studies possible in rodents and epidemiologic evidence from human populations.

A limited number of studies have examined the effects of exposure to maternal HFD and obesity during perinatal development on offspring cognition. However, the existing data come primarily from rodent studies and indicate that exposure to maternal HFD consumption and obesity is associated with cognitive impairments. A deficit in spatial memory measured using a Morris water maze was recently documented in adult male rats that were exposed to a diet high in saturated or trans fats during perinatal development [136]. This memory deficit was associated with inflammation in brain regions critical for cognitive function such as the hippocampus as evidenced by increased peripheral and hippocampal cytokine expression in response to a bacterial challenge and hippocampal microglial activation [136]. A second study confirmed these findings as impairments in spatial learning and memory were observed in adult rats [156]. The cognitive impairments observed in this model were associated with impairments in hippocampal development including decreased brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeletal-associated protein levels. A mouse study also observed that exposure to a diet with high lard content during perinatal development reduced both spatial memory and cognition in adult offspring [157]. A second mouse study found that diet-induced obese females had offspring with decreased BDNF synthesis in the hippocampus, which was associated with impaired dendritic arborization of hippocampal neurons [158]. These offspring were also identified to have delays in spatial learning when they were young. However, in this study cognitive

impairments were not evident in adult animals [158]. Male rat offspring exposed to maternal HFD consumption during gestation and lactation that continued consuming the HFD exhibited a decline in memory retention, but not acquisition in the Morris Water maze [159]. Maternal HFD consumption has also been associated with increased markers of oxidative stress and inflammation in the brain [159]. Overall, preliminary evidence from rodent studies indicates that exposure to maternal obesity and HFD consumption during early development may decrease offspring cognition. It is important that the impact of maternal diet and obesity is examined in larger animal models, such as NHPs, which share a similar trajectory of brain development and in which higher levels cognitive function can be assessed. Moreover, it will be important for future studies to parse out the contribution of pre- versus postnatal HFD to programming cognition. Lastly, though preliminary mechanistic targets have been identified such as reduced BDNF, increased oxidative stress, and inflammation, it is critical that mechanistic studies are expanded to enable the development of therapeutic interventions.

10.6 Maternal HFD Consumption Programs Brain Development

10.6.1 Melanocortinergic System

The hypothalamic melanocortinergic system is collection of neural circuits that are critical regulators of energy homeostasis [160], blood pressure regulation [161, 162], and sexual behavior [163]. The melanocortin system is comprised of a set of transmembrane receptors (MC1R–MC5R) [164] that are responsive to cleavage products of the precursor proopiomelanocortin. For our purposes, we will focus on alpha-melanocyte-stimulating hormones (alpha-MSH), which inhibit food intake, and agouti-related peptide (AGRP), which promotes hunger. These two peptides regulate food intake by acting on melanocortin receptor subtype 3 (MC3R) and melanocortin receptor subtype 4 (MC4R). As the melanocortin system is one of the primary regulators of energy balance, a number of studies have examined the impact of maternal obesity and HFD consumption on this system as a potential mechanism to explain the increased risk of obesity in offspring from obese mothers. In NHPs, we observe a reduction in the expression of AgRP mRNA and protein and an increased expression of POMC and MC4R in the arcuate nucleus of the hypothalamus (ARC) of fetal offspring [165]. Recent data from the model indicate that in juvenile NHP offspring, both maternal and postweaning HFD consumption suppress the density of AgRP staining in the paraventricular nucleus of the hypothalamus (PVH) and postweaning HFD consumption suppressed AgRP density in the ARC [80]. Many rodent studies also observe a programming effect of maternal HFD consumption during early development. However, these studies are inconsistent and report either an increase or a decrease in AgRP expression. In a rat model,

maternal HFD consumption-induced obesity was found to increase the mRNA expression of AgRP, POMC, and MC4R in the whole hypothalamus of fetal offspring [166]. However, another rat model examined the impact of exposure to maternal HFD consumption during the last 2 weeks of pregnancy and noted a decrease in the expression of AgRP and NPY in offspring at weaning [76]. A third rat study also noted that maternal HFD consumption decreased NPY and AgRP mRNA expression [167]. These differences between studies are likely due to differences in the composition of the experimental and reference diets and the length of exposure to the diets and thus the degree of maternal obesity and metabolic dysfunction. It is important to note that the melanocortinergic system develops rather late in development, occurring during the third week after birth in rodents [12, 13], and during the third trimester in humans and NHPs [14, 15]. These species difference in brain ontogeny makes the NHP model particularly important in the translation of findings to humans. The central melanocortin system appears to be impacted by inflammatory factors. Exposure of rodent hypothalamic explants to the inflammatory cytokine IL-1B results in a suppression of AgRP release and an increase in POMC release [168, 169]. Thus, we postulate that maternal obesity-induced inflammation impairs the development of the central melanocortin system impacting offspring physiology and behavior.

10.6.2 Maternal HFD Consumption Suppresses the Development of the Serotonin System

The serotoninergic system plays an essential role regulating numerous aspects of behavior and physiology including energy balance regulation and digestion. Serotonin (5-HT) is involved in neural development impacting neuronal growth, synapse formation, and migration of neurons [170, 171]. Decreased central serotonin levels are associated with mental health disorders including anxiety [172] and depression [173]. Reductions in brain serotonin are also reported in neurodevelopmental disorders such as ADHD [174] and ASD [42, 175]. Moreover, selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed to treat these mental health and neurodevelopmental disorders. During pregnancy, the serotonin system also plays a key role in regulating the maternal immune system to prevent allogeneic rejection of the fetus [176] and placental blood flow. Thus, changes in the development of the serotonin system due to exposure to maternal obesity and HFD consumption may underlie behavioral disorders.

Evidence from animal models of HFD-induced maternal obesity supports human evidence that impairments in the development of serotonin neural pathways are a potential mechanism for the changes in offspring behavior. In NHPs, we observe impairments in the development of the serotonin system in fetal offspring and increased anxiety in infant female offspring [135]. Recent data indicate that the female offspring exhibit an increase in anxiety behaviors into the juvenile time

period and that this is associated with a persistent suppression of serotonin synthesis in the dorsal raphe [137]. These findings are supported by similar findings in a rodent model. Murine offspring exposed to maternal HFD consumption were documented to have an increase in 5-HT1AR, the inhibitory autoreceptor, in the ventral hippocampus and increased anxiety behaviors [177]. Increased exposure to inflammation is documented in pregnancies complicated by obesity and HFD consumption [44, 178, 179], and the development of serotoninergic neural pathways is sensitive to inflammation [180]. Thus, we postulate that the increased inflammation induced by maternal obesity/HFD consumption impairs the development of the serotonin system leading to behavioral abnormalities in offspring. In our NHP model, we document that maternal HFD consumption increases inflammation in the placenta [50] and in the hypothalamus of the fetal offspring [165]. Given the similarities in the timing of brain developmental and physiology between NHPs and humans, a similar mechanism may contribute to the increased risk of psychiatric and neurodevelopmental disorders in offspring exposed to maternal obesity during perinatal development.

10.6.3 Programming of the Dopaminergic System by Maternal HFD

The dopaminergic system is another neural system that is critical in the regulation of behavior and physiology and appears to be impacted by exposure to maternal obesity and HFD consumption. Alterations in the dopamine (DA) system are postulated to underlie a number of neurodevelopmental (ASD [181–183], ADHD [184–186]) and mental health (schizophrenia [187–189], anxiety [190, 191], and depression [192, 193]) disorders. In NHPs, exposure to maternal HFD consumption was recently found to suppress offspring dopamine signaling in the prefrontal cortex as evidenced by a decrease in DA fiber projections and levels of the dopamine receptors 1 and 2 protein [73]. Evidence from rodent studies provides additional evidence that exposure to maternal HFD during gestation and lactation impairs the development of the DA system. In a rat model, perinatal exposure to maternal HFD consumption resulted in increased DA in the nucleus accumbens and reduced sensitivity to DA, as evidenced by reduced locomotor response to a psychostimulant [82]. Rat offspring from HFD mothers were also found to display an elevated DA response to acute stress and did not display the normal desensitization to repeated exposure to the stressor [194]. In a mouse model, maternal HFD consumption altered methylation and expression of DA genes [195]. Similar to the 5-HT system, the DA system is sensitive to exposure to maternal inflammation [196]. Thus, elevated perinatal exposure to inflammation associated with maternal obesity is thought to impact development of the DA system and increase offspring risk of developing psychopathology.

10.7 Maternal HFD Consumption Programming of the HPA Axis

Cortisol release from the hypothalamic–pituitary adrenal (HPA) axis plays a critical role in regulating psychological and physiologic stress. Stress triggers the hypothalamic paraventricular nucleus to release corticotropin-releasing hormone (CRH) and antidiuretic hormone (ADH) into the hypothamo-hypohyseal portal system triggering the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into systemic circulation. Circulating ACTH stimulates the release of glucocorticoids (primarily cortisol in humans and NHP and corticosterone in rodents). In addition to being stimulated by CRH, ACTH levels are also regulated by the hypothalamic suprachiasmatic nucleus (SCN) resulting in a circadian rhythm of ACTH and cortisol release, with levels of both hormones being lowest at night [197]. Interestingly, a number of studies find that the response of the HPA axis to stress also exhibits diurnal variation [197]. CRH is also expressed in areas of the brain important in behavioral regulation such as the amygdala and lateral bed of the nucleus stria terminalis [198] where it is postulated to regulate anxiety and fear. Given, the important role of the HPA axis in the regulation of behavior and physiology, it is important to examine the impact of maternal obesity and diet on the function of the HPA axis.

Human studies note an association between heightened activity of the HPA axis and mental health disorders including anxiety and depression [199]; thus, programming of the HPA axis by maternal HFD and obesity is a potential mechanism for the increase in anxiety observed in offspring exposed to maternal obesity and HFD. In NHPs, we note that maternal HFD consumption and obesity result in an increase in both acute stress response (plasma cortisol) and chronic stress response (hair cortisol) in infant and juvenile offspring [137]. This evidence is supported by rodent studies that also indicate an increase in corticosterone in offspring exposed to maternal HFD consumption. Male rat offspring exposed to a HFD during the last week of gestation and lactation exhibited elevated basal levels of corticosterone on postnatal day 10 [200]. Another study which examined the impact of HFD exposure during gestation and lactation noted that adult rat offspring had reduced basal corticosterone but an elevated and longer lasting corticosterone response to stress which was accompanied by an increase in anxiety behaviors [201]. This study also noted an elevated number of receptors for glucocorticoids in the amygdala [201]. As glucocorticoid action in the amygdala regulates CRH expression and anxiety-like behavior [198], this could be a mechanism by which exposure to maternal HFD increases anxiety in offspring. Glucocorticoid levels in the amygdala have not yet been examined in NHP exposed to maternal HFD. It will be important for future studies in NHPs to fully characterize the HPA axis and extrahypothalamic CRH expression and glucocorticoid receptors.

10.8 Mechanisms by Which Maternal HFD Consumption and Obesity Influence Offspring Physiology and Behavior

10.8.1 Inflammation-Induced Programming

As discussed above maternal obesity is associated with elevated levels of inflammatory factors such as c reactive protein, IL-6, IL-1β, and TNF-α [40]. Recent evidence indicates that many of these inflammatory factors can cross the blood placental barrier, triggering the release of additional inflammatory cytokines from the placenta that subsequently impact the developing fetus. Evidence from animal models extend these studies by documenting that maternal HFD consumption increases inflammatory markers, microglial activation, and changes the behavior of offspring. In NHPs, exposure to maternal HFD consumption causes elevated levels of circulating inflammatory markers in the fetus and increased microglial activation in the brain, which likely contribute to observed impairments in the development of the dopaminergic [73], melanocortinergic [165], and serotonergic systems [135] and a long-term impact on behavior and physiology [135, 165]. These findings are supported by evidence from a rat model that found that maternal HFD consumption results in increased microglial activation in the hippocampus and increased anxiety and impairments in spatial learning in adult male offspring [136]. A mouse study which examined both male and female offspring noted increased proinflammatory cytokines and microglial activation associated with increased anxiety behavior and impaired social behavior in female offspring exposed to HFD during gestation [81]. In this murine model, male offspring exposed to maternal HFD during gestation were noted to display hyperactivity. Placement of the dams onto a control diet during the lactation period was found to reduce the neural inflammation, social impairments, and anxiety observed in female offspring, but did not affect the hyperactivity observed in the male offspring [81], highlighting that the timing of the dietary exposure dramatically impacts the behavioral outcome and that various behaviors have different sensitive periods to maternal HFD developmental programming. In humans, exposure to elevated proinflammatory cytokines during perinatal development has been shown to impact brain development and increase risk for behavioral and metabolic disorders. Exposure of the developing fetus to an inflammatory environment is associated with prematurity, low birth weight [202], and increased risk of ADHD [203], ASD [204], and schizophrenia [205]. Also, exposure to increased levels of inflammatory markers during the neonatal period has been shown to increase risk for several serious metabolic diseases including heart disease, cardiovascular disease, type II diabetes mellitus, and hypertension [40]. Data from both human and animal studies demonstrate that exposure to elevated inflammatory factors during the perinatal period impairs the development of several neurotransmitter systems that regulate physiology and behavior such as the serotonin, dopamine, and melanocortin systems [40, 180, 206]. Exposure to inflammation is nonspecific

and is thus likely to impact many neural pathways. It is important that future research fully characterizes the impact of inflammation induced by maternal obesity and HFD consumption on the developing brain. Also, given the dramatic impact that exposure to inflammation has on offspring risk of metabolic and behavior disorders, it is critical that therapeutic interventions using anti-inflammatory agents are examined. A recent rodent study has examined one such possible therapeutic intervention, ursolic acid, which was observed to ameliorate the impairments in cognitive function observed with HFD consumption [157].

10.8.2 Programming by Excess Hormones and Nutrients

Maternal obesity or maternal overnutrition disrupts the normal development of many different organ systems in almost all mammalian species, as has been described in previous sections. Although obesity is an incredibly complex and multifactorial disease, there are several obvious changes in nutrients and hormones that have been demonstrated to direct or at least play a significant part in the maternal programming of the fetus. Often, these altered levels of hormones and nutrients act in concert to prepare the fetus for postnatal life. However, questions remain about the contribution of individual nutrients or hormones to maternal programming.

In maternal obesity, hyperglycemia is a hallmark of metabolic syndrome. To investigate whether high glucose intake during pregnancy in the absence of obesity can lead to programming changes, D'Alessandro et al. fed rats a high sucrose diet during pregnancy and lactation [207]. Despite not seeing any changes in body weights in the offspring, animals that were exposed to a high sucrose diet anytime during development demonstrated increases in blood glucose levels, as well as dyslipidemia with high circulating levels of very-low-density lipoproteins and triglycerides. This suggests that high sucrose exposure can program both glucose metabolism and hepatic lipid metabolism [207, 208]. Other studies in rodents have demonstrated that a similar model of sucrose consumption during pregnancy can program changes in the cardiovascular system [209]. There are currently no human situations where the contribution of just hyperglycemia during development can be studied to determine the effect on the developing fetus. Epidemiological studies in humans looking at the contribution of hyperglycemia in cases of nonobese gestational diabetes argue that just having hyperglycemia can alter the physiology of the offspring [210–212]. A large population study that underscored this finding was the HAPO study (Hyperglycemia and Adverse Pregnancy Outcome) in which the consortium confirmed that neonatal adiposity was correlated with maternal glucose levels [213], although this programming in neonates did not translate into an association with childhood obesity when the offspring reached the age of 5–7 years [214].

In the NHP, no current studies have investigated the direct role of glucose or fructose on the development of the offspring in the absence of a HFD. One study did

attempt to develop a model of type 1 diabetes in NHPs and determine the effect of hyperglycemia on offspring. The authors determined that the hyperglycemia, as a result of β-cell destruction with streptozotocin, results in large for gestational age offspring as well as hyperglycemia and hyperinsulinemia in the fetuses [215].

The experimental models for studying maternal obesity almost always utilize a diet that is high in saturated fat with simple sugars as the source of carbohydrate. Although some studies have addressed the contribution of simple sugars in developmental programming (see section above), there is little known on the direct and isolated effect of dyslipidemia on programming in the NHP. Research in our NHP model of gestational obesity has demonstrated that exposure to a diet high in saturated fats and sucrose resulted in significant elevated levels of triglycerides in the liver of the fetus [72]. This in utero exposure to high levels of lipids resulted not only in oxidative stress in the liver but also increases acetylation of histone H3, a hallmark of epigenetic programming [123]. Further, this study highlighted some of the molecular mechanisms involved in epigenetic programming by maternal obesity in NHPs. The importance of decreases in NAD-dependent protein deacetylase sirtuin 1 (SIRT1), an important player in epigenetic modifications, was underscored by the changes in known targets of SIRT1 like peroxisome proliferator-activated receptors gamma and alpha. Taken together, this study very elegantly showed that a maternal HFD, resulting in liver triglyceride levels threefold of normal, can result in epigenetic alterations that can have a deleterious effect on the future development of liver disease. It is also important to note that these changes were driven by the consumption of a HFD and were unrelated to maternal obesity.

Interestingly, when animals from a subsequent study were studied at 1 year of age, only offspring from mothers that demonstrated sensitivity to the maternal diet (insulin resistance) retained the increased levels of triglycerides in the liver [120]. It will be interesting to determine whether the epigenetic changes that were observed in the fetus persist in the animals after 1 year of age. Regardless, it is apparent that although consumption of a maternal HFD can result in epigenetic programming of the fetus, it requires insulin resistance in the mother to have dysfunctional lipid handling at 1 years of age. This observation clearly suggests that programming is a combinatorial process, which includes many different aspects of maternal obesity including nutrient excess, insulin resistance, and inflammatory processes.

10.9 Conclusions

Evidence from epidemiological studies and animal models indicates that perinatal exposure to maternal obesity and HFD consumption has a considerable impact on the physiology and behavior of the developing offspring. A number of mechanisms have been identified to contribute to maternal obesity and HFD consumption programming of offspring development including placental dysfunction and exposure to elevated levels of inflammatory factors, nutrients (glucose, triglycerides), and metabolic hormones (leptin, insulin) that impact the developing brain, liver,

pancreas, and cardiovascular system. Changes in these organ systems result in sustained alternations in the offspring physiology leading to susceptibility to obesity. Furthermore, impairments in the development of neurotransmitters systems important in behavioral regulation such as the serotoninergic, dopaminergic, and melanocortinergic systems lead to persistent changes in behavior including increased anxiety, impaired social behavior, and decreased cognitive function. The alarmingly high rate of maternal obesity and HFD consumption in Western nations places future generations not only at increased risk for obesity and metabolic disorders but also at heightened risk of developing neurodevelopmental disorders such as ASD and ADHD and mental health disorders such as anxiety. Given the substantial healthcare costs associated with each of these disorders, it is critical that future studies identify interventions that are efficacious in preventing and reducing the impact of maternal obesity and HFD consumption on offspring development.

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Chapter 11
The Impact of Maternal Obesity on Offspring Obesity via Programmed Adipogenesis and Appetite

Michael G. Ross and Mina Desai

Abstract Obesity and its related diseases are the leading causes of death in Western society. In concert with the epidemic of obesity among children and adults, there has been a marked and continuing increase in the prevalence of obesity and accompanying gestational diabetes among women presenting for prenatal care. Results from both human and animal studies indicate that in utero environment may contribute importantly to the developmental programming of adult obesity. Remarkably, considering cellular divisions necessary for organ and body growth (not including cell turnover), over 90 % of lifetime cell divisions occur by the time of birth. Thus, it should not be surprising that the maternal/fetal environment may alter cell signaling, epigenetic regulation, and organ development. As energy and nutrition balance are the ultimate endpoints of organ systems regulating energy balance, it would be further expected that the fetal nutrient environment may impact systems regulating food intake and energy storage. In this chapter, we present evidence of the effects of maternal obesity, gestational diabetes, and high-fat Western diets on the development of the hypothalamic appetite network and adipose tissue. Through the interplay of extracellular signaling factors, intracellular transcription responses, and nutrient-induced epigenetic alterations, the maternal environment can program fetal/newborn energy pathways resulting in a predisposition toward obesity. This predisposition is especially paramount within a postnatal environment that facilitates neonatal growth as well as access to energy-intense childhood and adult diets. These findings have great significance for prenatal, neonatal, and childhood care.

M.G. Ross, MD, MPH (✉) • M. Desai, MSc, PhD
Perinatal Research Laboratories, Department of Obstetrics and Gynecology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson Street Box 446, Liu Research Bldg, Torrance, CA 90502, USA

Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
e-mail: mikeross@ucla.edu

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Among US adults, 69 % are overweight (BMI 25 to <30 kg/m^2) and 36 % are obese (BMI ≥ 30 kg/m^2) [1]. Worldwide, nearly 1.5 billion people are overweight or obese. Obesity and its related diseases are the leading causes of death in Western society, with associated risks of hypertension, cardiovascular disease, stroke, and diabetes. Of concern to obstetricians, there is a marked and continuing increase in the prevalence of obesity and gestational diabetes among pregnant women (~30 %) [2, 3], a factor associated with both high birth weight newborns and a known risk factor for childhood obesity [4, 5]. Among women presenting for prenatal care, the incidence of obesity has doubled since 1980 [6]. Not only do women begin pregnancy at a higher body mass index, but, increasingly, women also gain excess gestational weight. Thus, clinicians caring for pregnant women are commonly caring for women who are overweight or obese.

As childhood obesity is a major risk factor for adult obesity [7], the 20 % incidence of childhood obesity [8] portends a further increase in the prevalence of adult obesity and diabetes mellitus [7]. Globally, the obesity observed in both developed and developing countries has been attributed to reflect societal, economic, and cultural problems. Accordingly, much attention has been focused on the role of environmental factors, including the availability of calorie-dense foods and lifestyles involving less physical work. However, these factors are unlikely to account for the dramatic increase in obesity during the past 60 years. This chapter presents evidence that the predisposition to obesity may be programmed or predetermined in utero.

The concept that the in utero environment programs obesity and obesity-associated disorders is supported by phenotype results of both human and animal studies and mechanistic insights from both in vivo and in vitro experiments. Based upon calculations of average cell weight, cell size, and organ growth, the newborn and adult body contain approximately 2.1×10^{12} and 3.7×10^{13} human cells [9], respectively. Beginning with a fertilized ovum, it requires over 41 cell division cycles to form a newborn and a total of 45 cycles for an adult. Thus, over 90 % of all cell divisions in a lifetime occur by the time of birth. It should, therefore, not be surprising that the maternal/fetal environment may alter cell signaling, epigenetic regulation, and organ development, with nutrient environment alterations specifically impacting energy regulating organs.

Initial experimental studies of fetal programming demonstrated that offspring exposed to maternal undernutrition during pregnancy were predisposed to developing adult obesity and metabolic syndrome, despite being born small-for-gestational age [10–13]. Maternal overnutrition, however, is perhaps more clinically relevant in today's Western society. The programming effects of maternal overnutrition may be associated with increased birth weight or newborn adiposity. Offspring of women with gestational diabetes are consistently larger than normal controls, with birth weight proportional to the mean maternal glucose levels

[14]. However, maternal overnutrition, resulting from increased pre- or early pregnancy body mass index or excessive gestational weight gain, has variable effects on birth weight [15–17]. Recent human studies have demonstrated that among these factors, maternal pre-pregnancy weight may be the most predictive of offspring obesity [18]. Whether the programming effects of gestational diabetes-associated macrosomia differ from that of maternal obesity alone is unknown at present. The Northern Finland Birth Cohort of 1986 demonstrated that the prevalence of overweight and abdominal obesity at age 16 years were highest in those exposed to both maternal prepregnancy overweight and gestational diabetes [overweight prevalence 40 % (odds ratio; OR 4.05), abdominal obesity prevalence 25.7 % (OR 3.82)]. Among mothers who were overweight prepregnancy, though not gestational diabetic, there remained an increased risk for offspring overweight and abdominal obesity (overweight OR 2.56, abdominal obesity OR 2.60). Surprisingly, in offspring of women with prepregnancy normal weight, the prevalence or risks of the outcomes were not increased by prenatal exposure to gestational diabetes [19]. Among a Jerusalem birth cohort of 1400 young adults (32 years of age), greater maternal prepregnancy BMI, independently of gestational weight gain, was significantly associated with higher offspring BMI, waist circumference, systolic and diastolic blood pressures, insulin, and triglycerides and with lower high-density lipoprotein cholesterol. Specifically, the offspring of mothers within the upper prepregnancy BMI quartile (>26.4 kg/m) have nearly a 5 kg/m higher BMI compared with the offspring of mothers with lower BMI quartile [20]. The development of metabolic syndrome among large-for-gestational-age (LGA) and appropriate-for-gestational age (AGA) children was examined in a longitudinal cohort study of Rhode Island (USA) children (age 6, 7, 9, and 11 years) born to mothers with or without gestational diabetes mellitus. Obesity (BMI >85th percentile) at 11 years was present in 25–35 % of the children, but rates were not different between LGA and AGA offspring. However, LGA status and maternal obesity increased the risk of metabolic syndrome approximately twofold [14]. In addition, exposure to maternal diabetes [OR 5.7 (95 % CI 2.4–13.4)] and exposure to maternal obesity [2.8 (1.5–5.2)] are independently associated with type 2 diabetes. These authors assessed that nearly 20 % of type 2 diabetes in youth could be attributed to intrauterine exposure to maternal obesity [21]. In addition to maternal obesity and maternal gestational diabetes, excessive gestational weight gain is associated with increased adiposity in offspring [22–24]. Regardless of the basis for overnutrition and whether birth weight is normal or increased, adult offspring of "overnourished" mothers consistently exhibit an increased risk of adult obesity and metabolic abnormalities [25–27], evidence of in utero programming.

Animal studies have begun to examine the mechanisms of fetal programming, with evidence that maternal obesity and a Western high-fat diet program fetal adipose tissue to promote increased adipogenesis, and hypothalamic neural pathways to promote appetite as compared to satiety [28]. Animal models of maternal overnutrition, including maternal obesity, replicate the human experience in that offspring are predisposed to adult obesity [28–30]. Several studies have confirmed that exposure to maternal high-fat diet during pregnancy may program adult

offspring obesity, hypertriglyceridemia and insulin resistance [28, 31, 32]. Furthermore, neonatal overnutrition (induced by nursing a smaller litter) also leads to rapid postnatal growth followed by hyperphagia and adult obesity [33]. This finding highlights the fact that interactions with the postnatal environment and neonatal growth rates may further modulate susceptibility to obesity, suggesting opportunities for prevention or mitigation.

Although a multitude of organ systems may be impacted by the maternal nutrient environment, this chapter focuses on the programming of appetite/satiety regulation and adipogenesis.

11.1 Appetite

Appetite regulation develops perinatally, and hence an altered environment during critical periods of development may program appetite and satiety mechanisms, thereby altering infant, childhood, and adult ingestive behavior. Numerous epidemiological and animal studies have demonstrated that in utero perturbations of the nutritional, hormonal, and/or metabolic environment as well as exposure to environmental toxins (e.g., endocrine disrupters) may alter the development of the appetite regulatory system, resulting in an increased risk of adult obesity.

Hypothalamic Sites of Appetite Regulation A complex circuit of hypothalamic nuclei regulate appetite and hunger, integrating actions of systemic and central appetite/satiety signals within several central regulatory sites. The predominant appetite regulatory site, the arcuate nucleus (ARC), receives input from peripheral (brain, pancreas, and adipocytes) and central sources [34]. The ARC contains two primary neuronal populations which regulate appetite: medial ARC orexigenic [NPY (neuropeptide Y) and AgRP (agouti-related protein)] and lateral ARC anorexigenic neurons [POMC (proopiomelanocortin) and CART (cocaine- and amphetamine-regulated transcript)]. Many of the ARC NPY/AgRP and POMC/CART neurons project to downstream neurons in the periventricular nucleus (PVN).

POMC neurons mediate anorexigenic responses by the release of alpha-melanocyte-stimulating hormone (α-MSH) which binds to PVN neurons expressing melanocortin-3 and -4 receptors (MC3/MC4-Rs). The orexigenic property of AgRP results from its competition with α-MSH at MC3/MC4-Rs. Among the five subtypes of NPY receptors, NPY-1R, which is expressed on PVN neurons, is primarily responsible for NPY-induced increases in food intake. In addition to the PVN, the ARC interacts with additional hypothalamic nuclei including the ventromedial nucleus, the lateral hypothalamus, the dorsomedial nucleus, and brainstem sites (e.g., locus coeruleus, nucleus of the solitary tract) [35, 36].

ARC neurons respond to blood-borne signals (e.g., leptin, insulin, and ghrelin) in addition to central neurotransmitters. Leptin is an adipose tissue peptide that is transported across the blood–brain barrier into the cerebrospinal fluid via a

saturable transporter system in the choroid plexus, a process that is mediated by a short form of the leptin receptor (ObRa) [37]. Insulin, which is secreted by the pancreas, gains access to the hypothalamus also by means of a saturable receptor-mediated process and diffusion from the median eminence [38]. Leptin serves as a long-term regulator of appetite/satiety and energy balance, whereas insulin acts more acutely as a satiety factor in response to meals. Both these anorexigenic factors stimulate the POMC/CART neurons and inhibit the NPY/AgRP neurons. Accordingly, ARC neurons exhibit high leptin and insulin receptor expression. Genetic mutations in the obese (ob) gene, which codes for leptin, or the diabetes (db) gene, which codes for the leptin receptor, lead to hyperphagia and obesity. Notably, central insulin deficiency also may result in hyperphagia.

ARC Neurogenesis ARC development is a relatively late process in rodents, rhesus monkeys, and humans, with final maturation not achieved until later stages of postnatal development [39]. However, the development of hypothalamic nuclei is initiated during fetal life, with continued neural development during the neonatal period [40, 41]. In rat hypothalamus, POMC neurons become detectable from gestational day E12.5, and NPY neurons first appear in the ARC at E14.5 [40]. A subpopulation of POMC neurons transition to NPY neurons; thus, the terminal peptidergic phenotype is not fully established until the postnatal period. Coinciding with ARC neuronal maturation, ARC projections in rodents are formed beginning in the second week of postnatal life [42].

The ARC neurons arise from neurogenic regions surrounding the third ventricle during fetal/neonatal life. During development, neuroprogenitor cells (NPCs) undergo extensive proliferation (two daughter NPCs), self-renewal (one NPC and one differentiated cell), and ultimate terminal division into cells destined for neuron, astrocyte, or oligodendrocyte fate [43]. NPCs migrate and populate the hypothalamic nuclei. The process of NPC differentiation, first to neurons, and secondly to the phenotype of appetite or satiety neurons, is regulated by a complex spatial/temporal interplay of pathways, including cell communication factors (e.g., Notch/Hes1), energy/nutrient sensors (e.g., SIRT1, AMPK), and a series of neuroregulatory basic helix-loop-helix (bHLH) transcription factors, such as Mash1 and Neurogenin-3 (Ngn3) [44], among others.

Following the differentiation of NPCs to neurons, those cells destined for the ARC further differentiate to express orexigenic (NPY, AgRP) or anorexigenic (POMC, CART) peptides. The transcription factor Mash1 is required for the normal development of POMC neurons [45]. Downstream from Mash1, Ngn3 also promotes the development of POMC neurons, while inhibiting NPY expression. Consistent with the critical role in ARC development, Mash1(−/−) mice demonstrate ARC hypoplasia with minimal expression of POMC neurons, while Mash1 (+/−) mice overexpress NPY neurons. Similarly, Ngn3(−/−) mice express markedly reduced POMC but increased NPY ARC neurons [46]. Thus, reductions in Mash1 and Ngn3 expression appear to shift ARC development toward a decrease in the POMC/NPY neuronal ratio [46]. Hes1, an upstream regulator which

promotes NPC proliferation while inhibiting differentiation, also acts as a transcriptional regulator (together with corepressors) of both Mash1 and Ngn3.

Mechanisms of Perinatal Appetite Programming Programmed appetite/satiety development resulting from maternal overnutrition may involve multiple interacting factors/pathways that influence offspring hyperphagia. Maternal and fetal hypothalamic energy/nutrient sensors may impact neuroendocrine signaling via inhibition/stimulation of transcription factors or epigenetic mechanisms. Subsequent effects on neurotrophic factors ultimately influence the final expression of hypothalamic ARC neuropeptides. Altered maternal nutritional alterations may bias the offspring regulatory network toward hyperphagia by increasing the production of and the sensitivity to the appetite peptide NPY and/or decreasing those of the satiety peptide POMC. Studies of programmed appetite in rodents demonstrate increased levels of hypothalamic NPY mRNA in fetuses [47] and adult offspring as well as in other regions (PVN and lateral hypothalamic area) [48]. Other studies demonstrate decreased ARC POMC Mrna [49]. In addition, exposure to gestational and lactational maternal diabetes also increases offspring NPY and AgRP as well as decreases POMC and α-MSH [50]. Overall, in response to maternal overnutrition, offspring exhibit an increased ratio of appetite to satiety gene expression, similar to that observed in response to Mash1 or Ngn3 knockouts (discussed above).

Both maternal and postweaning diets independently influence ARC formation, as hypothalamic development spans these periods. For example, maternal obesity combined with a postweaning high-fat diet increases ARC NPY signaling (PVN NPY1R), reduces POMC expression [51], and decreases sensitivity to leptin [26]. In response to a maternal high-carbohydrate diet, offspring demonstrate increased NPY release in the PVN [52]. Additionally, adult rats that are obese due to neonatal overfeeding demonstrate a reduced ARC neuronal response to leptin and insulin [53, 54]. Interestingly, rats that have a genetic predisposition to develop diet-induced obesity also have a preexisting reduction in central insulin sensitivity, and high-fat diets further reduce the sensitivity to insulin [55]. Notably, maternal undernutrition during gestation also causes offspring to have an impaired hypophagic response to insulin as adults [56].

Although insulin and leptin are important adult satiety factors, these peptides also have important roles during fetal life in the regulation of ARC neurogenesis. Both leptin and insulin induce NPC proliferation and promote NPC differentiation (dependent upon ex vivo NPC culture conditions). Exogenous insulin promotes cell growth and serves as a trophic factor in fetal neuronal cell culture [57]. Insulin potentiates greater NPC proliferation than does leptin and biases the differentiation of NPCs toward astrocyte lineage. Gestational diabetes may alter central insulin action either via central insulin resistance or potentially increased central insulin paralleling systemic levels. Either result may interfere with the ability of insulin to act as a neurotrophic factor, causing offspring to have impaired neuronal development specifically in the hypothalamic nuclei responsible for the regulation of appetite. These animals exhibit decreased neuronal cytoplasm in the ARC, the ventromedial nucleus, and the parvocellular division of the PVN, as well as an

increase in the glia-to-neuron ratio in the periventricular region of the hypothalamus [58]. In rat fetal brain cell culture, axonal growth is stimulated in response to insulin medium.

Following the observations that animals which are leptin-deficient or leptin-insensitive have decreased brain size and development [59], leptin has been recognized as a major neurotrophic factor during the development. In contrast to insulin, leptin promotes neuronal lineage [60], *ob*/*ob* mice have been shown to have significantly higher levels of oligodendrocyte precursor cells than wild-type mice [61], confirming the role of leptin as a regulator of neural progenitor fate. Leptin is well recognized to impact the postnatal development of ARC projection pathways [62]. Leptin preferentially increases AgRP/NPY inputs to the PVN, while POMC inputs are largely leptin independent [63]. In rodents, the leptin surge during the second postnatal week correlates with ARC to PVN axonal projections [64, 65] and influences ARC neuronal development [49]. Leptin-deficient (ob/ob) mice exhibit anorexigenic pathway axonal densities markedly less than controls [62]. Leptin treatment of *newborn* may rescue ARC projections [62]; however, offspring hyperphagia is not normalized, indicating that neural projections alone are not fully responsible for dysregulated energy intake.

Due to temporal specificity of neurodevelopment, critical neurotrophic or neurodifferentiation factor alterations may have varying effects dependent upon the gestational or newborn age. Thus, treatment of *ob*/*ob* leptin-deficient mice with leptin only restores ARC projections to the PVN if administered during postnatal days 4–12 [62]. In contrast, in control pups, leptin given between postnatal days 1 and 10 actually results in adult hyperleptinemia, leptin resistance, increased food intake, and excess body weight [66]. Thus, neonatal leptin excess can actually induce obesity. As macrosomic human infants have disproportionately elevated leptin levels [67, 68] in proportion to body weight and adiposity [69], it is feasible that neurogenesis is perturbed during both fetal and neonatal life. These findings point to the critical role of maternal nutrition preconception and during pregnancy/lactation as well as the longer term consequences of fetal macrosomia.

11.2 Adipose Tissue

In parallel to programming of appetite, maternal obesity/overnutrition may program fetal adipogenesis and lipogenesis. Adipogenesis, the process of cell differentiation by which preadipocytes become adipocytes, requires highly organized and precisely controlled expression of a cascade of transcription factors [70, 71] which, similar to neural development, may be influenced by the nutrient environment. Increase in adipose tissue mass or adipogenesis occurs primarily during the prenatal and postnatal development, though some adipogenesis continues throughout adulthood [72].

Adipogenesis The cellular development associated with adipose tissue growth involves both cellular hyperplasia (increase in cell number) and hypertrophy (increase in cell size) [72]. Hyperplasia (adipogenesis) involves the proliferation and differentiation of preadipocytes, whereas hypertrophy is the result of excess triglyceride accumulation in existing adipocytes due to a positive energy balance [73–75].

Adipogenesis occurs when committed preadipocytes (adipocyte precursor) undergo processes of differentiation, lipogenesis, and lipid accumulation to form mature adipocytes [76, 77]. Preadipocyte factor (Pref-1) maintains the stem pool and suppresses adipocyte differentiation by induction of its downstream target SOX9 (sex determining region Y-box 9) [78–80]. SOX9 in turn directly binds to the promoter regions of adipogenic transcription factors (CCAAT/enhancer binding family of proteins C/EBPβ and C/EBPδ) to inhibit their promoter activity, preventing adipocyte differentiation [80]. In contrast, upon suppression of SOX9, preadipocytes differentiate to adipocytes [81] with coordinated interaction of several adipogenic transcription factors (C/EBPβ, C/EBPδ, C/EBPα) [82, 83] which activate the peroxisome proliferator-activated receptors (PPARs), especially PPARγ_2 [84]. The principal adipogenic transcription factor, PPARγ_2, induces lipogenic transcription factor SREBP1 (sterol regulatory element-binding protein), thereby initiating both adipocyte differentiation and lipogenesis [85–87]. SREBP1 can also activate PPARγ, by both stimulating the production of an endogenous ligand [88] and by inducing PPARγ promoter activity [85, 88].

Lipogenesis The downstream targets of PPARγ and SREBP1 include lipogenic and lipolytic enzymes. The induction of lipoprotein lipase [89, 90] promotes fatty acid delivery to adipocytes, while induction of fatty acid transport protein [91] and acyl-CoA synthetase [92] results in enhanced fatty acid uptake by the adipocyte. These actions contribute to enhanced triglyceride synthesis and accumulation in adipose tissue [93]. The release of free fatty acid from adipocytes is facilitated by an intracellular lipolytic enzyme, hormone-sensitive lipase [94].

In addition to fatty acid uptake and storage, synthesis of fatty acids (via de novo lipogenesis) and triglycerides are equally important factors in fat accumulation. Triglycerides destined for fat storage in adipose tissue are composed of fatty acids from dietary sources and from de novo synthesis. De novo synthesized fatty acids can undergo modification through creation of double bonds via desaturation and/or further lengthening via chain elongation. While de novo synthesis and chain elongation promote energy storage, breakdown of fatty acids by chain shortening and β-oxidation promotes energy release. Since triglycerides become incorporated into adipose tissue for storage, an increase in the monounsaturated to saturated fatty acid ratio, therefore, increases propensity for fat storage [95]. Specifically, monounsaturated fatty acids which are the preferred substrate for triglyceride synthesis are a product of endogenous (de novo) synthesis from saturated fatty acid precursors [96]. The conversion of precursor (saturated) to product (monosaturated) fatty acids is catalyzed by the lipogenic enzyme, stearoyl-CoA desaturase enzyme-1 (SCD-1), which introduces a double bond at the Δ9 position. The product-to-

precursor ratio represents the desaturation index and which directly reflects SCD-1 expression/activity [97]. Notably, both the desaturation index and SCD-1 activity correlate with measures of adiposity [98].

Mechanisms of Perinatal Adipose Programming Mechanisms of perinatally programmed adipose tissue resulting from maternal overnutrition may involve multiple interacting factors/pathways that influence offspring adiposity and metabolic abnormalities. These include altered adipogenic and lipogenic signaling pathways, cellular growth and differentiation, regulatory hormones, energy sensors, and/or epigenetics. Altered maternal nutritional alterations may bias the offspring regulatory network toward increased triglyceride storage by promoting adipocyte number, adipocyte size, and/or availability of substrate for triglyceride synthesis.

Various animal models of maternal overfeeding and obesity (i.e., high-fat, high calorie, or cafeteria diet) before and/or during gestation and/or lactation have been utilized. Adipogenesis programming may occur in the presence or absence of increased newborn birth weight. In most cases, offspring of obese dams are predisposed to postnatal obesity. In rodent and sheep models of maternal obesity, enhanced offspring adipogenesis has been demonstrated in fetal life [99, 100] and in adults [101]. Programmed adipogenesis has primarily been attributed to upregulated PPARγ [27, 99, 102, 103]. Additional changes that facilitate triglyceride storage include adipocyte hypertrophy [27, 99, 100] enhanced lipogenic pathway as demonstrated by increased expression of lipogenic factors and enzymes (SREBP1, fatty acid synthase) [104], and reduced lipolytic capacity as evident by decreased expression of adrenoreceptors (β2, β3) [27], and hormone-sensitive lipase [105].

Metabolic Consequences of Programmed Adipose Tissue Adipokines secreted by adipose tissue regulate multiple metabolic pathways. For example, leptin regulates appetite, adiponectin and resistin impact on insulin sensitivity, TNFα and IL-6 are associated with inflammation, and angiotensinogen contributes to hypertension [106, 107]. Hence, increased adiposity causes aberrant adipokine secretion and subsequently a metabolic syndrome-like phenotype.

Maternal obesity/high-fat diet causes hypertensive phenotype in the high-fat offspring [28, 108], which may, in part, be mediated by the adipose renin-angiotensin system. Studies indicate that all components of the renin-angiotensin system are expressed in white adipose tissue from rodents [109, 110] and humans [111, 112], suggesting that the adipogenic RAS may be involved in the pathogenesis of obesity-related hypertension. Accordingly, studies have shown that adipose-derived angiotensinogen (precursor of vasoactive angiotensin II) can contribute to approximately 20 % of plasma angiotensinogen concentrations and can modulate blood pressure [112]. Overexpression of adipose angiotensinogen in mice induces hypertension with increased body fat and plasma angiotensinogen levels, indicating that an increased adipose tissue mass may result in higher circulating angiotensinogen levels, a finding confirmed in obese individuals [113].

Maternal obesity/high-fat diet is also associated with insulin resistance [28] and inflammation [101] which may in part be attributed to inflammatory adipokines

resulting from infiltration and expansion of macrophage [114]. Specific gene analysis of adipose tissue showed that the offspring exposed to maternal high-fat diet had increased mRNA expression of pro-inflammatory cytokine (TNFα), macrophage (CD68, MCP-1), and decreased glucose transporter (GLUT4) expression, suggesting that maternal obesity may affect fetal insulin sensitivity by altering inflammatory processes [25, 115].

11.3 Studies in Our Laboratory

We have examined both the phenotype and mechanisms of programming of maternal obesity/high-fat diet in rodent studies. Young female rats were weaned to a high-fat diet (60 % kcal) and continued on this diet throughout pregnancy and lactation [28]. Our results indicate a marked impact of maternal obesity/high-fat diet on offspring body composition and the risk of metabolic syndrome. Despite normal birth weight, all adult offspring mothers fed a high-fat diet exhibited increased body fat even those that received normal nursing by control mothers (i.e., exposure restricted only during pregnancy). This was accompanied by reduced lean body mass in all offspring of mothers fed a high-fat diet, particularly in those offspring that were exposed to maternal obesity/high-fat diet during pregnancy alone. Thus, in these offspring, increased body fat with a marked decreased in lean body mass resulted in normal body weight. These findings are similar to that described in humans, particularly in the Indian subcontinent, of the "thin-fat" phenotype in which individuals have a normal body weight, but markedly increased body fat and reduced lean body mass. Importantly, it was subscapular rather than abdominal visceral fat that was the preserved, suggesting increased subcutaneous adipose tissue. Similarly, Zambrano et al. [116] showed increased subcutaneous fat in offspring exposed to maternal obesity during fetal and nursing period.

Programming of Appetite Our studies show that increased offspring food intake appears to be a major contributor to excess body weight in males, though not in the females [28]. It is likely that in the female offspring either metabolic efficiency and/or energy expenditure may be programmed by maternal dietary changes in pregnancy and/or lactation. Consistent with this conclusion, studies on rodents and sheep offspring suggest a programming effect of maternal diet on energy expenditure in adult offspring [117].

In our studies, the hyperphagia exhibited by male offspring of mothers fed a high-fat diet is a result of programmed changes in appetite regulatory signal factors. At birth, the high-fat newborn males already have increased protein expression of hypothalamic appetite neuropeptide AgRP. Importantly, with continued exposure to maternal high-fat diet during nursing period, these offspring exhibit persistent increased AgRP with now decreased expression of satiety neuropeptide POMC, indicating impairment of both appetite and satiety pathways. To further examine mechanisms of nutritional programming, we determined hypothalamic protein

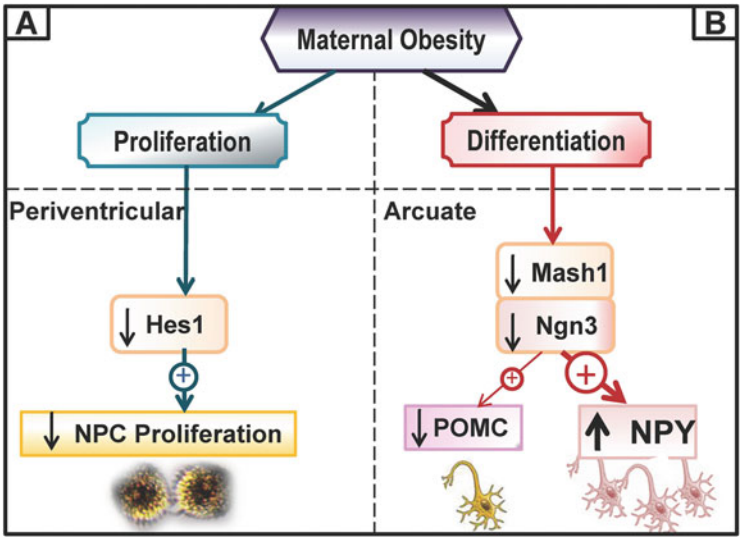

Fig. 11.1 Neural Progenitor Cell (NPC). (**a**) *Hes1*: Decreased Hes1 inhibits proliferation and reduces NPC pool. (**b**) *Mash1 and Ngn3*: Decreased Mash1 and Ngn3 promote NPY versus POMC neurons

expression of NPC proliferative/neurogenic factors implicated in production of AgRP/POMC neurons. Newborn males of mothers fed a high-fat diet had significantly decreased Hes1 and Mash1 though unchanged Ngn3. As adults, high-fat males showed decreased Hes1, Mash1, and Ngn3. These findings indicate an alteration in bHLH factors which regulate ARC neurogenesis in response to maternal high fat (Fig. 11.1).

As discussed above, leptin, which serves as a hypothalamic modulator of appetite/satiety in the adult, also has critical neurotrophic role during fetal life in the development of ARC pathways [118]. Our studies and others demonstrate that despite normal birth weight, high fat pups had lower plasma leptin levels [28, 119], which may play a role in developmentally programmed dysfunction of appetite/satiety pathways.

Programming of Adipogeneis and Lipogenesis Our studies show that enhanced adipogenesis also contributes to increased adiposity in the offspring of maternal obesity/high-fat diet pregnancies. The programmed changes include increased expression of adipogenic (PPARγ) and lipogenic transcription factors (SREBP1) in newborn and adult adipose tissue [120, 121] as well as increased expression of enzymes mediating fatty acid biosynthesis (fatty acid synthase, SCD-1) [104] (Fig. 11.2). The regulation of PPARγ transcription is not only dependent upon the energy status [122] and the availability of PPARγ ligands [123] but also by the presence of co-regulators [93, 124]. Our studies indicate that co-regulators of PPARγ are impacted by maternal obesity/high-fat diet. In the high-fat offspring,

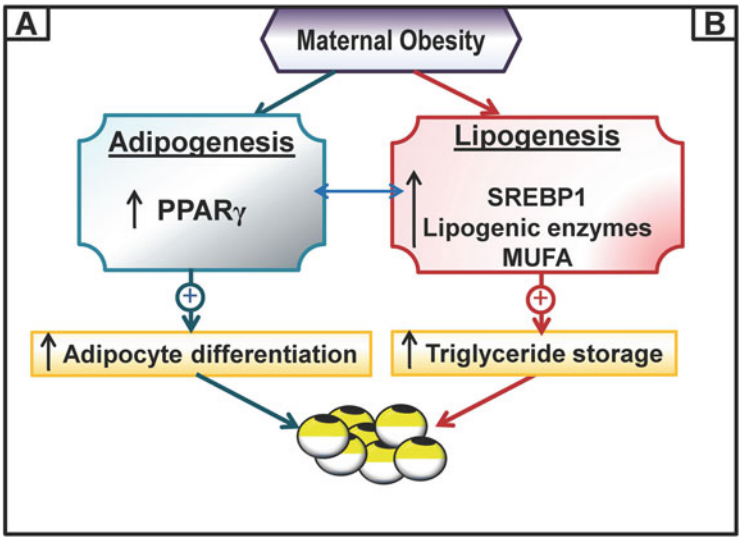

Fig. 11.2 Adipose Tissue. (**a**) *Adipocyte Differentiation*: Increased PPARγ promotes adipocyte differentiation and induces lipogenesis. (**b**) *Lipogenesis*: Increased lipogenic transcription factor (SREBP1), and enzymes (fatty acid synthase, SCD-1), and increased monounsaturated fatty acids (MUFA) facilitate triglyceride storage

the downregulation of co-repressor proteins and upregulation of co-activator are consistent with increased expression of PPARγ and their obese phenotype [125].

Contributing to adipogenesis/lipogenesis, maternal obesity/high-fat diet may markedly alter plasma fatty acids. We have shown that high-fat adult offspring demonstrate elevated plasma triglyceride levels, irrespective of period of exposure [28]. We have further explored mechanisms for programmed adiposity resulting from perturbations in fatty acid metabolism that promote fatty acid availability and triglyceride synthesis for fat storage. Our results demonstrate that exposure to maternal obesity/high-fat diet programs offspring fatty acid metabolism, providing another pathway leading to obesity. The adult offspring from maternal obesity/high-fat diet had increased plasma and liver desaturation index with upregulated liver SCD-1 protein expression, consistent with their obese phenotype [126].

We have further demonstrated that a high-fat diet during pregnancy and/or lactation is sufficient to induce upregulation of the rat adipose tissue angiotensinogen, likely contributing to the hypertensive phenotype [127]. Given the current global obesity epidemic and the increasing prevalence of obese women of reproductive age, obesity-mediated hypertension may present additional complication.

11.4 Conclusions: Clinical Implications and Conclusions

A major public health challenge in the twenty-first century is to devise an effective policy and practice to combat the epidemic of obesity across all spectrums of age groups. Prevention of childhood obesity remains a high priority for many health professionals. There is irrefutable evidence that departures from optimal growth in utero, whether from limited or excess nutrition, increase the relative risk of adult obesity and metabolic syndrome, in part a result of programming of both appetite and adiposity. Although food intake is a critical survival function, appetite and satiety demonstrate a remarkable heterogeneity among humans, with a spectrum contributing to both anorexia and hyperphagia. Whereas obesity was often viewed as dietary indiscretion combined with reduced energy expenditure, it is now recognized that enhanced appetite and adipogenesis may predispose to obesity. The maternal and thus fetal nutritional environment may respond to altered energy/nutrient levels during critical embryological and developmental periods so as to alter neurogenesis and adipogenesis. Through the interplay of extracellular signaling factors, intracellular transcription responses, and nutrient-induced epigenetic alterations, the maternal environment can program fetal/newborn energy pathways resulting in a predisposition toward obesity. This predisposition is especially paramount within a postnatal environment that facilitates catch-up or even excess neonatal growth as well as access to energy-intense childhood and adult diets. Collectively, these findings have great significance for prenatal, neonatal, and childhood care.

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Chapter 12
Developmental Programming of Nonalcoholic Fatty Liver Disease (NAFLD)

Kimberley D. Bruce and Felino R. Cagampang

Abstract Nonalcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease worldwide and is present in a third of the general population and the majority of individuals with obesity and type 2 diabetes. The less severe form of the disease is relatively common and can be somewhat benign. However, in certain individuals, the disease can progress to the more severe nonalcoholic steatohepatitis (NASH), resulting in a poor health, a poor prognosis, and a significant healthcare burden. In recent years, there has been a major research effort focused on identifying the factors that promote NALFD disease progression, and as a result there has been a significant advancement in our understanding of the interaction between nutrition and the molecular mechanisms that regulate hepatic lipid homeostasis. Nonetheless, the capacity of the maternal diet to alter these fundamental metabolic pathways and thus prime the development of severe fatty liver disease in the adult liver has proved to be one of the most striking findings from this body of research. Since the prudence of the maternal diet has wavered in recent years, this may explain why NAFLD—once commonly associated with older individuals—is now increasingly common in young adults, children, and adolescents. In the following chapter, we aim to review the current hypothesis surrounding the mechanisms that underlie the developmental priming of NAFLD. We will also explore how these novel insights have facilitated the emergence of promising new pharmacological and nutritional intervention strategies.

Keywords Nonalcoholic fatty liver disease (NAFLD) • Nonalcoholic steatohepatitis (NASH) high-fat diet • Pregnancy • Epigenetics • Circadian clock • Developmental priming

K.D. Bruce
Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO 80045, USA

F.R. Cagampang (✉)
Institute of Developmental Sciences, University of Southampton Faculty of Medicine, Southampton General Hospital (Mailpoint 887), Tremona Road, Southampton SO16 6YD, UK
e-mail: f.cagampang@soton.ac.uk

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12.1 The NALFD Spectrum

Nonalcoholic fatty liver disease (NAFLD), which was once thought of as a passive metabolic condition, describes a spectrum of disorders characterized by the accumulation of ectopic fat accumulation in the liver without significant alcohol use. At one end of the spectrum is simple steatosis, often termed NAFLD. Although many individuals with NAFLD remain stable, 25 % of these patients can progress to steatosis with inflammation, termed nonalcoholic steatohepatitis (NASH) [1]. This more severe form of the disease (NASH) can progress further still, with a significant proportion of individuals developing fibrosis (26–37 %) and cirrhosis [2]. NASH cirrhosis can eventually result in portal hypertension, liver failure, and ultimately death. Interestingly, a number of recent studies have shown that NASH cirrhosis is linked to hepatocellular carcinoma (HCC) [3]. Although the precise link between NAFLD and HCC is currently under investigation, early findings suggest that it involves alterations in major pathways that regulate hepatic metabolism, such as insulin resistance and cellular lipid metabolism. Since the development of NAFLD and HCC involves perturbations in the same molecular pathways, they are likely influenced by the same metabolic disorders such as obesity and type 2 diabetes (T2D). The rising prevalence of obesity-related disorders in many industrialized countries raises huge concerns regarding the concurrent rising incidence of NAFLD and HCC.

12.2 3-Hit Hypothesis

The precise interplay between factors that promote disease progression is still under investigation. Nonetheless, it is hypothesized that a 3-hit mechanism is involved in the pathogenesis and progression of NAFLD. The “1st hit” consists of hepatic triglyceride accumulation that may result simply from dietary or lifestyle factors. The “2nd hit” may include factors that promote disease progression such as pro-inflammatory cytokines, which in turn lead to steatohepatitis and/or fibrosis [4]. Recent work suggests that there are in fact a multitude of factors that may act as the 2nd hit to promote liver disease progression, including diets rich in saturated fat and cholesterol, diets low in polyunsaturated fat and fiber, diets during development, epigenetics, circadian rhythms, and disturbances in intestinal microbiota.

12.3 NAFLD Is the Hepatic Manifestation of the Metabolic Syndrome

The metabolic syndrome is a cluster of cardiometabolic conditions, which include obesity, insulin resistance, high blood pressure, and atherogenic dyslipidemia [5]. The present definition of metabolic syndrome does not include hepatic steatosis despite growing evidence suggesting that NAFLD is the hepatic manifestation of the metabolic syndrome [6]. While the existence of the metabolic syndrome remains controversial, features of the metabolic syndrome tend to aggregate in the same individuals, and over time the presence of multiple factors anticipates the onset of additional components [7]. Thus, the metabolic syndrome, and recognition of NAFLD as a primary feature, may serve an important utility in clinical practice as a predictor of progressive NASH and cardiometabolic disease.

12.4 The Incidence and Prevalence of NAFLD and NASH

The prevalence of NAFLD in the general population is variable and ranges from 9 to 37 % [8–10]. Current estimates state that NAFLD is the most common etiology of chronic liver disease in the USA and other developed countries [11, 12]. Specifically, in the USA recent estimates suggest that NAFLD affects 30 % of the general population, 58 % of overweight people, and 90 % of individuals who are considered morbidly obese [13]. As suggested by the natural history of NAFLD, the proportion of individuals with NASH is much lower and has been estimated to affect 5–7 % of the general population and as much as 34–40 % of patients who have elevated liver enzymes [14]. With the global rise of obesity, it is predicted that there will be greater rates of NAFLD progression and that NAFLD will be the most common etiology for liver transplantation in the twenty-first century [15].

12.5 Pediatric NAFLD

An increasing number of younger individuals are being diagnosed with NAFLD [16]. Recent estimates suggest that in Western societies, the number of children with NAFLD ranges from 3 to 10 % in the general population, and up to 70 % in children who are considered obese [17]. Alarmingly, the number of adolescents diagnosed with NAFLD has more than doubled in the last two decades [18], and like adults, pediatric NAFLD can also follow a severe disease progression to cirrhosis and end-stage liver disease [19], which is also predictive of features of the metabolic syndrome and intramyocellular lipid deposition [20]. Also similar to adults, both sex and race can be a risk factor for NAFLD onset, and its development appears to be more common in boys than girls [21]. However, unlike adults, in

pediatric NAFLD there is a unique deposition of fat in the periportal region [22]. While this difference is not well understood, it is clinically significant since periportal inflammation is often associated with more severe liver disease [23].

There is now a significant body of research which suggests that features of the metabolic syndrome including NALFD may have their origins very early in life. In humans, the liver itself begins to develop in the fetus at 4 weeks of gestation with the formation of the hepatic bud from the ventral endoderm, and gross morphogenesis is completed by the end of the first trimester with refined cellular development continuing throughout gestation [24, 25]. A plethora of genes and their transcription factors are involved in the development of metabolic processes, including gluconeogenesis, glycogenolysis, lipid oxidation, and de novo lipogenesis, and are already expressed in the fetal liver although not highly expressed until after birth [26]. The liver is also primary location of hematopoietic development from week 6 to 21 of gestation, with hematopoietic stem cells accounting for 60 % of total liver mass during peak hematopoiesis followed by regression to the fetal bone marrow by term [27]. The developing liver is therefore in a constant flux throughout gestation and is susceptible to adverse environment during this critical period of development such that the growing organism may undergo changes in its fundamental metabolic pathways in an attempt to adapt to its environment. Many of these changes persist into adult life and can increase the susceptibility of developing metabolic disease in later life stages. Thus, the metabolic health of the mother, whether inherent or acquired through imbalanced diet, may lead to a transgenerational amplification of metabolic disease, including NAFLD.

12.6 Conundrum of NAFLD Susceptibility in the Offspring: Is the "1st Hit" Down to Maternal BMI or Maternal Diet During Pregnancy?

Studies conducted in various animal models, including rodents, sheep, and nonhuman primates, have reported that consumption of a Western-style diet, mostly high-fat diet (HFD) during pregnancy, significantly increase NAFLD susceptibility in the adult offspring [28]. This is of particular relevance to humans in today's society, where abundance of food high in fat and calories coincides with increasing obesity epidemic that is occurring at a younger age. It is no coincidence that this epidemic is correlated with increasing number of obese women becoming pregnant and the onset of obesity-associated morbidities [29–31]. Although there is clearly an association between maternal obesity and subsequent childhood adiposity [32–35], it remains uncertain whether it is the consumption of HFD or the resulting obesity that leads to the development of NAFLD in the offspring.

On one hand, chronic consumption of a HFD, independent of maternal obesity and gestational diabetes, has been suggested to significantly increase the risk of NAFLD in the offspring. In a study conducted in a nonhuman primate, the Japanese

macaque, fetal offspring from both lean and obese mothers chronically consuming a HFD had significantly elevated liver triglycerides (TGs), suggesting an increased maternal lipid transfer to the fetus regardless of maternal obesity [36]. This was further substantiated by their findings of no change in mRNA or protein expression of lipogenic enzymes involved in de novo lipogenesis. These results therefore suggest that a developing fetus is highly vulnerable to excess lipids, independent of maternal obesity, increasing offspring risk to NAFLD. It was suggested that the increased lipid buildup in the fetal liver can cause lipotoxicity leading to increased macrophage infiltration and inflammatory cytokine production, the result of which causes premature gluconeogenic gene expression, steatosis, elevated triglyceride content, and oxidative stress that could persist into the postnatal period [37]. In another study, it was also suggested that maternal HFD feeding could increase apoptosis in the developing fetal liver contributing to the priming of the liver to NAFLD in later life [38]. However, others have countered that it is maternal obesity, and not the consumption of the HFD per se, which is the primary mechanism driving NAFLD susceptibility in the offspring. In a study conducted in rats, females were subjected to total enteral nutrition-based overfeeding to bypass the satiety response that limits ad libitum food intake, causing them to become obese prior to mating and this resulted in their offspring to be more prone to becoming obese when fed postnatally with a HFD [39]. In another study, rats dams fed a HFD but restricted to the caloric intake of pair-fed low-fat diet (LFD) mothers failed to become obese, and this prevention of maternal obesity resulted in normal body weight in the adult offspring [40]. Conversely, ad libitum maternal HFD feeding resulted in obese dams whose offspring were heavier in adulthood than offspring of non-obese dams. Although these studies show that maternal obesity rather than the HFD itself increased offspring body weight, it remains to be determined how this may lead to increased lipid accumulation in the offspring liver.

It is difficult to investigate NAFLD in neonatal studies due to the invasive nature of its definitive diagnosis. Thus, these kinds of studies have mainly been conducted in animal models, where maternal obesity is associated with NAFLD even before birth [28, 41, 42]. Evidence for a direct association between maternal obesity and offspring hepatic lipid accumulation in humans only recently came to light with the use of imaging technologies as a noninvasive means to screen for steatosis in newborn infants [43–45]. Maternal obesity is not only associated with greater morbidities in the mother but may also be responsible for accelerated hepatic fat accumulation in the offspring during early-life development. Interestingly, the aforementioned studies in the newborns found that neonatal hepatic fat did not correlate with newborn adiposity, suggesting that the drivers for hepatic fat storage and subcutaneous fat may be different and that factors associated with maternal obesity, such as excess serum lipids, could be associated with newborn hepatic fat accumulation [44, 45].

Pregnancies complicated by maternal obesity are often associated with gestational diabetes, which could serve as the catabolic switch that increases serum lipid levels and enhances placental lipid transport [46–48]. This excess lipid exposure may therefore utilize the fetal liver as ectopic sites of fat deposition and could

promote metabolic and cellular stress and inflammation in an organ not yet competent in handling such substrate overload.

12.7 The Role of Mitochondrial Dysfunction in Developmentally Primed NAFLD

Mitochondria are essential organelles that process glycolysis and lipolysis products to generate the cellular energy carrier ATP. They are the main energy source in hepatocytes and play a major role in oxidative metabolism and normal function of the liver. Mitochondria regulate cellular lipid metabolism, amino acid metabolism, cell proliferation, ion homeostasis, and even cell death pathways via reactive oxygen species (ROS) production. Therefore, it is no surprise that suboptimal mitochondrial function has been implicated in the development of chronic liver diseases including HCC and the NAFLD spectra. The mechanisms leading to altered mitochondrial energy metabolism and characterization of the transcriptional pathways that regulate mitochondrial biogenesis and function have been the subject of intense research focus. Recent findings have advanced our understanding and may offer important insights into possible therapeutic interventions aimed at improving hepatic pathophysiology.

In the fed state, food-derived NADH or flavin adenine dinucleotide (FADH2) acts as a hydrogen or electron donor and transfers the hydrogen/electron to an O_2 molecule, via redox components in the electron transport chain (ETC) complexes. This "oxidative phosphorylation" occurs in the inner mitochondrial membrane, where the majority of electron donors and acceptors are found, including cytochrome b, cytochrome b562 and b566, in ETC complex III [49]. In times of increased energy intake and metabolic demands, increased mitochondrial β-oxidation enhances the formation of NADH and FADH2 and increases the delivery of electrons to the ETC. Such an increase in electron flow through the ETC causes the buildup and leakage of electrons and ROS production [50]. Overproduction of ROS is considered as a major pathogenic agent of many metabolic diseases, including NAFLD.

The current hypothesis regarding the pathogenesis of NASH suggests that multiple "hits" are required for the disease to progress. While the "1st hit" may involve accumulation of fat in the liver, a growing body of evidence suggests that the second hit involves oxidative stress, lipid peroxidation, the production of malondialdehyde, 4-hydroxynonenal, pro-inflammatory cytokines, stellate cell activation, and fibrogenesis [51]. It is now fairly well established that mitochondrial dysfunction may be involved in at least one of these hits, due to their central role in the β-oxidation of free fatty acids (FFAs), ROS production, and lipid peroxidation [52]. In fact, a number of studies have reported defects in mitochondrial ETC enzymes in individuals diagnosed with NASH. Specifically, in patients with NASH, the activity of the ETC enzymes is markedly reduced and correlates

significantly with pro-inflammatory markers [53]. In addition, NAFLD is associated with ultrastructural mitochondrial abnormalities and depletion of mitochondrial DNA. Such changes in mitochondrial DNA have been shown to further suppress the expression of mitochondrial respiratory complexes I, III, IV, and V and exacerbate mitochondrial dysfunction [54]. While there are clear associations between mitochondrial dysfunction and NALFD pathogenesis, the mechanisms leading to improperly functioning mitochondria are not fully understood. It is plausible to suggest that nutrient excess may lead to increased ROS-mediated lipid peroxidation and mitochondrial dysfunction. In support of this hypothesis, NAFLD has been observed in the liver of obese sedentary and hyperphagic rats, characterized by reduced fatty acid oxidation, decreased cytochrome c protein content, and decreased carnitine palmitoyl-CoA transferase (CPT-1) activity [55]. Thus, it is thought that positive energy balance and nutrient excess may play a key role in NAFLD onset and progression.

Curiously, mitochondrial respiratory chain disorders are an established cause of liver failure in early childhood but have been underdiagnosed, partly due to underrecognition and partly due to the invasive nature of the investigations [56]. Since hepatic mitochondria are of maternal origin, they are a likely candidate vector for maternally inherited metabolic stress. Thus, mitochondria may be considered an important conduit for metabolic disease and a target for investigations into metabolic perturbations in offspring of obese mothers. Indeed, in recent years there has been a plethora of studies highlighting the role of mitochondrial dysfunctional in the molecular pathogenesis of developmentally primed NAFLD. Studies in rats have shown that adult offspring of mothers exposed to a HFD prior to conception, and throughout gestation and lactation, develop insulin resistance and features of NAFLD [57]. Similarly in mice, offspring of mothers fed a HFD, who are also fed a HFD in postnatal life, develop a more severe liver phenotype akin to human NASH [28]. In these studies, maternal HFDs have been linked to reduced ETC activity, which when coupled with further HFD challenge exceeds the liver's oxidative capacity, resulting in excessive fat accumulation and increased de novo lipogenesis, reduced β-oxidation, and inflammation [28].

These initial studies initiated an intensive research effort aiming to understand how maternal diets interact with mitochondrial function to reduce oxidative capacity. A number of studies have highlighted the role of mitochondrial Sirtuins and the acetylation of mitochondrial proteins in metabolic disease and aging. Mitochondrial protein acetylation regulates a number of enzymes involved in the TCA cycle, gluconeogenesis, and β-oxidation and is regulated (at least in part) by the mitochondrial class III NAD+-dependent deacetylate Sirtuin 3 (SIRT3) [58]. Since no significant changes in mitochondrial acetylation are observed in mice lacking both SIRT4 and SIRT5, SIRT3 is thought to be the primary mediator of mitochondrial protein acetylation [59]. Since mitochondrial acetylation is sensitive to nutrient status, and can be modulated in times of caloric restriction [60], SIRT3 seems a likely mediator of nutrient-derived mitochondrial stress. In fact, chronic (up to 16 weeks) feeding of a HFD has been reported to reduce SIRT3 activity and cause a threefold decrease in hepatic NAD+ levels. Chronic HFD feeding results in

hyperacetylation of mitochondrial proteins, which is associated with reduced protein activity and mitochondrial function. Interestingly, mice lacking SIRT3 demonstrate even greater hyperacetylation of mitochondrial proteins under HFD conditions and show a marked disruption in mitochondrial oxidative phosphorylation and ETC complex activity [60]. Importantly, this reduction in SIRT3 activity and abundance can be passed to the subsequent generation. For example, offspring of obese mothers have significantly reduced SIRT3 gene and protein expression [61]. One of the major consequences of reduced SIRT3 expression is reduced mitochondrial β-oxidation [61]. It is possible that impaired mitochondrial oxidative capacity creates a shunt of intermediary metabolites toward lipid storage and/or de novo lipogenesis, thus contributing toward the development of NASH in the offspring of obese mothers.

While the mechanisms leading to reduced SIRT3 activity are currently under investigation, a plausible explanation involves an altered availability of the essential cofactor NAD+, which in turn is able to directly affect SIRT3 function and abundance. This has implications not only for SIRT3 but for other NAD+-dependent Sirtuins, such as SIRT1, a protein long associated with metabolic health and longevity. During fasting, there is an increase in pyruvate and NAD+ levels that is able to facilitate SIRT1 activity and increase protein levels and (PMID: 15744310). Although it plays a number of intracellular roles, SIRT1 is also associated with mitochondrial function. When NAD+ levels are favorable, SIRT1 deacetylates and activates peroxisome proliferator-activated receptor alpha (PPAR-α), which in turn transcriptionally activates a number of genes associated with mitochondrial biogenesis, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG1α) [62], and mitochondrial oxidative metabolism [63]. Although the mechanism is not fully understood, it appears that PPAR-α and SIRT1 act upstream of a number of factors that are heavily associated with the onset of hepatic steatosis. Interestingly, mice that lack PPAR-α develop severe hepatic steatosis during fasting, an observation which is consistent with reduced mitochondrial capacity [64]. On the other hand, viral-mediated overexpression of SIRT1 is able to induce a gene cassette associated with a healthy, non-fatty liver. This included downregulated expression in a number of "lipogenic" genes associated with increased lipid accumulation, such as sterol regulatory element-binding protein 1c (SREBP-1c), fatty acid synthase (FASN), and the elongation of very long chain fatty acids protein 6 (ELOLV-6) [65].

Several studies have shown that a maternal HFD feeding and maternal obesity are both able to downregulate SIRT1, thus preventing the antagonism of lipogenic transcription factors and contributing to the developmental priming of fatty liver. In a recent study, in utero exposure to a maternal HFD, but not obesity per se, was linked to a decrease in SIRT1 gene expression and in vitro protein deacetylase activity in the offspring liver [66]. Moreover, a maternal HFD was associated with altered expression of SIRT1-regulated downstream lipogenic effectors, such PPAR-α, PPAR-γ, sterol regulatory element-binding protein F1 (SREBF1), cholesterol 7alpha-hydroxylase (CYP7A1), FASN, and stearoyl-CoA desaturase (SCD) in the offspring liver [66]. On the other hand, recent studies using models of maternal

obesity have reported that SIRT1 mRNA is unchanged in the livers of offspring of obese dams compared to offspring of normal weight dams. However, the downstream PPAR-α and SIRT1 gene cassette still becomes dysregulated, including blunted PCG-1α expression, which may prevent the mitochondrial biogenesis that necessitates HF catabolism, resulting in increased hepatic accumulation susceptibility to develop NAFLD [61].

12.8 Epigenetic Modifications Underlying NAFLD Development

Epigenetics refers to the heritable changes in gene expression that do not involve changes to the underlying genome, i.e., a change in the observable physical traits or biochemical characteristics of an individual (phenotype) without a change in its genetic makeup or genotype [67]. Conrad Hal Waddington first coined the term epigenetics in 1942, which was derived from the Greek word "epigenesis" to mean the influence of genetic processes on development [68]. Since then, research efforts have focused on unraveling epigenetic mechanisms involved in the regulation of gene expression. Although epigenetic change can occur naturally, it can also be influenced by several factors including age and environment factors including diet [69, 70]. Epigenetic aberrations are generally transient and non-heritable, but some are transmitted from one generation to the next (transgenerational), thus affecting the traits of the offspring without altering their DNA structure [71].

The process of regulating the expression of genes involves modification of chromatin structure, initiation and processing of transcription to generate messenger RNA (mRNA), and the translation of the mRNA into sequences of amino acids, which defines the protein [72]. Epigenetic mechanisms thus regulate the modification of the chromatin structure and the initiation of transcription to alter availability of genes to transcription factors required for their expression [73]. These epigenetic mechanisms include DNA methylation, posttranslational modification of histones, chromatin remodeling, and RNA-based mechanisms such as microRNA [74]. Recent studies have demonstrated that metabolic pathways perturbed by diets rich in saturated fat and cholesterol can trigger epigenetic changes, thereby modifying gene expression [75–77]. These epigenetic effects are increasingly recognized as crucial factors in the pathophysiology of NAFLD, and there are now a plethora of epigenetic changes associated with genes involved in NAFLD, in both animals and humans (Tables 12.1 and 12.2). Earlier studies in animal models have investigated the effects of maternal undernutrition on the epigenotype and metabolically perturbed phenotype of the offspring. The focus has now shifted to maternal obesity and its consequential effect on the offspring epigenome. One of the very early studies using an obese Agouti mouse have shown that genetic tendency towards obesity was progressively exacerbated when the Agouti allele was passed along successive generations [78].

Table 12.1 DNA methylation and histone modification in genes linked to development of NAFLD

Epigenetic mechanisms	Species	Target genes	References
DNA methylation	Mouse	MMTP, PPAR-α, INSIG, FASN	[79, 80]
	Rat	SREBPF2, AGPAT3, ESR1, FASN, CDKN1α, leptin, PPAR-α	[81–85]
	Humans	PGC1α, TFAM, MT-ND6, PC, ACLY, PGCG1, IGF1, IGFBP1, PRKCE, GALNTL4, GRID1, IP6K3	[86–88]
Histone modification	Mouse	ChREBP, CYP8B1, TNFα, CCL2, PPAR-α, ERO1α, LXRα, SIRT1, SIRT3, RORα	[89–96]
	Macaques	GPT2, DNAJA2, RDH12, NPAS2	[97, 98]
	Human	NER	[99]

ACLY ATP citrate lyase, *AGPAT3* 1-acylglycerol-3-phosphate O-acyltransferase 3, *CCL2* chemokine C–C motif ligand 2, *CDKN1a* cyclin-dependent kinase inhibitor 1a, *ChREBP* carbohydrate-responsive element-binding protein, *CYP8B1* sterol 12α-hydroxylase, *DNAJA2* DnaJ (Hsp40) homolog, subfamily A, member 2, *ERO1α* oxireductase endoplasmic reticulum oxidoreductin1α, *ESR1* estrogen receptor 1, *FASN* fatty acid synthase, *GALNTL4* putative polypeptide N-acetylgalactosaminyltransferase-like protein 4, *GPT2* glutamic pyruvate transaminase 2, *GRID1* glutamate receptor δ-1 IP6K3 Inositol hexaphosphate kinase 3, *IGF1* insulin-like growth factor 1, *IGFBP2* insulin-like growth factor binding protein 2, *INSIG* insulin-induced gene, *LXRα* liver X receptor α, *MT-ND6* mitochondrially encoded NADH dehydrogenase 6, *MTTP* microsomal triglyceride transfer protein, *NER* nucleotide excision repair, *NPAS2* neuronal PAS domain-containing protein 2, *SREBPF2* sterol regulatory element-binding transcription factor 2, *PC* pyruvate carboxylase, *PGC1α* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *PLCG1* phospholipase C-gamma-1, *PPARα* peroxisome proliferator-activated receptors α, *PRKCE* protein kinase C, epsilon, *RDH12* retinol dehydrogenase 12, *RORα* retinoic acid-related orphan receptor α, *SIRT1* sirtuin 1, *SIRT3* sirtuin 3, *TFAM* mitochondrial transcription factor A, *TNFα* tumor necrosis factor α

Table 12.2 MiRNA changes in NAFLD

Species	Upregulated MiR	Downregulated MiR	References
Mouse	miRNA-24, miRNA-33a, miRNA-34a, miRNA-122, miRNA-155, miRNA-181a, miRNA-182, miRNA-183, miRNA-192, miRNA-199a-3p/5p, miRNA-200b, miRNA-705, miRNA-1224	miRNA-92b-3p, miRNA-216, miRNA-302a, miRNA-328-3p, miRNA-467b, miRNA-484, miRNA-574-5p, miRNA-615-3p	[128–137]
Rat	miRNA-15b, miR-155, miRNA-200a/b, miRNA-429	miRNA-27, miRNA-122, miRNA-451	[124, 138–140]
Humans	miRNA-10b, miRNA-16, miRNA19a/b, miRNA-21, miRNA-27b-3p, miRNA-34a, miRNA-122, miRNA125b, miRNA-192-5p, miRNA-451, miRNA-1290	miRNA-28-3p, miRNA-99a, miRNA-132, miRNA-146b, miRNA-150, miRNA-181d, miRNA-197, miRNA-296-5p, miRNA-433, miRNA-511, miRNA-517a, miRNA-671	[118, 121, 141–149]

12.8.1 DNA Methylation in NAFLD

Epigenetic changes through DNA methylation refers to the addition of methyl groups to cytosine residues of DNA. In mammals, DNA methylation mainly occurs in regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide, or so-called CpG sites. These CpG sites tend to cluster together to form CpG islands. When a CpG island in the promoter region of a particular gene is methylated, expression of the gene is repressed or is turned off (Table 12.1). In livers of patients with NAFLD, there is evidence for increased methylation of the CpG island in the promoter region of PGC1α, a key transcription factor involved in mitochondrial biogenesis, fatty acid oxidation, gluconeogenesis, and lipogenesis [87]. Moreover, it has been shown that there is an inverse correlation between mitochondrial DNA (mtDNA) content and the methylation levels of the PGC1α promoter. Thus, the finding of a reduction in mtDNA content in livers of these NAFLD patients suggests that mitochondrial dysfunction associated with hepatic steatosis is due to liver DNA methylation of PGC1α. The mitochondria are also a major source and target of reactive oxygen species (ROS). The mtDNA-encoded NADH dehydrogenase 6 (MT-ND6) gene is a target of methylation in NAFLD [86]. In patients with NASH, it has been reported that MT-ND6 is highly methylated and the MT-ND6 gene is considerably reduced in their livers. Thus, DNA methylation of the mitochondrial gene may play an important role in the development and pathogenesis of NAFLD.

Differential methylation has also been identified in genes involved in metabolism and in insulin signaling when liver samples from NAFLD patients were analyzed by array-based DNA methylation and mRNA expression profiling [88]. The former includes pyruvate carboxylase (PC), ATP citrate lyase (ACLY), and Phospholipase C-gamma-1 (PLCG1), while the latter includes Insulin-like growth factor 1 (IGF1), Insulin-like growth factor binding protein 2 (IGFBP1), and Protein kinase C, epsilon (PRKCE). On the other hand, global hepatic DNA methylation can become progressively demethylated in mice that develop a fatty liver phenotype similar to human NASH induced by feeding a lipogenic methyl-deficient diet [100]. Thus, DNA methylation is particularly affected by the availability of *S*-Adenosyl-*L*-methionine (SAMe) and the dietary methyl donors including folate, betaine, and choline, which are associated with SAMe synthesis [101, 102]. SAMe influences the pathogenesis of NAFLD as a methyl donor in the synthesis of phosphatidylcholine, which is required for very-low-density lipoprotein (VLDL) assembly and hepatic triglyceride export. Evidence of a role for SAMe in NAFLD development has largely been based on animal studies. Methyl-deficient diets have been reported to result in the development of NAFLD in mice [103, 104]. These mice were found to have reduced concentration of hepatic SAMe and CpG island methylation of genes involved in DNA damage and repair, lipid and glucose metabolism, and the progression of fibrosis in their livers [105]. Inducing hepatic fat accumulation by feeding a HFD can be reversed by supplementation with methyl donors containing folic acid, choline, betaine, and vitamin B12. These

methyl donors reduced hepatic global DNA methylation and changed the methylation levels of CpG sites in the sterol regulatory element-binding transcription factor 2 (SREBF2), 1-acylglycerol-3-phosphate O-acyltransferase 3 (AGPAT3), and estrogen receptor 1 (ESR1) promoter regions [81]. Furthermore, methyl donor supplementation resulted in fatty acid synthase (FASN) DNA hypermethylation, leading to improvement of HFD-induced NAFLD [82]. Betaine supplementation was also found to restore methylation capacity by increasing SAMe concentration and genomic methylation level and the reduction in the methylation of the microsomal triglyceride transfer protein (MTTP) promoter [79]. This promotes hepatic triglyceride export and attenuates fat accumulation.

Feeding pregnant mothers a HFD can result in increased NAFLD susceptibility in the offspring in mice [28, 41], and DNA methylation plays an important role in this process. DNA methylation can be inherited from parents and passed to the next generation [106]. In rat offspring of HFD-fed mothers that develop NAFLD, it was suggested that hypomethylation of cyclin-dependent kinase inhibitor 1A (CDKN1a), an inhibitor of the hepatic cell cycle, and increased hepatic expression of the CDKN1a gene in early postnatal life contribute to predisposition to NAFLD in later life [83]. Interestingly, the hormone melatonin, which regulates the body's 24-h "clock," has been reported to reverse the methylation of leptin and prevent glucocorticoid-induced hepatic steatosis [84].

12.8.2 Histone Modifications in NAFLD

Evidence is now accumulating that histone modifications are also involved in transmitting an epigenotype with increased NAFLD risk (Table 12.1). Histone modifications mainly consist of acetylation, methylation, phosphorylation, and ubiquitylation. Histones are proteins that organize DNA strands into nucleosomes by forming molecular complexes around which the DNA winds, and modification of histone proteins can impact gene regulation by altering chromatin structure or recruiting histone modifiers. Most of the current evidence points to changes in histone acetylation in the development of NAFLD [97]. The modifying enzymes involved in histone acetylation are called histone acetyltransferases (HATs) and histone deacetylases (HDACs), and they play an important role in controlling histone H3 and H4 acetylation [107]. Histone H3 is primarily acetylated at lysines 9, 14, 18, 23, and 56 (denoted at H3K9, H3K14, H3K18, H3K23, and H3K56), while HDACs catalyze the hydrolytic removal of acetyl groups from histone lysine residues. There are four classes of HDACs, with. for example. HDAC1, HDAC2, HDAC3, and HDAC8 grouped as class I HDACs. In primates, hyperacetylation of H3K14 has been reported in the fetal hepatic tissue and this was accompanied by upregulated acetylation at H3K9 and H3K18 [97]. The same study also showed that the feeding the pregnant mother with a HFD can result in the depletion of HDAC1 protein in the fetal liver. These findings indicate that maternal obesity due to HFD feeding can already change fetal chromatin structure via histone modifications.

NAFLD development is also regulated by carbohydrate-responsive element-binding protein (ChREBP) by acting as transcriptional activator of lipogenic and glycolytic genes. A reduction in the activity of the HAT activator p300 was found to attenuate ChREBP-mediated hepatic steatosis in mice [89]. Furthermore, histone modification of genes that regulate bile acid synthesis and dietary cholesterol absorption was found to be linked to NAFLD development. Sterol 12-α-hydroxylase (CYP8B1) regulates bile acid synthesis and intestinal cholesterol absorption, and that histone acetylation of the gene promoter CYP8B1 was found to be induced following recruitment of cAMP response element-binding protein-binding protein (CBP) by the cholesterol-activated nuclear receptor and clock-controlled gene retinoic acid-related orphan receptor α (RORα) [90]. Thus, modifying RORα activity could potentially attenuate NALFD progression by histone modification. The link between histone modification and 24 h or circadian rhythms will be discussed further in the proceeding section of this chapter.

In mice, hepatic lipid accumulation due to a HFD was also reported to alter histone H3K4 and H3K9 trimethylation in PPARα and lipid catabolism-related genes increasing their expression levels and thus perpetuating further lipid buildup leading to hepatic steatosis and NAFLD progression [92]. The modifying effect of a HFD on histone has been reported to occur over generations. Offspring from pregnant mice with a HFD was found to have altered expression of genes involved in the upregulation of lipogenesis and ER stress due to reduced accumulation of methylated histones in liver X receptor α (LXRα) and oxireductase endoplasmic reticulum oxidoreductin1α (ERO1α) gene promoters [93]. In another study, maternal HFD feeding resulted in increased fetal hepatic acetylation of histone H3K14 and decreased SIRT1 expression [66]. Deacetylation by SIRT1 is responsible for the regulation of various proteins that are involved in the pathophysiology of NAFLD [108]. In the liver, SIRT1 is also reported to interact with the protein MENIN, and a reduction in MENIN gene expression particularly in aging accelerated hepatic steatosis following HFD feeding by recruiting SIRT1 to regulate CD36 expression and triglyceride accumulation via histone deacetylation [94]. Feeding a HFD in mice also induces hepatic mitochondrial protein hyperacetylation and downregulation of the major mitochondrial protein deacetylase of another sirtuin SIRT3, which resides at the mitochondria and modulates fatty acid oxidation [95, 109]. Hence feeding a HFD alters both SIRT1 and SIRT 3 expression via histone modification, and this impacts on lipid metabolism that is associated with NAFLD development.

12.8.3 MicroRNA Changes in NAFLD

Another epigenetic modification that is linked to NAFLD development is the alteration of microRNAs (miRNAs) (Table 12.2). MiRNAs are short, single-stranded RNA molecules approximately 22 nucleotides in length that can negatively modulate post-transcriptionally around 30 % of all mammalian protein-

encoding genes [110]. They induce gene silencing by binding to target sites found within the 3′UTR of the targeted mRNA, thus preventing protein production by suppressing protein synthesis and/or by initiating mRNA degradation [111]. MiRNAs play a key role in many important physiological processes such as cell proliferation, differentiation, apoptosis, and embryonic development, and that altered miRNA expression has been implicated in obesity, insulin resistance, T2D, and fatty liver disease [112, 113]. In patients with NASH, about 100 miRNAs that are involved in the pathogenesis of steatohepatitis, including the regulation of lipid and glucose metabolisms, oxidative stress, cellular differentiation, inflammation, and cell survival pathways, are differentially expressed [114, 115]. The most abundant miRNA in the liver is miRNA-122, which is a key regulator of glucose and lipid metabolism in adult livers [116, 117]. Serum miRNA-22 levels, which mainly circulate in argonaute 2-free forms, are significantly higher in mice with NASH [118]. In NAFLD patients, early studies found this miRNA to be significantly underexpressed in their livers [119, 120]. Further studies have shown that reduction in hepatic miRNA-122 was much lower in NAFLD patients with mild steatosis compared to those with severe steatosis, while patients with mild fibrosis showed higher serum and hepatic miRNA-122 levels than those with severe fibrosis [121]. Genetic deletions of miRNA-122 in mice also resulted in hepatic steatosis and inflammation [122, 123]. Besides miRNA-122, other miRNAs are reported to be involved in NAFLD development, including miRNA-21, miRNA-23a, miRNA-34a, miRNA-143, and miRNA-146b, which were found to be overexpressed in human NAFLD and NASH [120].

Diet fed to rats can cause considerable dysregulation of miRNAs and their target genes. In a study done in rats, HFD feeding was found to cause marked reduction in hepatic miRNA-122, miRNA-451, and miRNA-27 expression and increased expression of miRNA-200a, miRNA-200b, and miRNA-429 [124]. This study also showed changes in expression levels of proteins involved in regulating lipid and carbohydrate metabolism and signal transduction that are being regulated by these miRNAs in livers from the HFD-fed rats. These finding demonstrate that a HFD can alter the expression levels of miRNAs and some of their targets, contributing to the development of fatty liver and progression of nutritional steatohepatitis. Nevertheless, there is a paucity of information on whether maternal nutrition during pregnancy impacts on the hepatic miRNA status in their offspring. Our own study in mice shows that in livers of offspring mothers fed a HFD during pregnancy had markedly increased hepatic expression of key genes including those regulating fetal growth, such as insulin-like growth factor-2, and fat metabolism, including peroxisome proliferator-activated receptor-alpha and carnitine palmitoyl transferase-1a [125]. These changes were accompanied by reduced expression of miRNAs involved in developmental timing (let-7c) and fat oxidation (miRNA-122). More recently, it has been reported that in livers of weaned offspring of mouse dams fed a HFD during pregnancy and lactation, the expression of miRNA-122 was reduced but that of miRNA-370 was increased [126]. Moreover, miRNA-370 is involved in metabolism by activating lipogenic genes indirectly through miRNA-122 [127]. Thus, changes in key metabolic genes and miRNAs in the liver of offspring

from dams fed a HFD may alter early fetal growth and fat metabolism increasing offspring NAFLD susceptibility in later life.

12.9 Disruption of the Circadian Clock and NAFLD Development

A wide array of physiological processes is expressed in a rhythmic pattern with duration of about 24 h, coinciding with the day–night cycle. These 24 h rhythms are termed "circadian" and are regulated by an endogenous circadian clock network composed of key "clock" genes. Circadian rhythms are entrained by the light–dark cycle but can also be influenced by environmental temperature and food availability. The central circadian clock network is found in the hypothalamic region of the brain called the suprachiasmatic nuclei (SCN). It is now well established that clock genes are found and rhythmically expressed in most organs and tissues, including those involved in metabolism such as the liver, muscle, and adipose tissues. The generation of circadian rhythms is through a series of autoregulatory transcriptional and translational interactions [150, 151]. The key clock genes are circadian locomotor output cycle kaput (CLOCK) and brain and muscle aryl 1-hydrocarbon receptor nuclear translocator-like 1 (BMAL1), which form a heterodimer complex that activates transcription of other clock genes, including Period (PER1, PER2, PER3) and Cryptochrome (CRY1 and CRY2). The translated PER and CRY proteins form complexes and translocate back to the nucleus where they then negatively regulate CLOCK and BMAL1 activity. Though the central circadian clock network regulates circadian processes such as the sleep/wake cycle, body temperature, blood pressure, and hormone secretion, at the whole body level, it is the intrinsic clock gene network in the liver that determines hepatic clock function. Nevertheless, the systemic cues, such as light–dark cycles, fine-tune hepatic rhythms.

The circadian clock network in the liver regulates a plethora of genes and nuclear receptors that are important in several metabolic pathways, such as the metabolism of glucose, fatty acids, cholesterol, and amino acids [152–156], and in the detoxification of xenobiotics [157]. Thus, an intact circadian clock is essential for the maintenance of body homeostasis, and disruption of the clock network at the central and organ level leads to desynchronization of metabolism and consequently the development of obesity and fatty liver disease. In healthy individuals, there is a nycthemeral rhythm in de novo lipogenesis associated with the sleep–wake cycle and the feeding–fasting cycle [158]. At night when individuals are normally asleep and are therefore in the fasted state, de novo lipogenesis supplies less than 5 % of fatty acids to the hepatocyte. During the day when individuals are normally in the feeding state, characterized by high insulin levels, insulin stimulates de novo lipogenesis, supplying approximately a quarter of the free fatty acids to

hepatocytes. This nycthemeral rhythm in de novo lipogenesis is absent in NAFLD patients [158].

Early studies have suggested mutations in the core clock genes are linked to NAFLD development. Mice with mutations in clock genes have provided key insights into the interdependence between the circadian clock and metabolism. Altering key components of the clock network, for example, in the CLOCK mutant mice, give rise to the development of metabolic pathologies including obesity and hepatic steatosis [159]. Moreover, mice deficient in CLOCK and BMAL1 exhibit suppressed diurnal variations in glucose and triglyceride levels, which were amplified by feeding a HFD [153]. This observation has been extended to findings in humans, where common genetic variations of the CLOCK gene are reported to be linked to susceptibility to NAFLD [160, 161]. These studies show that CLOCK variant haplotype frequencies significantly differ between NAFLD patients and controls.

The epigenetic modifications associated with NAFLD development involve the circadian clock network. CLOCK itself possesses histone acetyltransferase (HAT) activity, and this HAT activity is necessary for CLOCK-BMAL1-dependent transactivation of clock-controlled genes and, therefore, downstream circadian clock function [162, 163]. In addition, it has been shown that activation of several CLOCK-BMAL1 target genes involves changes in histone H3 acetylation in the PER1, PER2, and CRY1 promoter regions [164]. Thus, clock-mediated epigenetic processing is upstream of several cellular metabolic cascades associated with hepatic liver accumulation. PPAR-α, for example, is a nuclear receptor that regulates the transcription of genes involved in lipid and glucose metabolism following binding of endogenous nonesterified free fatty acids (NEFAs). The CLOCK-BMAL1 heterodimer mediates transcription of the PPAR-α gene and an increase in PPAR-α protein, which subsequently binds to the PPAR response element (PPRE) and activates the transcription and translation of BMAL1, demonstrating the reciprocal link between circadian and lipid metabolic processes [165, 166]. Studies have also shown that there is a daily whole-genome cycling of the activating chromatin mark H3K4me3 (histone H3 trimethylated at lysine 4) and the inhibitory chromatin mark H3K9me3 (histone H3 trimethylated at lysine 9) in the mouse liver [167], suggesting that these activation marks are regulated in a circadian manner at thousands of gene loci. In the same study, the histone-remodeling enzyme mixed lineage leukemia 3 (MLL3) was also found to modulate hundreds of epigenetically targeted liver circadian output genes, especially those in the one-carbon metabolism pathway [167]. This suggests that MLL3 is a clock-controlled factor that could potentially regulate circadian epigenomic profiles and is thus a good candidate linking the circadian clock and liver diseases.

HDAC3 occupancy on genes involved in lipid metabolism in the mouse liver was also shown to have a pronounced circadian pattern, which peaks during the day and is at its nadir at night [168]. This circadian pattern was found to be inversely associated with the genome-wide histone acetylation and RNA polymerase II recruitment at the same sites, suggesting that HDAC3 is involved in circadian epigenomic remodeling that leads to transcriptional repression of hepatic lipogenic

genes during the day but allows transcriptional activation of these genes at night. The genomic binding sites of REV-ERBα were also found to significantly overlap with those of HDAC3 and its binding partner, the nuclear receptor corepressor (NCoR), especially on genes involved in fatty acid synthesis, and that there is a close correlation between signal intensities of REV-ERBα binding and those of HDAC3-NCoR at the same sites. In the HDAC3 liver-specific knockout mice, depletion of HDAC3 in the liver switches metabolic precursors for lipid synthesis and storage within lipid droplets and away from hepatic glucose production by sequestration of lipids in perilipin 2-coated droplets and this contributes to the development of steatosis [169]. Thus, a loss in the circadian rhythm of REV-ERBα binding should result in de novo lipogenesis and development of hepatic steatosis in a similar manner as was found in the HDAC3 liver-specific knockout mice. This was indeed the case [168] and implies that circadian epigenomic remodeling controlled by HDAC3 is largely directed by REV-ERBα.

Disruption of circadian clock function caused by chronic lifestyle disturbances, such as professional jet lag (night workers) or long-term shift work, is also suggested to contribute to the manifestations of fatty liver disease [170, 171]. A mouse model of shift work appears to share the same mechanism in humans where timed sleep restriction resulted in disruption of circadian rhythms of genes in the liver that are involved in glucose and lipid metabolism, including BMAL1, PER1, REV-ERBα, and the D site of albumin promoter binding protein (DBP) [172]. It is interesting to note that timed food access was able to restore molecular rhythms in the liver and metabolic function under sleep restriction conditions, suggesting that hepatic circadian desynchrony marks an early event in the metabolic disruption associated with chronic shift work. Thus, strengthening circadian clock network in the liver by minimizing food intake during night shifts may counteract the adverse physiological consequences frequently observed in human shift workers. In another mouse study, increased lipogenesis brought about by timed sleep restriction was found to be blunted in PER1/2 double mutant animals [173]. Although this was examined at the adipose tissue, it suggests that the absence of a functional clock in these double mutants may also protect these mice from sleep restriction-induced metabolic reprogramming that may include the development of NAFLD.

Altered nutrition during critical developmental periods could lead to disruption of the circadian clock network modulations in the rhythm of expression and increased NAFLD susceptibility in later life. This notion is now being supported by results of recent investigations. In utero exposure to maternal HFD has been shown to upregulate the expression of fetal hepatic circadian-associated neuronal PAS domain-containing protein 2 (NPAS2), at least in part, through hyperacetylation of histone H3 at lysine 14 [98]. In another study in mice, offspring exposed to HFD both in utero and in postnatal life develop NAFLD, and this was accompanied by the disruption of rhythmic pattern in expression of the key clock genes BMAL1, CLOCK, PER1, PER2, CRY1, and CRY2 in the offspring liver [174]. Hypermethylation of the promoter regions for BMAL1 and PER2 and altered 24-h rhythmicity of hepatic pro-inflammatory and fibrogenic mediators were also observed in these offspring. Thus, exposure to HFD in utero may alter the hepatic

circadian clock network during development, resulting in the disruption of rhythmic patterns in metabolic processes leading to NAFLD development. It will be of interest to examine whether the REV-ERBα/NCoR/HDAC3-mediated epigenomic remodeling is involved in the HFD-induced modulation of the activity of other transcription factors involved in lipogenesis such as the SREBPs and PPAR-γ.

12.10 Developmental Priming of NAFLD as a Marker of Premature Metabolic Decline

As previously described throughout this book, there is a wealth of data from both human and animal studies to suggest that poor nutritional exposures during early life increase the risk of developing features of the metabolic syndrome in later life. Collectively, these findings demonstrate that early dietary exposures can accelerate the onset of conditions traditionally associated with aging such as insulin resistance, type 2 diabetes, obesity, hypertension, CVD, and NAFLD [175]. This suggests that nutritional challenges that are imposed during critical periods of development and plasticity are able to set the trajectory of "metabolic aging" throughout the life course. While the mechanisms that link early nutrition to longevity are currently under investigation, preliminary findings highlight changes in cellular processes with established roles in aging, such as reduced longevity-associated Sirtuin proteins, altered epigenetic regulation of key metabolic genes, and maternally inherited mitochondrial dysfunction [175].

SIRT1 is a longevity-associated lysine deacetylase, a crucial sensor of cellular metabolism, and a central molecule connecting various metabolic processes in the liver. As the nexus of metabolism and aging, SIRT1 protects cells against oxidative stress, regulates glucose/lipid metabolism, and promotes DNA stability by binding to and deacetylating several substrates [176]. During aging and the onset of age-related disorders, including metabolic diseases, cancer, and neurodegenerative conditions, Sirtuin abundance and activity is reduced [177]. Thus, it has long been hypothesized that SIRT1 may play a role in the developmental priming of fatty liver. Indeed, in utero exposure to a maternal HFD has been shown to increase fetal histone acetylation with a concomitant decrease in SIRT1 expression and activity, implying that SIRT1 is a likely molecular mediator of the fetal epigenome and metabolome, and with additional implications for hepatic SIRT1 in premature aging of the liver [66].

There is also a strong association between SIRT3 and longevity [178]. As previously described, several studies have also shown that the mitochondrial Sirtuin SIRT3 may be also perturbed by maternal obesity, with detrimental consequences for offspring liver function. Recent human studies have shown that obese pregnant women display decreased skeletal muscle mitochondrial ETC activity and reduced mitochondrial antioxidant defense, concomitant with reduced SIRT3 activity, suggesting that reduced SIRT3 plays a role in the increased oxidative stress often

observed in pregnancies complicated by obesity and gestational diabetes [179]. Such a decrease in this antioxidant capacity is likely to impair the defense system in the offspring liver. A recent rodent model has also demonstrated that maternally derived SIRT3 aberrations in the liver may be a conduit for suboptimal liver function in the offspring. Specifically, offspring of HFD dams show reduced SIRT3 expression, which leads to impaired hepatic fatty acid oxidation [61]. These observations suggest that SIRT3-mediated mitochondrial dysfunction may be key underlying mechanism that reduces hepatic fatty acid oxidation and antioxidant defense system, contributing to the metabolic aging of the liver and the premature onset of severe fatty liver disease.

While further studies are needed to ascertain the effect of early diet exposure on liver function and ultimately life span, reduced Sirtuin abundance is a likely candidate that mediates detrimental effects on both metabolism and longevity. Much of the research aiming to understand the mechanisms by which the maternal diet can prime the development of fatty liver disease has focused on SIRT1 and SIRT3. However, recent data suggest that other longevity-associated Sirtuins such as SIRT6 and its cofactor FOXO3 are also involved in the pathophysiology of NAFLD. For example, SIRT6 and FOXO3 may transcriptionally and epigenetically regulate proprotein convertase subtilisin kexin type 9 (PCSK9) expression and LDL-cholesterol homeostasis [180]. In particular, hepatic SIRT6 deficiency leads to elevated PCSK9 gene expression and LDL cholesterol. Since the ability of monoclonal antibodies that inhibit PCSK9 and dramatically lower LDL cholesterol has received much attention of late, the role of SIRT6 in this process is an exciting research avenue. Thus, Sirtuin proteins present a promising target for pharmacological intervention to prevent the developmental priming of NAFLD, and further investigation is needed to determine the role of other Sirtuin proteins and their transcriptional cofactors.

12.11 Potential Strategies to Delay and Reverse the Developmental Priming of NAFLD

Current efforts to ameliorate NAFLD or T2D with pharmacologic agents have been met with limited success. It is likely due to the fact that many treatments have focused on treating the end-stage disease and not the mechanisms that are central to the disease pathogenesis itself. While interest in the “developmental priming” phenomena has led to important nutritional and education guideline reforms during pregnancy and early life, arguably some of the most important outcomes have been due to scientific findings using preclinical disease that have provided unrivaled insight into the molecular pathogenesis of disease. Research into the developmental priming of NAFLD has been a particularly intensive and has highlighted a number of key mechanisms that are critical in the molecular pathogenesis of the disease, namely mitochondrial dysfunction, oxidative stress, lipid peroxidation, and de novo

lipogenesis and epigenetics. We are now seeing a new wave of innovative interventions that target these key pathways to prevent, delay, and reserve the onset of NAFLD.

12.11.1 Enhancing Mitochondrial Metabolism

As previously described, a number of studies have highlighted the role of suboptimal mitochondria in developmentally primed NASH. It is therefore interesting that a number of proof-of-concept studies have explicitly shown that increased mitochondrial efficiency can promote NAFLD reversal. In a recent study, a liver-targeted derivative of mitochondrial protonophore 2,4-dinitrophenol (DNP) was shown to enhance hepatic mitochondrial uncoupling and ameliorate NAFLD and T2D in the rat [181]. The thermogenic effect of this drug has long been known, and DNP has been investigated since the early twentieth century for its ability to promote weight loss. However, production of the drug ceased in the USA in the late 1930s following numerous reports of deaths in individuals taking DNP. In the aforementioned studies, the molecule used was targeted specifically to the liver, significantly reducing its toxicity, while retaining its potent mitochondrial uncoupling effects in the liver [181]. Subsequently, Perry et al. further improved the safety and efficacy of DNP by developing a version of the drug with lower peak plasma concentrations and sustained-release pharmacokinetics called CRMP (controlled-release mitochondrial protonophore) [181]. In rat models, CRMP produces mild hepatic mitochondrial uncoupling and reduced hypertriglyceridemia, insulin resistance, hepatic steatosis, and diabetes [182]. These data support the notion that mild hepatic mitochondrial uncoupling may be a safe and effective therapy for the related epidemics of metabolic syndrome, T2D and NASH. Whether this strategy could be employed in models of maternally inherited stress remains to be seen. One problem may lie within the already damaged mitochondrial pool in the livers of offspring from obese mothers. While mitochondrial uncoupling may increase oxidative metabolism in a healthy mitochondrial pool, CRMP may not have the desired effect in the dysfunctional mitochondrial pool in the developmentally primed liver and may in fact exacerbate oxidative stress. Needless to say, further research is needed to ascertain the effect of mitochondrial uncouplers on already suboptimal mitochondria.

12.11.2 PUFA Supplementation

The presence of inflammation within the liver is a key marker of NAFLD progression and NASH onset. In light of this, interventions that promote an anti-inflammatory state would be a suitable strategy to limit disease severity. Although a number of studies have shown that maternal obesity can cause inflammation in the

offspring, our understanding of the effectiveness of anti-inflammatory agents administered during pregnancy is limited. Nonetheless, preliminary studies have shown that dietary supplementation with polyunsaturated fatty acids (PUFAs) may have an anti-inflammatory effect. PUFAs exist as either omega 6 [n-6; linoleic acid (LA)] or omega 3 [n-3; alpha linolenic acid (ALA), eicosapentanoic acid (EPA), and docosahexanoic acid (DHA)] fatty acids [183]. It is generally accepted that eicosanoid signaling molecules derived from n-6 PUFAs are more immune-reactive than eicosanoids derived from n-3 PUFAs, considered to be anti-inflammatory [184]. Importantly, HFDs are predominantly n-6 PUFA rich and are relatively deficient in n-3 PUFAs, thus being a potential contributing factor to the pro-inflammatory state of obesity. Studies have shown that both EPA and DHA may have anti-obesogenic effects and may be able to prevent diet-induced obesity (DIO) [185]. Moreover, in a rat model of HFD feeding, dietary supplementation with krill-derived oils (KO) rich in EPA and DHA was able to increase fatty acid oxidation and inhibit lipogenesis in the liver, preventing hepatic steatosis. It is noteworthy that these effects may have mitochondrial origins since KO supplementation was associated with a significant increase in the activity of CPT-I, suggesting that the flux of fatty acids entering the mitochondria for oxidation may be enhanced by EPA and DHA [186]. Interestingly, CPT-I is known to be transcriptionally regulated by the member of the PPAR nuclear receptor family [187], of which n-3 PUFAs are a known agonist. It is therefore likely that PPAR signaling is an important mechanism in the insulin-sensitizing effects of n-3 PUFAs. In support of this notion, the hepatic insulin-sensitizing effects of n-3 PUFA supplementation were absent in PPAR-alpha knockout mice compared to wild-type controls [188].

The evidence from animal models regarding the potential of PUFAs as therapeutic agent to treat NAFLD is quite convincing; however, there is less data to support the notion that PUFA supplementation during pregnancy may have positive outcome on liver function in the resulting offspring. However, studies using Fat-1 transgenic mice, which are able to covert endogenous n-6 PUFA to n-3 PUFA, have shown that offspring of HFD-fed mothers who possessed the Fat-1 transgene were protected against hepatic fat accumulation, suggesting that increased relative n-3 fatty acids can ameliorate the developmental priming of fatty liver disease [189]. This lack in our understanding strongly suggests that further studies are warranted that specifically determine the effect of n-3 PUFA supplementation during development on hepatic fat accumulation and disease progression in offspring.

12.11.3 Sirtuin Activators

There has been considerable attention focused on the Sirtuin proteins that have been repeatedly implicated in the benefits to health and longevity associated with fasting and caloric restriction. It is therefore unsurprising that in models of maternal

obesity, a state of nutritional excess, reduced Sirtuin abundance and activity has been repeatedly observed in the developmental priming of NAFLD. In response, a number of studies have assessed the role of Sirtuin activators, such as resveratrol, in animal models of maternal obesity and maternal HFD feeding. For example, in a nonhuman primate model, resveratrol supplementation was able to improve both maternal and fetal hepatic fat accumulation [190]. While these proof-of-concept studies are promising, further studies in humans that better determine safety and efficacy are much needed.

12.11.4 Metformin

Metformin is a commonly used as an insulin-sensitizing agent that is able to suppress hepatic gluconeogenesis. Although still subject of intense investigation, current thinking suggests that the molecular mechanism of metformin involves inhibition of the mitochondrial respiratory chain (complex I), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP), activation of protein kinase A (PKA), inhibition of mitochondrial glycerophosphate dehydrogenase, and activation of AMP-activated protein kinase (AMPK) [191]. Probably one of the best-studied effects is AMPK activation, which is thought to stimulate ATP-producing catabolic pathways and to inhibit ATP-consuming anabolic processes such as gluconeogenesis. Indeed in the liver, activated AMPK reduces hepatic gluconeogenesis via the phosphorylation of CREB-binding protein (CBP) and the dissociation of the gluconeogenic transcriptional complex CREB–CBP–TORC2 [192]. Metformin-induced activation of AMPK decreases fatty acid and cholesterol synthesis at least in part by reducing acetyl-CoA carboxylase (ACC), 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, fatty acid synthase (FASN), and inhibiting SREBP-1c [193]. In support, in the obese leptin-deficient Ob/Ob mice, a proxy model for hepatic steatosis, metformin treatment was able to reverse hepatomegaly, hepatic fat accumulation, and ALT abnormalities [194]. Clearly, metformin administration may be a suitable intervention to ameliorate the increased fat accumulation and gluconeogenesis that occurs during the developmental priming of NAFLD.

Metformin has been given to pregnant women since the 1970s [195] and is increasingly used as an alternative treatment of infertility and gestational diabetes [196–198]. However, its effect on offspring metabolic health is the current focus of research. In a study conducted in rats, diet-induced obesity during pregnancy enhanced fetal and placental cytokine production, which was reduced by maternal metformin treatment [199]. It remains to be determined whether this reduction in maternal and fetal inflammation impacts on NAFLD susceptibility of the adult offspring. In another study in mice, maternal metformin treatment was found to significantly improve glucose tolerance in HFD-fed offspring [200]. Nevertheless, it still remains to be determined if the improved metabolic profile in the metformin-exposed offspring also protects them from developing NAFLD. Several clinical

trials are currently under way to examine the effect of maternal metformin treatment during gestational diabetes in the offspring. In an earlier trial conducted in women with gestational diabetes, Rowan et al. have shown that children exposed to metformin had more subcutaneous fat at 2 years of age without the expense of the total amount of fat compared to those exposed only to insulin [201]. These changes in the fat distribution were suggested to provide a protection against later accumulation of ectopic fat, but can only be validated when these children have become adults. Findings from the recent Efficacy of Metformin in Pregnant Obese Women, a Randomised controlled (EMPOWaR) clinical trial, however, were discouraging and showed that metformin did not affect birth weight percentile in obese pregnant women and suggested that metformin should not be used to improve pregnancy outcomes in obese women without diabetes [202]. However, it is important to remember that birth weight is not the only important marker for long-term health in the offspring, but liver fat accumulation and function should also be considered.

In summary, while there are a number of strategies showing promising clinical outcomes, the capacity to reverse the developmental priming of NAFLD has not been demonstrated. Several studies have shown that proof-of-concept interventions during critical periods of development or plasticity may be able to ameliorate or reverse the effects of maternal obesity. However, their specific effect on liver function requires further investigation. It is important to note that while many of the interventions described target common metabolic pathways, the exact mechanisms are distinct (i.e., promotion of mitochondrial uncoupling via DNP, versus mitochondrial complex inhibition via metformin). A therapy that is beneficial for one individual may exacerbate the condition in another. Thus, the patient should be metabolically assessed as thoroughly as is reasonably possible before a pharmacological intervention during pregnancy is recommended.

As a group, pregnant women are extremely compliant to healthcare recommendations in order to do the very best for their developing baby and thus are likely to strongly adhere to suggested lifestyle and nutritional regimes during pregnancy. Therefore, identification of suitable intervention, or indeed preventative, strategies during pregnancy has huge clinical potential for both the current and the future generations.

12.12 Conclusion

The prevalence of maternal obesity is rapidly increasing worldwide, and as a consequence of developmental priming, the features of the NAFLD are also increasing in the next generation. The exact pathogenesis of NAFLD is likely multifactorial and adverse in utero events very likely play a role. Exposure to excess maternal lipids during pregnancy can already promote fetal mitochondrial dysfunction, oxidative stress, a disrupted circadian clock network, and premature gluconeogenesis, glycogenolysis, lipid oxidation, and de novo lipogenesis, thus priming the offspring liver to increase susceptibility to postnatal nutritional insults

resulting in NAFLD development. Thus, reduced oxidative capacity of the liver not only contributes to liver disease progression but also to whole-body hyperlipidemia, insulin resistance, and consequent metabolic syndrome and T2D.

Although further studies are still needed in both human and animal models to better understand the role of prenatal events in the pathogenesis of NAFLD, potential treatments are already emerging that benefit not only the obese pregnant mothers but also the future metabolic health of the offspring.

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Chapter 13
Maternal Metabolic State and Cancer Risk: An Evolving Manifestation of Generational Impact

Frank A. Simmen, Melissa E. Heard, John Mark P. Pabona, Lorenzo M. Fernandes, Charles P. Mercado, and Rosalia C.M. Simmen

Abstract Metabolic stress in the early-life environment as a consequence of maternal overnutrition and obesity leads to an increased risk of adult metabolic syndrome in offspring. Given the greater risk for cancer development at a number of tissue sites for obese individuals, exposure of the highly developmentally "plastic" fetus and neonate to a dysregulated maternal endocrine milieu may similarly result in increased cancer susceptibility as adults. In rodent models, from which this concept has gained the most direct experimental support, the feedforward circuitry for cancer propensity appears to be generationally transmitted, in part, via epigenetic biochemical marks. Here, we review the current state of this nascent field with attention given to tissues that are likely impacted by the

F.A. Simmen, PhD (✉) • R.C.M. Simmen
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA

The Winthrop P. Rockefeller Cancer Institute, Little Rock, AR 72205, USA

Interdisciplinary Biomedical Sciences Program, The University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA
e-mail: simmenfranka@uams.edu

M.E. Heard • C.P. Mercado
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA

J.M.P. Pabona
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA

Department of Internal Medicine, Harlem Hospital Center, Columbia University Medical Center, New York, NY 10037, USA

L.M. Fernandes
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA

Interdisciplinary Biomedical Sciences Program, The University of Arkansas for Medical Sciences, 4301 W. Markham Street, #505, Little Rock, AR 72205, USA

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recent epidemic of maternal obesity. We highlight current thinking on underlying molecular mechanisms and discuss how such knowledge may be used to design interventional strategies for obese pregnant women to counter increased risk for malignancy in their offspring.

Keywords Maternal • Obesity • Overweight • Dietary fat • Fetus • Placenta • Lactation • Cancer • Programming • Epigenetics • Breast • Liver • Colon • Rectum • Soy • Blueberry

13.1 Introduction

Metabolic stress in the early-life environment that is imposed by maternal overnutrition and obesity is known to lead to an increased risk of adult metabolic syndrome in human, primate, and rodent offspring [1–3]. Given the increased risk for oncogenesis at a number of tissue sites for obese or overweight individuals [3, 4], it follows that exposure of the highly developmentally "plastic" fetus and neonate to a dysregulated maternal endocrine milieu may similarly result in increased cancer susceptibility. The latter is in accord with growing evidence and hence acceptance for the concept of the early origins of chronic diseases, initially forwarded by Prof. Barker and colleagues [5]. In rodent models, from which this concept has gained the most direct support due to the ease of experimental manipulation, the feedforward circuitry for cancer propensity appears to be generationally transmitted, in part, via a spectrum of epigenetic biochemical pathways. In this chapter, we review the existing state of this field with attention given to tissue sites that are likely to be impacted by the recent epidemic of maternal obesity. We further highlight current thinking in the field on underlying molecular mechanisms and discuss how such knowledge may be exploited to design interventional strategies for obese pregnant women to counter increased risk for malignancy in their offspring.

13.2 Oncogenic Risks Promoted by Maternal Metabolic Dysfunction due to Obesity

13.2.1 Breast Cancer

In rats, consumption of a maternal high-fat (HF) diet during pregnancy and lactation consistently increased the incidence of experimentally induced mammary tumors in female offspring [6–9]. A surprising exception to the latter is a recent report of reduced mammary cancer incidence in offspring of rat dams consuming lard-based diet (60 % fat-derived) during pregnancy or lactation and which occurred despite the offspring's elevated serum levels of the obesity-associated adipocytokine leptin [10]. Leptin is considered to be an oncogene by virtue of its

ability to enhance the proliferation and metastasis of estrogen receptor-positive breast cancer cells [11] and to mediate obesity-mediated breast cancer progression, the latter via effects on breast cancer stem-like cells [12]. Using MMTV-Wnt-1-Tg mice, an estrogen receptor-positive breast cancer model, our laboratories have recently reported the increased mammary tumor incidence and decreased mammary tumor latency (time to tumor appearance) in female progeny of dams consuming HF diet during pregnancy and lactation [13]. While consumption of HF diet did not induce obesity in dams, it significantly elevated several systemic metabolic indices including blood glucose and serum biomarkers of oxidative stress. To the best of our knowledge, this is the first and only report to date demonstrating that in utero plus lactational HF diet exposure leads to increased mammary tumor risk in a mouse model. Epidemiologic data linking maternal obesity and obesogenic diet with breast cancer risk of daughters are not currently available. However, several studies, albeit indirect, suggest the possibility of an association. Poor maternal nutrition (i.e., malnutrition) during pregnancy (the Dutch Famine paradigm) has been implicated as a positive risk factor for breast cancer in female offspring [14]. While counterintuitive to the idea of HF-diet promotion of breast cancer risk, these results support the notion that maternal dietary intake can program breast cancer susceptibility. In another study, maternal BMI favored increased obesity in children [15], and childhood obesity increases risk for subsequent adult obesity and breast cancer [3]. Finally, a prospective 54-year follow-up of 9300 daughters exposed in utero to the endocrine-disrupting chemical DDT provided evidence for maternal endocrine perturbation as a predictor and marker of breast cancer risk [16]. While endocrine disruption caused by environmental carcinogen(s) and HF-diet exposure are likely to disrupt genetic programs in fetal and postnatal breast epithelium by distinct mechanisms, these findings provide compelling evidence for the concept of aberrant targeting of the developing fetal mammary gland and its functional maturation as being important to later breast cancer susceptibility.

13.2.2 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a prevalent cancer with an increasing global incidence. HCC is caused by hepatitis viruses B and C, chronic alcohol abuse, and nonalcoholic fatty liver disease (NAFLD), the latter a frequent comorbidity to obesity. Nonalcoholic steatohepatitis (NASH) is an advanced progressive form of NAFLD that is considered to be an immediate precursor condition to cirrhosis and HCC in an increasing number of individuals [17]. Obesity and diabetes are risk factors for NASH and HCC. Consistently, maternal HF diet during rodent pregnancy leads to a NAFLD-like syndrome in offspring [18–21]. This syndrome is associated with altered expression of hepatic fatty acid metabolic genes [22], whole body and liver insulin resistance [19], an induced senescence pathway [23], and disrupted circadian rhythms and associated gene expression [20] in hepatocytes. Collectively, these data raise potential concerns for trans-generational deleterious

effects of maternal obesity and HF diets on offspring liver health as reflected by the fatty liver phenotype. The effects of maternal obesity or maternal HF diet on risk of HCC in adult children or in primate offspring have yet to be explored. However, the latter goal is worth undertaking given the emerging literature with rodent models coupled with the dramatic rise in the USA and worldwide of reproductive-aged obese/overweight women with potential to become pregnant.

13.2.3 Colorectal Cancer

Obesity and metabolic syndrome are well-established risk factors for colorectal cancer (CRC) in humans and in rodent models [3]. To the best of our knowledge, there are no publications that have directly evaluated the effects of maternal HF diet or maternal obesity on CRC risk in offspring. However, several published studies provide support for a significant impact of maternal and postnatal nutritional status on predisposition to CRC in adult progeny [24–27]. Work from our laboratories showed that maternal dietary protein type, casein versus soy protein (with all other diet constituents kept constant), could influence colon cancer initiation in adult rat progeny [24, 25]. Further, analyses of individuals in the Netherlands who experienced starvation during the Dutch Famine of World War Two found reduced risk for CRC as adults; this was associated with an altered global DNA methylation state [26]. These data imply that CRC risk can be influenced by gestational and neonatal metabolic environments, may have epigenetic underpinnings, and is likely established during cancer initiation and progression, raising the potential of a shift in disease occurrence to a younger age in obese patients or patients born of obese mothers, relative to those with normal, healthy bodyweights.

13.3 Molecular Mediators and Mechanisms

13.3.1 Insulin and the IGF–IGFBP System

Insulin as well as the IGF/IGFBP system has been functionally associated with the increased incidence of breast, liver, and CRCs in obese individuals [3, 4]. Given that diet/nutrition can elicit long-lasting changes in the insulin and IGF–IGFBP systems [3, 25], it is not surprising that these pathways are considered as prime mediators of maternally transmitted generational effects. In humans and rodents, in utero exposure to maternal obesogenic diet often leads to a state of insulin resistance and hyper-insulinemia in adult offspring [1, 28]. In a study from our laboratories, female offspring of mouse dams consuming HF diet throughout pregnancy and lactation exhibited increased insulin resistance [13]. Gestational exposure is an important developmental stage for eliciting this effect based on a study showing

that mice born from HF-fed dams and immediately cross-fostered to dams fed control diet continued to manifest insulin resistance as adults [29]. In another study, dams exposed to HF diet prior to mating through pregnancy and lactation transmitted propensities for hyperinsulinemia to F1 and F2 generations, invoking epigenetics as contributory to the observed trans-generational effects [30]. The latter findings are remarkable (and disturbing) given that they portend future generations with dysregulated insulin signaling consequent to the current trend of obese/overweight females of reproductive age. In rats and mice, maternal obesity induced by HF diet during pregnancy led to gender-specific alterations in circulating levels and hepatic expression of components of the IGF–IGFBP system in the offspring [31, 32]. The latter if found to be true for humans may provide a rationale for gender-selective susceptibilities in certain cancer types and further support the adverse outcomes of maternal metabolic perturbations due to overnutrition.

13.3.2 Programming of Adipose Tissue and Adipocytokine Genes

The elevated risks for cancers such as those of the breast, liver, and colorectum with overweight/obesity are correlated with altered serum leptin and adiponectin levels (i.e., increased leptin/adiponectin ratio) and attendant increased pro-inflammatory state [3, 11, 12, 33–35]. In rats, an obesogenic diet that was initiated prior to mating and continued through pregnancy and lactation led to greater white adipose tissue mass in both male and female adult offspring [1, 36]. Similarly, in a recent study from our laboratories, C57BL/6J mouse dams fed HF diet (CHF) during pre-pregnancy, pregnancy, and lactation, despite showing comparable blood glucose and insulin levels as those of control diet (CAS)-fed dams (Fig. 13.1), elicited increased measures of poor metabolic status and adiposity in their young adult offspring (Fig. 13.2). These data indicate that subtle metabolic perturbations in mothers can translate to dramatic outcomes in progeny. Indeed, offspring of CHF-fed dams showed higher body weight at weaning, increased blood glucose levels, and more retroperitoneal tissue mass (normalized to body weight) and, conversely, lower ovarian weight (normalized to body weight) than same-aged progeny of CAS-fed dams (Fig. 13.2). The lack of comparable effects of exposure to CHF-fed dams on gonadal fat tissue mass by contrast to retroperitoneal fat mass implies that anatomically distinct fat depots may respond differently to metabolic perturbations induced by maternal HF diet. This observation has physiological importance since different adipose tissue depots are not the same with respect to their profile of secreted cytokines and growth factors and, therefore, may have distinct contributions to cancer risk. A significant finding from this study is that early exposure to maternal HF diet selectively elicited early negative effects on the progeny [e.g., greater retroperitoneal fat mass, lower ovarian weight at weaning (PND21)] that were not further modified by additional HF-diet exposure during

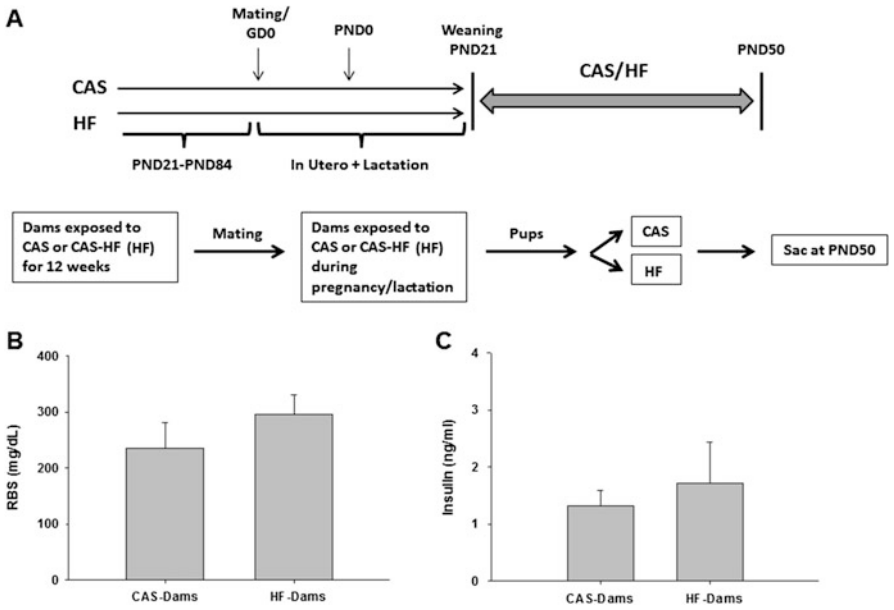

Fig. 13.1 C57BL/6 J mouse model of maternal high-fat diet and dietary consequence on maternal serum parameters. Animal procedures were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee. (**a**) Female mice were fed AIN-93G diet with casein as the sole protein source. Diets provided 17 % kcal from fat (CAS) or 45 % kcal from fat (HF) [13]. PND: postnatal day. (**b**) Random blood sugar (RBS; glucose) of mouse dams ($n = 4$–5 animals per diet group) measured at the time of weaning of their pups (**a**). RBS indicates that glucose concentrations were evaluated in sera without mice being subjected to fasting. Glucose concentrations did not differ as a function of diet ($p > 0.05$). (**c**) Serum insulin levels of mouse dams ($n = 4$–5 per diet group) measured as in (**b**) did not also differ with diet ($p > 0.05$). For (**b**) and (**c**), bars are means ± SEM. CAS/HF refers to the shift in diet from CAS to HF as indicated in Fig. 13.1. *Sac* sacrifice, *SEM* standard error of the mean

postnatal life (CHF, weaning to PND50; Fig. 13.2). The maternal HF-diet effect of lowering ovarian tissue weight in the female offspring is an interesting and novel finding that warrants follow-up for ramifications to offspring's hormonal profile, fertility, cancer predisposition, and epigenetic events during oogenesis. These collective findings indicate that maternal HF diet can establish a fairly rigid program that may not be easily readjusted (or reversed), thereby supporting the importance of intervention and/or prevention in the mother to positively affect progeny's health predisposition.

A number of studies in rodent models have specifically linked maternal HF-diet effects to perturbations in adiponectin and leptin expression in adipocytes. For example, C57BL/6 mice born from HF-diet fed dams and subsequently cross-fostered to dams that were fed control diet displayed reduced serum adiponectin levels and larger adipocyte cell sizes as adults [29]. HF-diet exposed offspring mice were also reported to display greater serum leptin levels [30, 37, 38]. In our own studies, we observed that female offspring exposed to maternal HF diet had

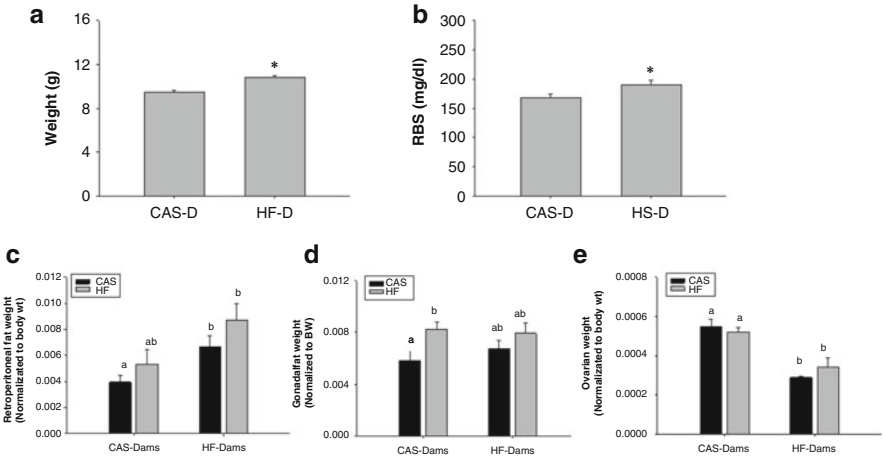

Fig. 13.2 Maternal high-fat diet (paradigm in Fig. 13.1) elicited significant effects in mouse offspring. (**a**) Maternal HF diet increased pup weaning weights; combined data for males and females are presented. CAS-D and HF-D denote PND 21 pups from CAS- and HF-fed dams; $n = 26$ and $n = 25$ pups, respectively. (**b**) Maternal HF diet increased blood glucose (RBS) of PND 21 pups; combined data for males and females are presented. CAS-D and HF-D, $n = 26$ and $n = 25$ pups, respectively. *, $p < 0.05$). Maternal HF diet increased retroperitoneal (**c**) but not gonadal adipose tissue depot weight (**d**) and decreased ovarian tissue weight (**e**) in offspring at PND 50. All tissue weights were normalized to body weight. Postweaning HF diet further increased the weight of the gonadal fat depot but only in the offspring of control diet-fed dams (**d**). Bars (means ± SEM) with different superscripts differ ($p < 0.05$). CAS/HF refers to the shift in diet from CAS to HF as indicated in Fig. 13.1

accelerated mammary gland development and greater mammary gland adiposity (i.e., higher percentage of larger adipocytes in the mammary fat pads) [13]. Larger adipocytes synthesize and secrete (locally and systemically) more leptin and less adiponectin than smaller adipocytes and have been linked to an increased state of insulin resistance [39]. Maternal HF diet also was associated with lower serum adiponectin and lower skeletal muscle adiponectin receptor 1 gene expression in offspring [36]. Whether maternal HF diet similarly affects adiponectin receptor expression (and, hence, adiponectin resistance) in mammary, liver, and colon tissues has not been evaluated; however, since these tissues may be direct targets of maternal metabolic perturbations, cancer risk in adult human offspring may stem in part from these tissues' dysfunctional adiponectin signaling.

13.3.3 Balance of PTEN/Akt Signaling

As mentioned above, there is convincing evidence for maternal dietary programming of insulinemia and hyperglycemia in offspring [3, 13]. Insulin signaling via Akt is opposed by the expression and actions of the protein Phosphatase and Tensin

Homolog deleted in chromosome ten (PTEN), an essential tumor suppressor in multiple tissues, including breast, liver, and colon. Work from our laboratories has linked increased mammary gland expression of PTEN with exposure to mammary cancer-preventive diets in rodents and to dietary bioactive components added to breast cancer cells in vitro [40–42]. Of note, female offspring of mouse dams consuming HF diet during pregnancy and lactation had lower PTEN expression in mammary glands [13]. In a study from another laboratory [43], maternal HF diet caused an increase in prostate epithelial cell proliferation in adult male mouse offspring, partly as a consequence of activated Akt and deactivated PTEN. Significantly, these programmed effects were greatest in aged offspring, leading the authors to speculate on these finding's relevance to increased risk for prostate cancer in older men. Endometrial cancer and obesity are also highly associated [44]; the linkage has been attributed partly to a lack of PTEN's opposing action on Akt signaling in endometrial epithelial cells. Thus, it is of great interest to relate the current epidemiology of endometrial and prostate cancers to maternal metabolic status and relative PTEN expression in cancer-susceptible tissues of progeny.

13.3.4 Oxidative Stress

It is conventionally assumed that increased levels of oxidative stress promote cancer development. In a study from our laboratories, we found that mouse dams consuming HF diet during pregnancy showed increased serum oxidative stress biomarkers when these parameters were measured immediately after their pups are weaned [13]. Maternal HF diet also elicits an increase in levels of oxidative stress biomarkers in offspring's sera [45, 46] and in mammary tumors [13]. These effects may be due, in part, to a repression of hepatic antioxidant defense gene expression with corresponding resultant decreases in systemic antioxidant capacity in progeny [23]. In this regard, it is tempting to speculate that increased tissue oxidative stress may underlie, in part, incidence of NAFLD in offspring from HF-diet fed dams.

13.4 Trans-generational and Epigenetic Aspects of Dietary Programming

In rodent models, the adverse physiological and endocrine effects of maternal obesity and maternal HF-diet consumption can be transmitted to subsequent generations, even in the absence of additional chronic dietary insult to the progeny and with obvious ramifications for cancer risk [30, 31, 47]. While not the topic of this review, paternal obesity (by itself and in combination with maternal obesity) and paternal HF diet may also contribute to an adverse physiological state with

increased cancer predisposition in offspring [48, 49]. In the specific case of the rat and maternal HF diet, increased mammary cancer risk transmitted across several generations has been linked to an altered mammary gland DNA methylation state [50]. Other studies have linked epigenetic changes to trans-generational effects of maternal HF diet on somatic growth, insulin resistance (increased), circulating IGF-I (increased), and mammary gland cell cycle-related gene expression of progeny [31, 51]. Of particular note, HF-diet consumption by pregnant Sprague–Dawley rats during pregnancy and lactation programmed the long-lasting repression of the mammary gland-expressed p16 (INK4a) gene in offspring; this was associated with reduced acetylation of histone H4 at this gene's promoter to maintain a gene-repressive chromatin conformation [51].

In the study of Zhou et al. [21], the authors demonstrated that offspring's phenotype of fatty liver was mediated in part by the stable induction of hepatic PEPCK gene expression, via epigenetic histone modifications at its promoter. Two other papers revealed the involvement of DNA methylation and histone acetylation and methylation in the programming of liver phenotypes in rat and mouse progeny as a consequence of maternal high-fat consumption [52, 53]. Maternal HF diet effects on leptin and adiponectin gene expression in the white adipose tissue of F1 and F2 offspring are associated with decreased acetylation and increased methylation of H3K9 at the adiponectin gene promoter and increased methylation of H4K20 at the leptin gene promoter [37]. These studies provide mechanistic insights into the long-lasting, multigenerational effects of maternal diet and obesity on groups of genes whose selective changes in expression may become cancer promoting and which, if occurring in humans, may lead to devastating consequences on generational health.

13.5 Potential Interventional Strategies

There is a lack of understanding as to how many generations the increased cancer risks (due to obesity and HF diet) are transmitted. However, in an insightful paper, a normal gestational diet for three generations was required to completely abolish the effects of a maternal HF diet on the altered leptin and adiponectin gene expression in white adipose tissue of mouse progeny [47]. The antidiabetes drug metformin was previously suggested [3] as a candidate interventional agent for administration to pregnant obese/overweight mothers to counter the predicted elevated cancer risk in offspring, the latter based on studies with rodent models. Specifically, metformin when given only during gestation was found to negate the increased adiposity and glucose intolerance typically observed for adult offspring mice born from HF-fed mothers and who were later provided high-fat diet as young adults [54]. However, this same group reported that metformin provision to pregnant mothers on regular (non-high fat) diet did not elicit the same effects on progeny; indeed, offspring gained more body weight and showed higher mesenteric fat weight when they were provided high-fat diet as young adults [55]. The potential widespread clinical use of

metformin has been recently evaluated in two randomized, double-blinded, placebo-controlled studies of obese pregnant women without diabetes [56, 57]. Metformin was found to have no significant effects on mean birthweight at delivery in both studies, while reducing maternal gestational weight gain [56, 57]. The incidence of side effects varied in the two studies, however, with one reporting no significant difference [56], while the other indicating these to be higher in the metformin group than in the placebo group [57]. Thus, metformin's use for improving progeny outcomes in obese women without diabetes requires more extensive clinical evaluation.

HF diets induce a state of oxidative stress in both the maternal and fetal compartments and in the blood circulation of adult progeny. Thus, conceptually, consumption of diets enriched in antioxidants may help counter long-lasting effects of maternal diet and obesity on adiposity, hyperinsulinemia, hyperleptinemia, insulin resistance, and oxidative stress in offspring. Proof of principle for this concept has been achieved with two such factors, namely quercetin and grape skin extract [45, 46]. Moreover, the highly bioactive phenolic compound resveratrol, when given to adult progeny of HF-fed rat dams, successfully reversed their hyperleptinemia [58]. Supplementation of the mother's diet with soy protein or whole blueberry powder inhibited mammary tumorigenesis in rat and mouse progeny [59–61]. Since these diet additives possess significant antioxidant capacity, they may similarly prove useful as dietary supplements in paradigms of maternal (and paternal) HF diet and obesity to mitigate cancer risk in offspring. Interestingly, supplementation of maternal diet with blueberry at amounts comparable to two cups of blueberry/day (5 % blueberry powder added to casein-based diet) lowered progeny's body weights, serum insulin, and serum leptin/adiponectin ratio [61]; thus, the beneficial effects of maternal blueberry consumption are supported by induction of anticarcinogenic adiponectin and, conversely, repression of pro-carcinogenic leptin in offspring. Also, maternal consumption of soy protein isolate or blueberry powder led to elevations in expression of the tumor suppressor PTEN in mammary glands of offspring [59, 61]. Future studies should address the promising protective effects of soy protein isolate and blueberry powder in the context of maternal HF-diet consumption or obesogenic conditions to provide strategies that can be easily incorporated in dietary programs of pregnant obese/overweight mothers.

Provision of soy protein isolate to pregnant mothers with metabolic dysfunctions may also prove efficacious for offspring's liver heath. In work from our laboratories, intervention with soy protein during pregnancy only or throughout pregnancy and lactation led to reduced liver steatosis in 3-month-old rat offspring (Fig. 13.3). These data localized the effect of dietary soy protein to gestation, since the magnitude of hepatosteatosis observed in offspring exposed only during this developmental period did not differ from that of pregnancy plus lactation-exposed progeny. This diet-switching paradigm is highly applicable for assessing effects of dams fed soy protein isolate in the context of HF diets on progeny and, by extension, other preventative dietary strategies for suppressing NAFLD and the associated HCC in offspring born of HF-fed mothers.

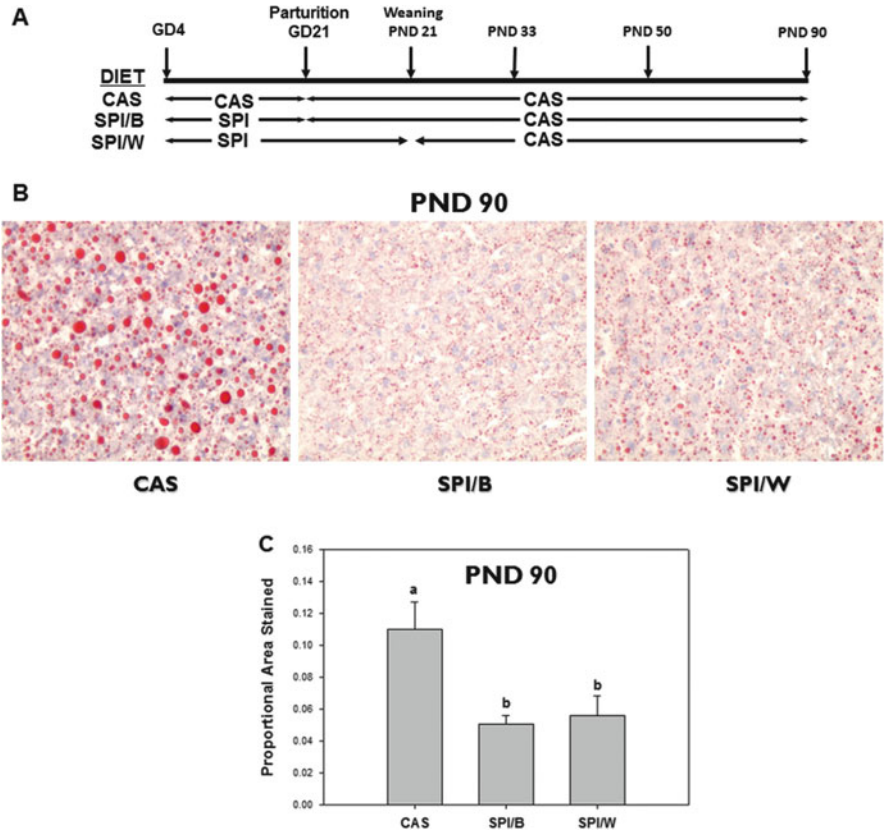

Fig. 13.3 Effects of maternal dietary intervention on hepatosteatosis in offspring. Animal procedures were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee. Diets contained either casein (CAS; 20 % w/w) or soy protein isolate (SPI; 20 % w/w) as sole protein source and were formulated according to the AIN-93G diet formula, except that corn oil replaced soybean oil [25, 57]. (**a**) Pregnant Sprague–Dawley rat dams at gestation day 4 were placed on CAS or SPI diets. Rats were provided food and water ad libitum. At birth, litters were culled to an average of five males and five females per lactating dam (CAS: 22 dams; SPI/B: 22 dams; SPI/W: 14 dams). (**b**) Lipid bodies in the left lateral lobe of livers of postnatal (PND) day 90 rat offspring were assessed by staining of OCT-embedded tissue sections with Oil Red O (ORO) followed by counterstaining with Phoenix Blue. Representative ORO-stained liver section from an animal (PND90) of each of the three dietary groups is shown. (**c**) Quantification, by image analysis, demonstrated lower lipid droplet area (reflecting less droplet size and/or number) in livers of SPI/B and SPI/W, compared to CAS animals at PND 90 ($n = 6$–8 animals/diet group/age). Bar graphs (expressed as mean ± SEM) represent the proportion of area stained with Oil-Red-O. Different superscripts indicate significant differences between diet groups ($p < 0.05$)

Lastly, we note the potential for dietary supplementation of pregnant mothers with methyl nutrients and folic acid to counter cancer risk in offspring [27, 62]. While these supplements have not been examined with respect to maternal and/or

offspring obesity, they have been shown to oppose experimentally induced tumor genesis in offspring of rat dams fed laboratory chow diets.

13.6 Conclusions

Much has been learned from using rat and mouse models on whether and, to a limited extent, how maternal diet and maternal obesity can influence progeny's cancer risk. Such studies have also indicated that these early maternal effects can be amplified by postnatal exposure to HF diet [63, 64]. These data in total imply a feedforward circuitry of generational influence with potentially devastating consequences. Figure 13.4 provides our working model that incorporates potential contributing factors acting on the maternal side and in the fetal/neonatal offspring that may predispose the latter to cancer susceptibility. Emerging data implicate epigenetic signals acting at multiple levels in the transmission of phenotype from mother to offspring, but the complexity of the signaling process prevents complete understanding on whether the epidemiology of human cancers is governed by similar mechanisms. We wish to emphasize two other points in Fig. 13.4. First, the nature of the placentation process and, hence, the feto-placental-maternal interface is to some degree, species-specific. This means that results from rat and

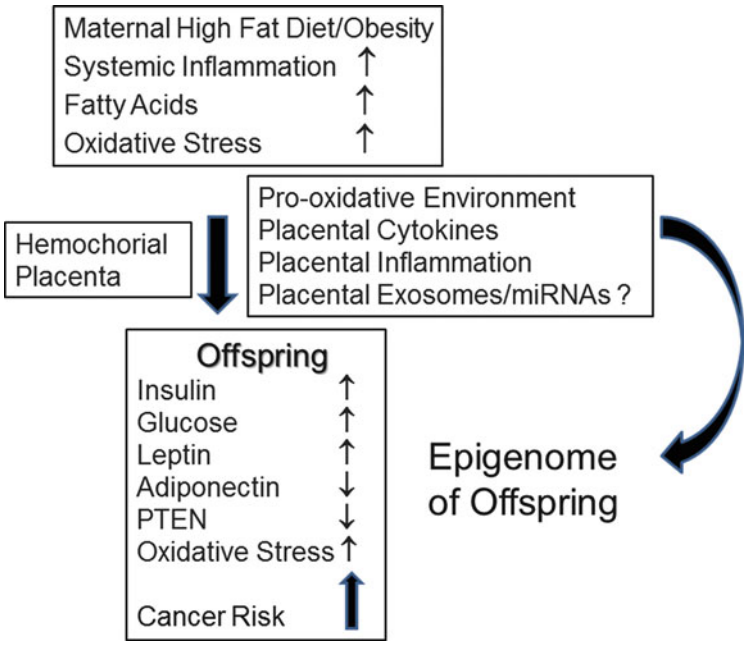

Fig. 13.4 Postulated model for maternal HFD/obesity effects in adult offspring to increase cancer risk at multiple tissue sites

mouse models may not be entirely applicable to humans, although all manifest a hemochorial placenta, when examining trans-generational aspects of cancer risk. Second, it seems a good possibility that placenta-elicited exosomes containing miRNAs and other noncoding RNAs may constitute a treasure trove of biomarkers for offspring cancer risk. The contributions of lactation and of colostrum/milk constituents to cancer risk in offspring are relatively unexplored. Evolving studies on the use of cord blood and resident stem cells and placenta from obese and normo-weight women and their daughters at delivery for biomarker evaluation provide promising avenues for dissecting linkages and mechanisms. In the short term, however, it is important that public health recommendations about lifestyle modifications that can be easily and widely implemented be put in place for pregnant mothers. Only then can we exploit and augment the benefits of scientific inquiry to mitigate the alarming trend of adverse effects of poor maternal metabolic history in cancer etiology.

Acknowledgments

Disclosure of Potential Conflicts of Interest

Authors disclose no potential conflicts of interest.

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Chapter 14
The Influence of Maternal Obesity on Offspring Cardiovascular Control and Insights from Rodent Models

Paul D. Taylor

Abstract Human cohort studies of mother–child associations around pregnancy suggest that pre-pregnancy body mass index (BMI) is causally associated with cardiometabolic risk factors in young adult offspring. Corroborative evidence in mammals for the influence of maternal obesity on offspring cardiovascular function is provided by obese pregnancy models in sheep and non-human primates, whilst more mechanistic studies in rodents suggest that perinatal exposure to the metabolic and hormonal milieu of maternal obesity may permanently change the central regulatory pathways involved in cardiovascular development and control. Shared central pathways of leptin and insulin signalling play an important role in the hypothalamic control of appetite and energy expenditure via sympathetic innervation of metabolically and thermogenically active tissues such as brown adipose tissue (BAT), but are also involved in sympathetic activation of non-thermogenic tissues, including the kidney, and central selective leptin sensitivity is implicated in obesity-related hypertension. In rodent studies, maternal obesity confers persistent sympathoexcitatory hyper-responsiveness and hypertension to the exposed offspring which appears to be mediated by neonatal hyperleptinaemia associated with permanently altered hypothalamic structure and function. Indeed, the neurotrophic role of leptin in hypothalamic development and aberrant cardiovascular control is evidenced by a rat model of experimental neonatal hyperleptinaemia in which leptin administration in naive pups during the critical period of postnatal hypothalamic plasticity leads directly to permanent cardiovascular dysregulation and hypertension. This chapter will discuss the epidemiological evidence and mechanistic insight from rodent studies on the influence of maternal obesity on offspring cardiovascular control.

P.D. Taylor, PhD (✉)
Division of Women's Health, Faculty of Life Sciences and Medicine, Women's Health Academic Centre KHP, King's College London, 10th Floor, North Wing, St Thomas' Hospital, 1 Westminster Bridge, London SE1 7EH, UK
e-mail: Paul.taylor@kcl.ac.uk

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14.1 Introduction: Prevalence and Relevance of Maternal Obesity in Pregnancy

The Health Survey for England (2013) currently estimates the rate for obesity is 26 % for men and 24 % for women. More recent projections suggest that by 2030, 41–48 % of men and 35–43 % of women could be obese if current trends continue (*Statistics on Obesity*, *Physical Activity and Diet*: *England 2015*) [1]. The prevalence of maternal obesity is rising in line with the general population trends, and has more than doubled in the past 20 years, with an estimated 20 % of pregnant women classified as obese in the UK [2, 3]. Recent evidence addressing longevity and morbidity associated with maternal obesity indicates an increase in all-cause mortality in adult offspring and increased mortality from cardiovascular events in particular [4]. It is, therefore, critical that we understand the consequences of the obesity epidemic not least in terms of the impact on the cardiovascular and metabolic health of the future generations. Whilst human mother and child cohort studies indicate associations between maternal factors in obese pregnancy with childhood cardiovascular outcomes, they are by their nature limited in their potential to assign cause and effect due to residual confounding factors around shared genetic, environmental and social influences in childhood. Animal models have proved invaluable in dissecting out the various developmental influences and mechanistic pathways that conspire to affect changes in offspring phenotype and predispose to cardiovascular morbidity and mortality. This chapter will discuss the evidence from animal studies that maternal obesity predisposes offspring to cardiovascular dysfunction in later life. It will illustrate the potential mechanisms involved in the developmental programming of hypertension in particular, which is arguably the single best predictor of premature death from cardiovascular disease.

14.2 Clinical and Epidemiological Evidence for the Influence of Maternal Obesity on Offspring Cardiovascular Function

Maternal obesity in pregnancy, whether characterised by pre-pregnancy BMI $>30\ kg^2$ or excessive gestational weight gain (GWG), is now considered the single biggest obstetric risk factors and is associated with an increased incidence of all common complications of pregnancy affecting maternal morbidity and mortality outcomes [3, 5, 6]. Increased rates of Caesarean section (CS) in obese pregnancy,

now the most common surgical procedure performed in women of reproductive age [7] and longer stays in neonatal intensive care units, also have healthcare and health economics implications [3, 8]. Caesarean section may carry its own inherent risk of long-term cardiometabolic risk centred around mode of delivery and suboptimal microbial colonisation of the neonatal intestinal tract by the maternal microbiome [9]. However, there is increasing evidence that maternal obesity per se is a risk factor for obesity and related disorders in the next generation [10–12]. Some commentators have referred to the 'transgenerational acceleration' of obesity, an independent relationship between maternal body mass index (BMI) and adiposity in children. There is now widespread concern that exposure to the metabolic milieu of maternal obesity and associated gestational diabetes mellitus (GDM) may initiate developmental changes which set metabolic and cardiovascular development on a trajectory for both childhood obesity and hypertension [13–15].

14.2.1 Maternal BMI GWG and Blood Pressure in Offspring

Whilst the underlying mechanisms are not yet understood, numerous studies have demonstrated the association between maternal BMI and offspring adiposity or BMI, supporting the observation that 'maternal obesity begets offspring obesity' [16–18]. Recent meta-analyses estimated that maternal pre-pregnancy obesity confers a threefold increased risk of obesity in the child [19], whereas GWG (above Institute of Medicine Guidelines, in the USA) is associated with a more modest 33 % increased risk. Moreover, both maternal pre-pregnancy obesity and GWG (especially in first trimester) are also associated with other cardiovascular risk factors in children including adverse lipid profiles, insulin resistance and inflammatory markers [20–23]. Together with increased risk of obesity in children born to obese pregnant women, these cardiovascular risk factors will contribute to the elevation of blood pressure in childhood, and for the most part, the reported association between maternal pre-pregnancy BMI and GWG with offspring blood pressure appears to be largely mediated by these cardiovascular risk factors especially child's current BMI [24], suggesting perhaps not surprisingly that maternal obesity begets obesity-related hypertension in the child. However, there is emerging evidence from both human and animal data for an independent relationship between maternal BMI and offspring blood pressure.

Wen et al. (2011) studied over 30,000 mother–child pairs in the Collaborative Perinatal Project and investigated the influence of childhood BMI status in the association between childhood systolic blood pressure (SBP) and pre-pregnancy BMI [25]. Higher offspring SBP was significantly associated with pre-pregnancy overweight and obesity (vs. normal weight); however, the relationship attenuated to null after adjustment for childhood BMI. Hence, a child's current BMI may largely mediate the associations of maternal pre-pregnancy BMI with offspring blood pressure. Similarly, the Jerusalem Perinatal Family Follow-up Study, a birth cohort of 1400 young adults born in Jerusalem who had extensive archival data and

clinical information, reported that both pre-pregnancy BMI and GWG were independently associated with cardiometabolic risk factors in adulthood, including systolic and diastolic blood pressure [26]. Again, after adjustment for offspring adiposity the observed association was lost, indicating that the relationship between maternal obesity and offspring blood pressure appears to be driven mainly by current offspring adiposity. Of course that is not to say that both obesity and hypertension could not be programmed concurrently through shared causal pathways, e.g. in the hypothalamus, which regulates both blood pressure regulation and body weight through energy balance.

In support of an independent association, between maternal obesity and offspring blood pressure, the Amsterdam Born Children and their Development (ABCD) Study recently reported that pre-pregnancy BMI, in over 3000 women, was positively linearly associated with offspring diastolic (DBP) and SBP age 5–6 years [24]. After adding birth weight and child BMI to the model, the independent effect size of pre-pregnancy BMI on SBP and diastolic blood pressure decreased by approximately 50 %, indicating that child's current BMI partly mediated the association. However, the relationship still held, indicating for the first time an independent relationship between maternal BMI and childhood blood pressure. Respiratory sinus arrhythmia (RSA, a derivate of parasympathetic activity) was positively associated with pre-pregnancy BMI but disappeared after adjusting for possible confounders.

So what are the potential mechanisms? Clearly, there is something about the obese and diabetic milieu in pregnancy which is influencing the development of the fetal/neonatal heart and cardiovascular system to increase blood pressure and cardiovascular disease risk. Candidate 'vectors' in the transmission of obesogenic and cardiovascular risk to the developing fetus or neonate include those hormonal and metabolic elements that can cross or signal via the placenta or be mediated as milk-borne factors. Such factors may include, but are not limited to, macronutrients such as glucose and lipids (fetal overnutrition hypothesis), which via increased placental transfer can trigger a reactive hormonal response in the developing fetus to activate insulin, leptin and glucocorticoid signalling pathways. Similarly, immune and inflammatory mediators may also interfere with developmental processes during periods of developmental plasticity. In humans, the first trimester seems particularly susceptible to the effects of excessive GWG on childhood cardiometabolic outcomes. The Generation R study, from Rotterdam, The Netherlands, recently reported that increased weight gain in the first trimester of pregnancy was associated with increased risks of childhood overweight and clustering of cardiometabolic risk factors, largely mediated by childhood adiposity [21]. Childhood diastolic blood pressure at 4 years of age, and increased adiposity from 2 years of age, has also been associated with rapid weight gain in the first trimester [27]. Interestingly, the first trimester is associated with accumulation of maternal fat depots and may provide insight into the kind of developmental signals that might arise for excessive weight gain during this critical period for placental development. Indeed, leptin may play an important role in early placentation by stimulating several genes involved in angiogenic signalling pathways and fatty acid

metabolism [28]. Moreover, elevated serum leptin levels in the first trimester have been associated with placental disease and pre-eclampsia in lean women [29]. In the rodent, trans-placental passage of I^{125} leptin from the maternal to the fetal circulation increases tenfold in late gestation, consistent with leptin's putative role as a fetal growth factor [30]. A similar elevation in fetal cord blood leptin concentration occurs in human pregnancies towards term [31].

14.2.2 Insight from Intervention Studies Designed to Improve Maternal Metabolic Profiles in Obese Pregnancy

Most studies examining the consequences of maternal obesity for offspring cardiometabolic outcomes have been observational in nature and therefore are subject to the influence of confounding variables which can affect the outcome e.g. maternal education, socioeconomic factors, lifestyle factors, ethnicity and genetics. Intervention studies, especially randomised control trials (RCTs), have greater validity to establish cause and effect, in that putative mediators can be modulated to influence a given outcome. Interventions, therefore, designed to improve GWG, glucose homeostasis and/or metabolic profiles in obese pregnancy would be hypothesised to improve fetal and childhood cardiometabolic outcomes. However, to date, very few relevant studies in obese pregnancy have been reported which might provide mechanistic insight and potentially inform policy for effective intervention strategies [32, 33]. RCTs that have attempted to address the consequences of maternal obesity and weight gain in pregnancy are logistically quite difficult and have tended to focus on determinants of energy balance such as diet and exercise as lifestyle interventions. Studies have been of varying quality with little consensus on the core outcomes affecting maternal and fetal health. A recent systematic review and meta-analysis identified 44 relevant randomised controlled trials, involving 7278 women, that had diet or lifestyle interventions in pregnancy and reported obstetric outcomes [34, 35]. Overall, there was 1.42 kg reduction (95 % confidence interval 0.95–1.89 kg) in GWG with any intervention compared with control. Combining interventions, there were no apparent effects on birth weight or the incidence of large for gestational age (LGA) or small for gestational age (SGA) babies between the groups, although physical activity intervention alone was associated with reduced birth weight (mean difference −60 g, −120 to −10 g). Dietary intervention resulted in the largest reduction in maternal GWG (3.84 kg, 2.45–5.22 kg), with improved pregnancy outcomes compared with other interventions, although the overall evidence rating was low to very low.

Certainly, diet and lifestyle interventions in pregnancy can reduce GWG and influence fetal outcomes; however, there is some controversy around intervention targeting weight gain in pregnancy due to potential adverse fetal outcomes. Current UK guidelines, contrary to IOM (USA) guidelines which provide ranges of

recommended weight gain based on pre-pregnancy BMI, do not advocate targeted weight management during pregnancy. A consensus statement from the ILSI Europe Workshop Obesity in pregnancy concluded that the evidence available on short- and long-term health impact for mother and child currently favours actions directed at controlling pre-pregnancy BMI women of reproductive ages. The consensus called for more randomised controlled trials to evaluate the effects of nutritional and behavioural interventions on pregnancy outcomes [6]. Those RCTs which have reported to date suggest that diet and exercise interventions in obese and overweight pregnancy can be effective in changing maternal behaviour, but that diet and exercise alone may not be sufficient to prevent pregnancy outcomes such as gestational diabetes and pre-eclampsia; improvement in metabolic profiles may still be beneficial to longer-term offspring cardiovascular health [36–38].

A recent 'diet and exercise' lifestyle intervention in 157 obese and 97 lean pregnant women, conducted in Odense and Aarhus University Hospitals in Denmark, reported on offspring metabolic risk factors at 2.8 years of age [39, 40]. The outcome measures were BMI Z-score, abdominal circumference, blood pressure and fasting plasma glucose, insulin, high-density lipoprotein and triglycerides. No differences were observed between the intervention and control obese groups, or between the obese and lean groups. The authors concluded that early childhood metabolic risk factors were largely unaffected by lifestyle interventions in obese pregnant women. This relatively small negative study is the first of its kind and the results of larger ongoing RCTs are eagerly awaited. It should also be noted, despite strong evidence from meta-analyses [19, 41], that not all mother–child observational cohort studies have supported an association between maternal obesity or GWG and increased cardiovascular risk [14, 42]. However, many of the mother–child cohort studies were cross-sectional and reported a relatively low prevalence of obesity in their pregnant populations, which may have masked associations with childhood outcomes.

There are two large RCTs currently evaluating the efficacy of dietary and lifestyle interventions in obese pregnancy: the UK Pregnancies: Better Eating and Activity Trial (UPBEAT, NIHR programme; ISRCTN89971375) and the LIMIT trial in Adelaide, Australia (ACTRN12607000161426). As well as reporting on pregnancy outcomes [36–38], both studies now have the invaluable opportunity to follow up the children to investigate long-term cardiovascular and metabolic development. The wealth of data from these highly characterised pregnancies will allow detailed investigation of the potential benefits of intervention and the relationship between maternal metabolic profile and offspring cardiometabolic health. A subsidiarity study, UPBEAT Tempo Heart, funded by the British Heart Foundation is currently ongoing and is specifically investigating cardiovascular structure and function in neonates and 3-year-old children born to the UPBEAT participants. Various state of the art neonatal magnetic resonance imaging modalities and cardiac and vascular ultrasound techniques will, for the first time, investigate the consequences of maternal obesity for infant cardiovascular development related to targeted modulation of the maternal metabolic profile.

In addition to the very valuable RCTs conducted, some rather elegant 'sibling pair' studies, performed in children born to mothers before and after bariatric surgery for extreme obesity, have provided strong evidence for an association between maternal and offspring cardiometabolic risk factors [43, 44]. The prevalence of overweight and obesity was higher in the children born before, compared to those born after maternal biliopancreatic diversion bariatric surgery. At the time of follow-up, children born after maternal surgery (AMS) exhibited threefold lower prevalence of severe obesity, greater insulin sensitivity (homeostasis model assessment of insulin resistance index), improved lipid profile (cholesterol/high-density lipoprotein cholesterol and high-density lipoprotein cholesterol) and lower C-reactive protein and leptin, than children born before maternal surgery. These studies, therefore, powerfully demonstrate the benefits of weight reduction in obese pregnancy for offspring cardiometabolic risk sustained into adolescence and most likely attributable to an improved intrauterine environment. More recent mechanistic studies from the Canadian Institutes of Health Research suggest that improved maternal gestational metabolic profile (lipid and carbohydrate metabolism) interacts with offspring gene variations to modulate gene expression levels and ameliorate cardiometabolic risk profiles in those siblings born AMS [45–47]. Specifically, improvements in cardiometabolic risk markers in siblings born after as compared to those born before maternal weight loss surgery may be mediated through differential methylation of genes involved in immune and inflammatory pathways. Although sibling studies such as these help to minimise residual confounding through shared genetic background and social environment, one caveat is the potential influence of an altered postnatal 'maternal' nutritional environment pre- versus post-maternal surgery. Although impressive, these intervention studies are still essentially observational and do not carry the same weight of evidence as randomised controlled trials in establishing causality.

Animal models can, to a large degree, avoid confounding variables associated with human epidemiological studies. Rodent studies in particular provide mechanistic insight into the effects of obesity in pregnancy and have generated testable hypotheses that can be back-translated to human studies.

14.3 Insight from Animal Models into the Effects of Maternal Obesity on Offspring Cardiovascular Development and Control

Numerous animal models have been developed to recreate the conditions described in the early epidemiological association studies that generated the DOHaD hypothesis and allow investigation nutritional and hormonal factors that can shape offspring phenotype [48, 49]. Animal studies have certain advantages over the human mother–child cohort studies, which as we have seen are limited in terms of establishing cause and effect. Rodents are mammals and share all but 1 % of our

genes and have highly conserved physiological systems and similar placentation which, despite altricial versus precocial species differences in the developmental stage at birth, make them an excellent model for human pregnancy. Rodent models are particularly amenable to developmental programming and life-course studies due to the relatively short life cycles. Rats and mice reach sexual maturity in a little over 1 month of age, which means that the consequences of environmental influences in development on the adult phenotype can be studied within a reasonable timeframe. Rodent models can avoid many of the residual confounding observed in human population studies by reducing genetic variability in subjects (through the use of inbred strains and genetically identical animals) and tightly controlling environmental conditions, e.g. standardising animal husbandry. Experimental diets can be tested that could not ethically be tested in human cohorts. Moreover, rodent models facilitate the investigation of underlying physiological, cellular and molecular mechanisms during critical periods of development not easily available to clinical researchers.

14.3.1 Animal Models of Maternal Overnutrition and Obesity in Pregnancy

As with the early epidemiological studies which focused on the developmental programming effects of famine and low birthweight, much of the basic science research in developmental programming of cardiovascular function has focused on maternal undernutrition (for reviews, see [50, 51]). Relatively few studies have examined the effects of maternal obesity or overnutrition on blood pressure and by far the majority have been in rats and mice. Maternal overnutrition in rodents has been found to result in increased SBP in the offspring with some gender differences depending on the model employed (for reviews, see [52–54]. There are many routes to obesity; however, diet-induced obesity is normally preconditioned in female rats and mice by the ad libitum introduction of a highly palatable semi-synthetic high-fat diet or ‘chow’ in which carbohydrates are replaced with dietary fats and simple sugars to promote weight gain. Alternatively a highly palatable ‘cafeteria’ diet or ‘junk food’ diet has been employed, high in saturated fat, simple starches and sugars often reported to mimic the Western diet [55, 56]. The addition of simple sugars in particular appears to stimulate appetite and increase calorific intake, which is normally under tight homeostatic control in rodents. Sugar, either in the chow or presented as sugar water [57], appears to affect a more rapid shift towards a positive energy balance and development of obesity. Obesity in pregnancy is a risk factor for gestational diabetes in human pregnancy and in obese rodent dams also there is a degree of gestational diabetes apparent with maternal hyperinsulinaemia and glucose intolerance in pregnancy and/or lactation [58–63].

14.3.2 Cardiovascular Dysfunction in Animal Models of Maternal Overnutrition and Obesity

The early rodent models of overnutrition involving a high-fat diet in pregnancy [60, 61, 64–70] showed deleterious consequences for cardiometabolic function in the progeny of fat-fed animals, exhibiting many facets of the metabolic syndrome including hypertension. Similar corroborative findings were subsequently reported by different groups all over the World employing subtly different rodent models [53, 54, 71, 72]. Offspring of diet-induced obese mice (OffOb) develop systolic and mean arterial hypertension which deteriorates with age as measured by 24 h ambulatory blood pressure radio-telemetry. Hypertension at 3 months of age was associated with resistance artery endothelial dysfunction, a criteria for metabolic syndrome and another risk factor for cardiovascular disease [60]. The attendant complications of metabolic syndrome may play a significant part in the aetiology of the hypertension in this model. Visceral adiposity and insulin resistance develop with age; hence, there is a likely component of obesity-related hypertension in mature adult mice (for review, see [73]). However, it is technically possible to measure ambulatory blood pressure in very young offspring of obese rat dams employing mouse radio-telemetry technology in neonatal rats, and blood pressure is already elevated in juvenile offspring of obese dams prior to the development of offspring obesity [74]. Basal night-time (active phase) mean arterial pressure (MAP) was elevated in the offspring of obese dams (OffOb) relative to offspring of controls (OffCon; MAP, males: OffOb, 121.8 ± 0.6 mmHg vs. OffCon, 115.0 ± 0.5 mmHg, $n = 6$, $p < 0.01$; females: OffOb, 125.4 ± 0.4 mmHg vs. OffCon, 114.4 ± 0.5 mmHg, $n = 6$, $p < 0.001$). Blood pressure response to a brief restraint stress is also exaggerated in OffOb mice which implicates hypersensitivity of the cardiovascular stress response and the sympathetic nervous system (SNS).

14.3.3 Autonomic Nervous System Dysfunction in Offspring of Obese Rodents

Early-onset juvenile hypertension in offspring of the obese dams is associated with marked perturbations in the autonomic control of blood pressure. Power spectral analysis of the heart rate variability (HRV) derived from continuous waveform analysis of the blood pressure telemetry record revealed a significant increase in the sympathetic component of the autonomic control of blood pressure, as indicated by the ratio of low-frequency (LF) to high-frequency (HF) oscillations at 30 and 90 days of age. The parasympathetic component of ANS control of blood pressure was also significantly reduced at 90 days whereby high-frequency heart rate oscillators were strongly attenuated in offspring of obese rats versus offspring of control. This could contribute to a further increase in blood pressure. In the time-

domain parasympathetic indexes, the standard deviation of normal to normal intervals and root mean square of successive differences were also reduced, confirming the parasympathetic dysfunction shown by power spectral analysis. Consistent with the observed increase in basal sympathetic tone and the increased cardiovascular reactivity to stress, renal tissue norepinephrine content and renin expression were markedly raised in OffOb compared with OffCon. Similarly, the pressor response to a leptin challenge was enhanced in OffOb rats (Delta MAP: OffOb, 9.7 ± 0.8 mmHg vs. OffCon, 5.3 ± 1.3 mmHg; $n = 8$; $p < 0.05$). Leptin increases blood pressure through an increase in hypothalamic and nucleus of the solitary tract (NTS) efferent sympathetic tone via the brainstem and renal nerve [75], and both systemic and central administrations of leptin increase renal nerve activity and MAP in the rat [76] (Fig. 14.1).

The observed hypertension, which persisted into adulthood, was abolished by alpha- and beta-adrenergic blockade indicating sympathetic involvement. Moreover, OffOb rats demonstrate reduced baroreflex sensitivity, with attenuation of the tachycardia and bradycardia responses to sodium nitroprusside and phenylephrine, respectively, resulting in a decreased slope of the curve of HR against MAP.

Taken together these observations suggest the developmental programming of a primary hypertension of sympathetic origin in the offspring of obese dams arising from persistent sympathoexcitatory hyper-responsiveness acquired in the perinatal period. Leptin also triggers sympathetic hyperactivity, but intriguingly, the juvenile offspring of obese rats display very specific and highly divergent responses to a leptin challenge in terms of appetitive behaviour, suggestive of selective leptin responsiveness in pathways originating at the level of the hypothalamic nuclei.

14.3.4 Selective Leptin Responsiveness in Offspring of Obese Rodents

Leptin is an adipokine peptide hormone released from fat cells in proportion to their size, as they become enlarged with stored triglyceride. Leptin is critical to the regulation of energy balance and feeds back to the appetite regulatory centres in the brain to report a positive energy balance. Activation of leptin receptors (LepR) at the arcuate nucleus (ARC) of the hypothalamus activates satiety pathways to inhibit further food intake, but also promotes energy expenditure via sympathetic stimulation of the metabolically active tissues such as brown adipose tissue (BAT) involved in thermoregulation [77]. SNS stimulation by leptin also appears to have an important role in cardiovascular control [77]. Leptin infusion or leptin overexpression in genetically modified mice increases renal sympathetic nerve activity (RSNA) and elevates blood pressure and heart rate [73, 78–80]. In the ARC, leptin stimulates the expression of pro-opiomelanocortin (POMC) and activates POMC neurons to release melanocyte-stimulating hormones (MSH) that act on secondary neurons expressing melanocortin receptors (MC4R) in the

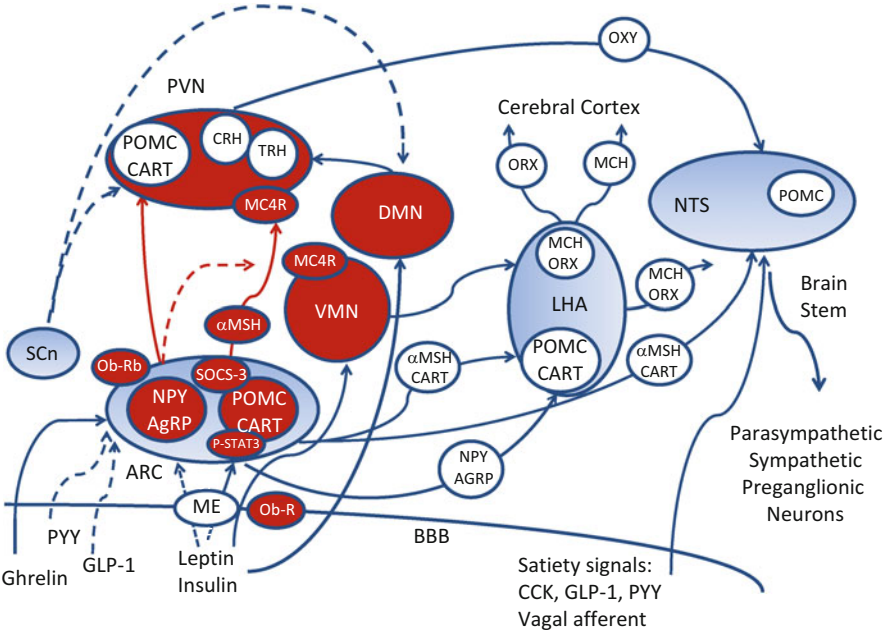

Fig. 14.1 The hypothalamic nuclei and related brain regions. The arcuate nucleus (ARC) is located in the hypothalamus, close to the median eminence (ME) where the blood–brain barrier (BBB) is incomplete allowing blood-borne signals to reach ARC neurons. Leptin, insulin and ghrelin are the most important hormonal satiety signals and are also actively transported across the BBB where they activate anorexigenic neurons coexpressing alpha melanocyte-stimulating hormone (αMSH) and cocaine- and amphetamine-regulated transcript (CART) and inhibit orexigenic neurons coexpressing agouti-related protein and neuropeptide Y (AgRP and NPY). Both populations of neurones project widely throughout the brain. CART is also expressed in the paraventricular hypothalamic nucleus (PVN) and LHA. αMSH is cleaved from the precusor polypeptide proopiomelanocortin (POMC) along with other peptides such as β-endorphin and ACTH. The ARC integrates this information together with inputs from brainstem areas and signals other hypothalamic nuclei such as the ventromedial hypothalamic nucleus (VMN), dorsomedial hypothalamic nucleus (DMH) and PVN, to reduce food intake. Signals from the ARC to the PVN and the lateral hypothalamic area (LHA) also increase feeding. Divergent projections from the orexin containing neurons (ORX) and melanin-concentrating hormone (MCH) neurons in the LHA ascend to the cerebral cortex and descend to the brainstem and spinal cord. Oxytocin containing neurons (OXY) of the PVN innervate vagal preganglionic parasympathetic neurons involved in gastrointestinal control (OXY). Hormones from the gastrointestinal tract including cholecystokinin (CCK) and glucagon-like peptide (GLP-1) modulate these processes through shorter-term changes in satiety and hunger. Inputs from the suprachiasmatic nucleus (SCn) to the PVN and DMN also regulate diurnal feeding patterns. The three possible outputs from the hypothalamus that regulate food intake and energy expenditure are activation of motor neurons via the brain stem; activation of neuroendocrine neurons in the PVN that secrete corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) to activate the pituitary axes (e.g. hypothalamic–pituitary–adrenal and hypothalamic–pituitary–thyroid axis activation result in secretion of glucocorticoids and thyroid hormone); autonomic nervous system both sympathetic and parasympathetic e.g. influencing heart rate and blood pressure and thermogenesis in metabolically active tissues

paraventricular nucleus of the hypothalamus and related brain regions (NTS) to increase sympathetic activity.

Leptin deficiency, on the other hand, in both humans and animals, causes obesity in the absence of hypertension [81, 82]. In established obesity, chronic hyperleptinaemia can lead POMC neurons to become unresponsive to leptin with the loss of the anorectic actions of leptin, yet with preservation of the pressor effects on blood pressure, effectively a state of acquired *selective leptin resistance*, which has been hypothesised to underlie obesity-related hypertension [43, 73, 83]. Offspring of obese rats also appear to exhibit a developmentally programmed early leptin resistance as juvenile animals which precedes the onset of obesity and hyperleptinaemia. Administration of exogenous leptin at a dose (10 mg/kg i.p.) which inhibits 24 h food intake and promotes weight loss in control animals had no apparent effect in 30-day-old offspring of obese rat dams [70, 84, 85]. Following similar administration of leptin in young OffOb rats, phosphorylated-STAT3, a marker of leptin signalling, was selectively reduced in the ARC, but not in other hypothalamic nuclei [70]. The dorsomedial hypothalamus (DMH) and ventromedial hypothalamus (VNH) in addition to the PVN also express LepR and have been implicated in leptin's ability to stimulate BAT and cardiovascular control independently of MC4R signalling (Fig. 14.1). This provides a possible explanation for selective leptin resistance in chronic obesity and also potentially the selective leptin responsiveness observed in young offspring of obese rats which was not obesity related, but a direct consequence of early-life 'exposure' to maternal obesity.

14.3.5 Neuronal Development of the Neonatal Brain: A Role for Leptin

This then begs the question, what is it about the immediate maternal environment that gives rise to the primary programming of sympathetic hypertension in offspring of obese rodents? Hypertension appears to arise as a direct consequence of in utero or postnatal exposure to maternal obesity and is not the result of increased adiposity in the offspring, which only becomes evident in older animals. Intriguingly, the observed alteration in central leptin sensitivity in offspring of obese rodents may provide a clue, especially when we consider the critical role that leptin plays in development of the CNS and the neonatal hyperleptinaemia associated with maternal obesity in rodents.

The apparent primary programming of a sympathetically mediated hypertension secondary to maternal obesity in young offspring of obese rodents may arise not only from perturbation of central leptin sensitivity, but also through dysregulation of the normal neurotrophic action of leptin resulting in both structural and functional deficits in leptin signalling during neuronal development [70].

Maternal obesity in rodents is associated with marked hyperleptinaemia in the neonate during a critical period in brain development when leptin is thought to play

a permissive neurotrophic role in establishing the neural circuitry of the hypothalamic nuclei [86]. Leptin, possibly in concert with other neurotrophic factors including insulin and corticosterone, appears to be critical during this period of developmental plasticity for promoting neural growth of the hypothalamic nuclei involved in both appetite and blood pressure regulation.

A physiological postnatal surge in the plasma leptin concentration was first described in neonatal rats by Ahima and colleagues in 1998 and has since been described by others in both rats and mice [87–92]. The leptin surge peaks during the second postnatal week in rodents (postnatal day 10) before returning to normal levels at weaning. Leptin signalling pathways are incomplete at this stage of development and pups are able to maintain a high level of food intake despite higher plasma leptin levels. The physiological role of the leptin surge appears to be in orchestrating hypothalamic neuronal outgrowth and connectivity between hypothalamic nuclei [92, 93].

In a landmark paper, Sebastian Bouret *and colleagues* (2004) first described the neurotrophic action of leptin in leptin-deficient (ob/ob) mice. Bouret initially observed incomplete formation of the neural projections between the arcuate nucleus (ARC) to the paraventricular hypothalamic nucleus (PVH) of the hypothalamus in the hyperphagic and obese ob/ob mice [86]. Bouret *and colleagues* were able to restore normal hypothalamic development by giving neonatal mice replacement leptin treatment critically during the second postnatal week. Leptin treatment in adult (ob/ob) mice had no apparent effect again highlighting the early postnatal period as being critical to hypothalamic development in rodents. Similar attenuation of hypothalamic neural projections from the ARC is also observed in DIO rats genetically predisposed to develop diet-induced obesity [94]. These two genetic models of hyperphagia and obesity [94, 95] also show reduced immunoreactivity for agouti-related peptide (AgRP) containing neurons in the PVH which originate in the ARC [94, 95]. Reduced density of arcuate projections and AgRP-containing neurons in the hypothalamus may permanently influence the structure and function of neural circuits involved in both energy balance and autonomic regulation of cardiovascular control (Fig. 14.1). Moreover, AgRP is the endogenous antagonist of MC4R, and reduced antagonism would increase melanocortin signalling at sites relevant to blood pressure regulation to promote hypertension [96].

It seems likely that the exaggerated and prolonged neonatal leptin surge reported in offspring of obese rat dams may have precipitated the attenuated AgRP immunoreactivity reported in the PVH at postnatal day 30. It is tempting to speculate that similarity in neonatal AgRP neural development between this model and Bouret's study in ob/ob mice which lack leptin [86] is a consequence of leptin resistance in the former and leptin deficiency in the latter. These studies, and others since Bouret's pivotal study, which have focused on the neurotrophic action of leptin and the influence of early nutrition, seem to indicate a permissive role for leptin and leptin signalling in the normal development of the neonatal hypothalamus and may provide the strongest mechanistic link between maternal obesity and permanent programming of cardiovascular control [63, 72, 94, 97–106].

14.3.6 Nutritional Impact on Leptin Signalling in Development

Little has been reported on the origins or determinants of the leptin surge in rodents. In neonatal OffOb rats, the plasma leptin surge is matched by a similar profile of adipocyte leptin mRNA expression, suggesting that neonatal adipose tissue is the source of the plasma leptin surge, as has been suggested by others [87, 89, 91]. Maternal nutritional status has been shown to affect the timing of the neonatal plasma leptin profile [91, 107], which is thought to reflect the differentiation of pre-adipocytes into mature adipocytes which can then produce leptin [108]. Factors such as insulin which affect maturation of the pre-adipocytes may therefore influence the timing of the leptin surge [109]. Indeed, in offspring of obese rats there is a peak in the neonatal plasma insulin profiles in response to elevated glucose concentrations in the milk, which appears to precede the neonatal plasma leptin surge [110].

Whilst several models of developmental programming report maternal nutritional modulation of neonatal leptin profiles associated with changes in offspring cardiometabolic phenotype [91, 105] pharmacological manipulation of the leptin surge in rodents provides further support for the role of leptin in shaping cardiometabolic outcomes [91, 107, 111, 112]. A greater understanding of the determinants of the neonatal leptin surge and the comparative physiology in humans, which is poorly understood, might inform interventions to reduce the risk of obesity and hypertension.

14.3.7 Experimental Neonatal Hyperleptinaemia in Rodents

To investigate the cardiovascular consequences of the exaggerated and prolonged leptin surge observed in offspring of obese rat dams, we treated naive rat pups with exogenous leptin to mimic leptin concentrations over the same time course [113]. Neonatal rats born to control dams were treated twice daily either with recombinant rat leptin (10 mg/kg i.p.) or saline vehicle control from postnatal day (PD) 9–15. Cardiovascular function was assessed remotely by radio-telemetry. In juvenile leptin-treated animals SBP was raised by 13 mmHg compared to controls. The cardiovascular response to a brief restraint stress and a pharmacological challenge to a bolus dose of leptin was enhanced in the leptin-treated animals. Power spectral analysis of HRV derived from the blood pressure waveform of the telemetry record confirmed heightened sympathetic drive contributing to hypertension in the leptin-treated animals. Analysis of tissue catecholamine levels at 30 days of age showed a twofold elevation in renal noradrenaline concentrations in the leptin-treated animals. Hypertension in the leptin-treated animals was normalised following mixed alpha and beta blockade (terazosin and propranolol). Hypertension and heightened sympathetic tone were observed independent of changes in adiposity and/or hyperleptinaemia suggesting a direct influence of neonatal leptin exposure on the developing pathways of blood pressure control [113].

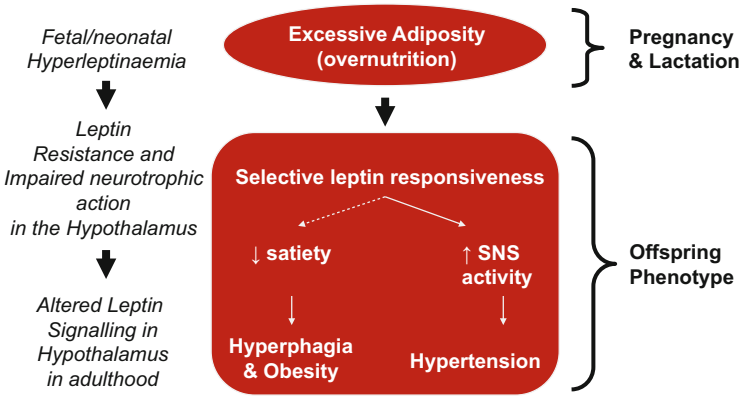

Fig. 14.2 Developmental programming of obesity and hypertension secondary to maternal obesity and/or neonatal hyperleptineamia. The schematic shows the proposed developmental origins of 'selective leptin responsiveness' in which the anorexic actions of leptin are lost whilst the pressor effect of leptin is enhanced

Leptin-treated animals were 'leptin resistant' at 30 and 90 days of age, with no response in feeding behaviour or weight loss following a leptin challenge. Similar studies have reported hypothalamic leptin resistance following leptin administration in neonatal rats [107, 114] and mice [91]. However, the studies by Samuelsson et al. provide the first direct evidence that exposure to hyperleptinaemia in early development causes adulthood hypertension of sympathetic origin and supports a role for leptin in the cardiovascular phenotype of acquired 'selective leptin responsiveness' secondary to maternal obesity in the OffOb model (Fig. 14.2). Comparing the two phenotypes, however, the offspring of the obese rat dams appear to have a more pronounced hypertension and more robust cardiovascular phenotype than the leptin-treated rats [84]. This suggests that other factors relating to maternal obesity contribute the OffOb phenotype in addition to neonatal leptin exposure [113]. Neonatal hyperinsulinaemia may also have a part in aberrant hypothalamic development [115] and may influence offspring cardiovascular control secondary to maternal obesity and glucose intolerance [60, 61]. Indeed, maternal high-fat feeding during lactation in mice causes offspring obesity associated with severely impaired POMC and AgRP projections to PVH, which is prevented by specifically knocking out insulin signalling in POMC-specific insulin receptor-deficient mice [116].

14.4 Identifying the Site of the Hypothalamic Lesion in Offspring of Obese Rodents

14.4.1 A Role for Hypothalamic Melanocortin Signalling

Rodent studies of maternal obesity and experimental hyperleptinaemia indicate that increased sympathetic nerve activity (SNA) is an important mediator of

hypertension since alpha- and beta-adrenergic receptor blockade and renal denervation ameliorate the elevation of blood pressure in these models [60, 74, 84]. However, the molecular mechanisms and neuronal pathways are yet to be fully elucidated. We can hypothesise that the 'selective leptin responsiveness' observed and the exaggerated pressor response to leptin identifies the hypertensive lesion in the hypothalamic leptin signalling pathways that regulate sympathetic efferent activity. Leptin activates POMC neurons in the arcuate nucleus, which release peptide melanocyte-stimulating hormones (MSH) that act on MC4R expressing neurons in the PVH and other brain regions to increase SNA [117, 118]. Humans with loss-of-function mutations of MC4R are obese but have normal blood pressure [119]. Moreover, the SNA responses to acute leptin are abolished in MC4R-deficient mice [120] and by central administration of the MC4R antagonist SHU9119 [119]. We have reported that hypothalamic MC4 mRNA expression is increased in adult offspring of obese rats compared to controls and that central administration of the MC3/4R antagonist SHU9119 decreases MAP to a greater degree in offspring of obese rats compared to controls [121]. Maternal obesity appears to result in increased MC4R signalling which contributes to hypertension in this rodent model. It is tempting to speculate, therefore, that increased signalling via MC4R in the PVN or possibly in the brainstem mediates the primary sympathetic hypertension in offspring of obese pregnant rats. Indeed, recent unpublished observations by Samuelsson and colleagues on the effects of maternal obesity imposed on the genetic background of MC4R null mice mouse model indicate that hypertension in offspring of diet-induced obese is dependent on the presence of functional MC4R in the PVH [122]. Heterozygote loxTB Mc4r mice were mated with Sim1-Cre genetically modified heterozygote loxTB Mc4r littermates [123] to generate WT, homozygous loxTB MC4R (MC4R null mice) and Sim1-Cre, loxTB MC4R (MC4R–PVH) offspring in which MC4R is re-expressed specifically in the PVN. These studies identify MC4R signalling in the paraventricular hypothalamus as the primary lesion in the development of sympathetic hypertension secondary to both maternal obesity and experimental neonatal hyperleptinaemia (Fig. 14.3).

14.4.2 Leptin Receptor Signalling Beyond the Arcuate Nucleus

As evidenced in chronic obesity and potentially in offspring of obese rodents, POMC neurons in the arcuate nucleus may become leptin resistant; however, leptin can act independently of MC4R signalling to affect changes in cardiovascular control. The DMH is intimately involved in the activation of BAT and regulation of the cardiovascular system and both humans and animals with loss-of-function mutations in leptin and LepR are obese but not hypertensive. Selectively blocking the action of leptin in diet-induced obese mice using specific antibodies, antagonists

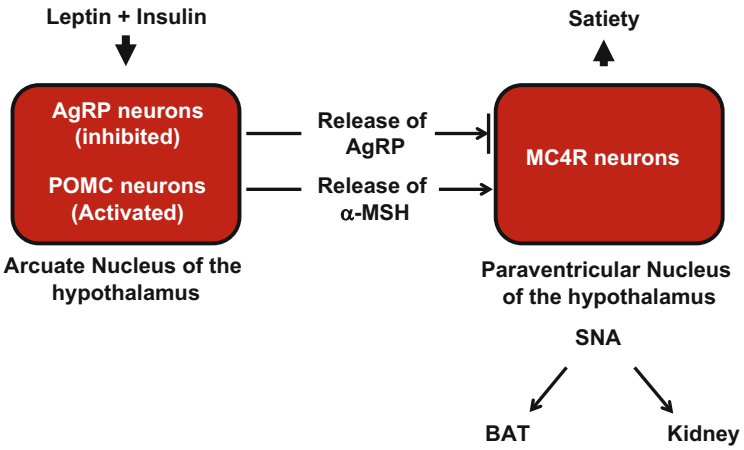

Fig. 14.3 Leptin signalling, blood pressure and MC4-R pathway. Leptin and insulin act synergistically to activate shared central sympathoexcitatory pathways which are mediated by the melanocortin 4 receptor (MC4R) and the PI3 kinase pathway. AgRP is the endogenous antagonist of MC4R, and reduced antagonism would increase melanocortin signalling at sites relevant to blood pressure regulation to promote hypertension

or inhibiting activity of LepR expressing neurons originating in the DMH prevents the elevation in HR and BP in diet-induced obese mice [124, 125] although the effects on RSNA were not reported. Re-instating LepR in the DMH of obese LepR-deficient mice elevates BP. Hence, the DMH, independent of MC4R signalling, represents another potential candidate site for the hypertensive lesion and leptin hyper-responsiveness in offspring of obese mice.

14.4.3 The Gut Microbiome and Epigenetic Modifications Arising from Obesity in Pregnancy

Whilst the aforementioned alterations in hypothalamic structure and function secondary to maternal obesity may be mediated through classical processes of developmental neuroendocrinology, they do not exclude a potential role for novel molecular mechanisms which may shape gene expression during critical periods of developmental plasticity. The gut microbiome is emerging as a key player in obesity research and presents yet another very immediate environmental stimulus to developmental programming through epigenetic modification of gene environment and expression and shaping offspring phenotype in development and beyond. Moreover, the transfer of gut microbiota from mother to baby that occurs during normal vaginal delivery presents another vector for inheritance of epigenetic traits that may influence obesity and cardiometabolic risk. From the obstetric perspective, there has been much recent interest around mode of delivery establishing

phenotypic traits in the offspring. Obesity is a major risk factor for Caesarean section, and babies born particularly by pre-labour caesarean section (CS) can develop acute and chronic physiological changes which are hypothesised to reflect aberrant microbial colonisation of the infant intestinal tract [126, 127]. Associated phenotypic changes range from altered feeding behaviour, metabolism and blood pressure to type II diabetes, immune-related conditions and neurological and stress-related problems [128]. Mode of delivery and antibiotic use in late pregnancy are potential confounders in studying the effect of obesity in mother/child cohorts, associated with as much as 46 % increased risk of childhood obesity [129].

The gut microbiota with an estimated biomass of 2–3 kg in humans contains a unique group of symbiotic micro-organisms both bacteria and viruses with a combined gene pool or 'microbiome' far in excess of the human genome. Besides the more mundane yet essential biological functions such as the digestion of complex carbohydrates, these microbial genes influence innate and adaptive immunity and may have key regulatory functions in metabolic pathways in health and in disease [130–132].

Some elegant inoculation studies in animals demonstrate the power of the gut microbiota to affect metabolic and cardiovascular phenotype. Toll-like receptors of the innate immune system are critical for both colonisation and homeostasis of the human microbiota. Genetically altered mice specifically lacking TLR 5 exhibit hyperphagia, obesity and hypertension associated with a dysbiotic gut microbiota. Remarkably, transfer of faecal material from affected mice to germ-free wild-type mice (treated with antibiotics) confers similar features of the metabolic syndrome in recipient mice [133]. Diet also has an important role in shaping the composition of the gut microbiome, with high-fat Western diets favouring a more 'Bacteroides' enterotype associated with obesity [134–136]. These studies therefore highlight the importance of the healthy colonisation and maintenance of the microbiota and imply that diet and/or obesity in pregnancy could set up a dysbiosis in the offspring through the inherited microbiome.

14.4.4 The Gut Microbiota and Cardiovascular Control

Emerging evidence suggests that gut microbiota influence blood pressure regulation and salt sensitivity through interactions with genetics, epigenetics diet and lifestyle factors and the widespread use of antibiotics [137]. Human essential hypertension along with several models of hypertension in rodents [the spontaneously hypertensive (SHR) and Dahl salt-sensitive rat] is associated with altered microbiota and the relative ratio of gut species *Firmicutes* and *Bacteroidetes* [138, 139]. Fermentation products derived from gut bacteria can influence blood pressure control via modulation of sympathetic pathways of energy expenditure and catecholamine metabolism in the gut, together with intestinal and renal ion transport which can influence salt sensitivity.

Short-chain fatty acids (SCFA) derived from gut bacteria can also modulate renal sensory nerves to affect renin release and blood pressure via the gastro-renal axis [140]. SCFA can increase energy expenditure via the gut–brain axis stimulating the SNA via G protein-coupled receptor—GPR41 and elevating blood pressure [141].

Hypertension is affected by low-grade inflammation which can arise from compromised microbiome diversity. Pre-eclampsia is characterised by hypertension and inflammation in pregnancy and is improved by long-term probiotic use [142]. Probiotics may therefore find prophylactic use in blood pressure control via epigenetic modification of the complex regulatory pathways involved in hypertension.

Animal studies addressing the probiotic modulation of the microbiome in obese pregnancy and epigenetic effects on the offspring are ongoing in our laboratory, and it remains to be seen whether the colonisation and the diversity of intestinal microbes is a modifiable risk factor for offspring cardiovascular outcomes secondary to obese pregnancy.

14.5 Translation from Animal Models Back to Human Obesity in Pregnancy

The rodent models of obesity in pregnancy share many similarities with the metabolic profiles in obese pregnant women including insulin resistance and maternal hyperglycaemia leading to a reactive fetal hyperinsulinaemia [143]. Obese pregnant women, like their obese rodent counterparts, also demonstrate hyperleptinaemia, and cord blood leptin is raised in babies born to obese women [143]. Thus, in common with the neonatal rodent, the fetus of an obese woman is exposed to both hyperinsulinaemia and hyperleptinaemia [144]. It is important, however, in extrapolating to humans from rodents, to acknowledge that as an altricial species, rodents give birth to young at a less advanced stage of development compared to human infants (precocial) and that the period of hypothalamic plasticity that occurs in rat pups postnatally probably equates to the third trimester in human pregnancy. However, there is evidence from non-human primates that this developmental window may extend into the suckling period [99, 145]. Studies of human fetal brain development are understandably limited, but there is evidence that neural projections begin to develop between hypothalamic nuclei from 21 weeks of gestation [146]. It seem likely, therefore, that the critical window for hypothalamic developmental in humans is quite broad and that there is potential for exposure to the adverse neurotrophic effects of pathological levels of leptin in obese pregnancy both antenatally and potentially post-partum via mother's milk.

In comparison with rodents, a leptin surge has not been described in human development as such; a developmental role for leptin might be suggested by the

unexplained high concentration of leptin in fetal cord blood, which falls rapidly post-partum [31], and is related to birth weight [147, 148]. Recent studies provide strong evidence for a positive correlation between maternal and fetal plasma leptin concentrations (and a negative correlation between fetal leptin and insulin sensitivity), evidence of the maternal–fetal transmission of this potentially critical neurotrophic factor [149].

The hyper-reactivity of the SNS in offspring of obese rodents has not been established in human studies. Whilst the ANS has not been extensively studied in the children of obese women, a correlation has been observed between fetal cardiac sympatho-vagal activation during labour and maternal BMI [150]. The ABCD study of 3074 women reported that pre-pregnancy BMI was positively linearly associated with offspring blood pressure, but not with sympathetic or parasympathetic drive in 5–6 year olds. However, only a small proportion (5 %) of the women studied were clinically obese [24]. Ongoing studies will characterise ANS as part of a follow-up study of neonates and 3-year-old children born to obese pregnant women participating in the UPBEAT RCT (UK pregnancy and better eating trial) compared with offspring born to lean control mothers.

14.6 Conclusions

The prevalence of maternal obesity in the UK has more than doubled in the past 20 years and is predicted to rise with in line current secular trends. Not only is maternal obesity the single biggest obstetric risk factor for adverse pregnancy outcome, but it carries with it reduced life expectancy in adult offspring through increased cardiovascular mortality. Maternal obesity, in particular pre-pregnancy BMI, is associated with childhood and adult hypertension; however, the extent to which elevated blood pressure and other cardiovascular risk factors are dependent on the offspring BMI requires further studies in younger children. Ongoing RCTs of diet and lifestyle interventions aimed at modulating the determinants of obesity in pregnancy have the greatest potential to establish cause and effect and inform intervention strategies to improve offspring cardiovascular outcomes. Animal studies, which to a large extent avoid confounding variables, support a strong association between maternal obesity and offspring blood pressure. This involves early activation of the SNS and suppression of parasympathetic drive, which is independent of offspring adiposity and, therefore, secondary effects of obesity-related hypertension. Evidence suggests the developmental programming of a primary hypertension secondary to maternal obesity and neonatal hyperleptinaemia which is of autonomic nervous system origin and associated with selective leptin responsiveness at the level of the hypothalamic nuclei. Altered leptin signalling in the hypothalamus may arise through neonatal hyperleptinaemia during the critical developmental window of neuronal development and hypothalamic plasticity when leptin appears to have a permissive neurotrophic role in normal physiological development. Leptin resistance arising from downregulation of LepR during this

period may produce suboptimal neural outgrowth and connectivity between the hypothalamic nuclei resulting in altered structure and function of pathways involved in energy expenditure and blood pressure control. In situ hybridisation experiments that can map LepR expression within the highly heterogeneous neuronal populations and cell types with the ARC will identify the aetiology involved. Meanwhile, preliminary studies not yet published from our laboratory, employing Cre-lox technology, identify a role for the hypothalamic melanocortin system in the origins of the hypertension secondary to maternal obesity and experimental neonatal hyperleptinaemia. These studies pinpoint MC4R in the PVH as the primary lesion in the autonomic hypertension in these models. The intestinal microbiota sometimes referred to as the 'second brain' presents some exciting new pathways in the gut–brain axis which can influence physiology through innate immune and metabolic systems to potentially influence developmental programming of the central nervous system. The colonising gut flora present a plausible vector in the transmission of maternal epigenetic traits to offspring and it remains to be seen whether the microbiota colonisation and the diversity of intestinal microbes might be a target for intervention in maternal obesity.

Finally, whether increased fetal exposure to leptin secondary to maternal obesity influences human hypothalamic development to a similar degree remains to be seen, but evidence from non-human primates supports translation of similar underlying cellular and molecular mechanisms. In fact, it would be surprising if the neurotrophic effects of leptin and the consequences of leptin overexposure on hypothalamic development were not conserved across mammalian species. However, at present, the prospect of pharmacological interventions in leptin signalling during pregnancy seems unlikely. Therefore, diet and lifestyle interventions that modulate adiposity levels and reduce possible fetal leptin overexposure remain the best option for intervention.

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Chapter 15
Maternal Obesity Effects on the Risk of Allergic Diseases in Offspring

Katharine C. Pike and Liesbeth Duijts

Abstract Parallel increases in prevalence of both obesity and allergic disease have occurred in recent decades, suggesting that these conditions may be causally linked. Allergic diseases are often established in childhood. Factors acting in early life, including the prenatal period, might influence the risk of developing these conditions. Epidemiological data partially support associations between maternal obesity and allergic disease. These associations appear largely restricted to asthma, not all of which is allergic. Maternal obesity could directly influence respiratory or immune development predisposing to asthma and, potentially, other allergic diseases via immune modifying effects of adipokines, epigenetic effects, or effects upon the maternal and fetal microbiomes. Indirect effects of maternal obesity which might, in turn, influence the risk of developing allergic disease include pregnancy complications and obesity in offspring. Finally, associations between maternal obesity and allergic diseases in offspring might reflect shared genetics, gene–environment interactions or confounding by shared diet or habitual activity. Given the considerable impact of allergic asthma and other allergic diseases upon individuals and health-care systems, identifying a causal pathway between maternal weight and allergic diseases would be of great importance for public health. Further research is needed to identify the underlying mechanisms, effect modifiers and long-term consequences into adulthood.

Keywords Obesity • Allergy • Asthma • Rhinoconjunctivitis • Atopic dermatitis • Epigenetics • Adipokines • Microbiome • Programming

K.C. Pike (✉)
Respiratory, Critical Care and Anaesthesia Section, University College London, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
e-mail: k.pike@ucl.ac.uk

L. Duijts
Department of Pediatrics, Divisions of Respiratory Medicine and Neonatology, University Medical Center Rotterdam, Erasmus MC—Sophia Children's Hospital, P.O. Box 2060, 3000 CB Rotterdam, The Netherlands

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15.1 Introduction

Allergic diseases, including allergic asthma, allergic rhinoconjunctivitis and atopic eczema have increased in prevalence in many Westernised countries over the last half century [1]. More recently, similar increases have occurred in developing countries, particularly those undergoing socio-economic change and urbanisation [2, 3]. From the 1990s onwards, much interest has focused upon the concept of 'developmental programming', whereby environmental exposures acting in early fetal life, or even around conception, might lead to developmental adaptations which influence disease risk throughout life. Increased obesity might be one factor underlying the increase in allergic disease that appears to accompany urbanisation and attainment of a Westernised lifestyle. Maternal pre-pregnancy obesity has been demonstrated to be associated with adverse pregnancy outcomes, including pregnancy-induced hypertension, pre-eclampsia, gestational diabetes and need for assisted delivery or caesarean section [4]. There is also evidence that maternal pre-pregnancy obesity or greater gestational weight gain is associated with an increased risk of preterm birth and, compared to offspring of mothers of normal weight, a greater risk of being born either low birthweight or large for gestational age [4–6]. Maternal pre-pregnancy obesity might adversely affect the pulmonary development of the fetus, leading to relatively smaller airways, and impaired lung function. Alternatively, maternal pre-pregnancy obesity might affect the development of the fetal immune system. These developmental effects could subsequently lead to an increased risk of allergic diseases.

15.2 Ecological Observations

In the late 1990s, the International Study of Asthma and Allergies in Childhood (ISAAC) reported prevalences for asthma approaching 40 % in young teenagers in regions of the UK, New Zealand and Australia [7]. Similarly, these and other Westernised countries have also reported high prevalences of allergic asthma, allergic rhinoconjunctivitis and atopic eczema in children [8] and in adults [9]. While these increases appear to have plateaued in the developed world, increases are now occurring in developing nations where living conditions and lifestyle are becoming more like those in developed countries [2, 3]. A number of European studies have shown significant variation in the burden of allergic diseases within populations relatively similar in terms of genetic make-up, but living under very different economic and environmental circumstances. For example, the incidence of asthma, rhinitis and atopic sensitisation amongst East German children was found to be substantially lower compared to those growing up in the more affluent West Germany [10]. Similar findings have been reported comparing children living in Eastern Europe to those living in Scandinavia [11]. The results of longitudinal studies in developing countries also suggest an increase of allergic

diseases as countries become more affluent. Urban and richer middle class Ghanaian children have been found to be more likely to develop atopy or exercise-induced asthma than poorer children or those living in a rural environment, for example [12].

Changes in employment, diet and activity levels, which together contribute to increased prevalence of obesity, are potentially important contributors to the association between assuming a Westernised lifestyle and acquiring an increased predisposition to allergic disease. This is exemplified by data collected from the Inuit population of Greenland at the end of the last century, during a period of rapid urbanisation. Parallel increases occurred in obesity and sensitisation to aeroallergens during this period [13–15]. Moreover, in England during a period of rapidly increasing asthma prevalence, the prevalence of adult obesity increased from 15 % in the early 1990s to almost 25 % in 2001 [16]. Similar increases in prevalence occurred in the USA, where between 1960 and 1994 the prevalence of obesity rose from 12.8 to 22.5 % [17]. Whilst this ecological data cannot be used to infer causality, these findings suggest that obesity and allergic diseases, mostly asthma, have increased in parallel in developed and, more recently, in developing countries. Since many allergic diseases such as allergic asthma, allergic rhinoconjunctivitis or atopic eczema have their inception in childhood, the factors most likely to influence the likelihood of an individual developing these diseases are those acting early in life. Recent data demonstrate that over half of women of childbearing age in England are obese or overweight [18]. Moreover 20–40 % of women in Europe and the USA gain more than the recommended weight during pregnancy [19]. Together these epidemiological observations support the hypothesis that temporal changes in obesity amongst mothers might contribute to the increasing rates of allergic diseases experienced by their children.

15.3 Cohort Association Studies

Since 1996, many prospective cohort studies conducted in Europe, and the USA, have sought to examine the relationships between maternal obesity and childhood allergic diseases. Given the difficulties associated with interpreting body mass index (BMI) during pregnancy, cohort studies have most frequently measured pre-pregnancy BMI. Studies that focused upon wheezing outcomes observed that increased pre-pregnancy maternal BMI (i.e. obesity, mostly defined as $\geq$30 kg/m^2) compared with normal weight was associated with a 1.52–3.52-fold increased odds of wheezing before 3 years of age [20–28]. A meta-analysis of 85,509 subjects participating in European birth cohorts observed that maternal overweight (BMI 25–29.9 kg/m^2) and pre-pregnancy obesity (BMI $\geq$30 kg/m^2) were equally associated with any wheezing and with recurrent wheezing of at least four episodes before age 2 years [odds ratios (OR) (95 % CI): 1.08 (1.05, 1.11) and 1.19 (1.12, 1.16), respectively) and (OR (95 % CI): 1.12 (1.08, 1.17) and 1.16 (0.97, 1.39), respectively] [29]. Multiple socio-economic and lifestyle factors were taken into account

as well as maternal hypertensive disorders, pre-eclampsia and gestational diabetes. Mediators such as birthweight, gestational age, mode of delivery and breastfeeding moderately changed the results.

For asthma, maternal pre-pregnancy obesity, compared with normal maternal weight, was associated with a 1.52–3.40-fold increased odds of asthma between age 6 years and adolescence [26, 30–34]. Results of these previous studies were confirmed by a meta-analysis of 108,321 subjects participating in observational studies within Europe and the USA. Maternal pre-pregnancy overweight/obesity was associated with an increased risk of ever asthma or wheeze [OR (95 % CI): 1.31 (1.16, 1.49)] and recurrent asthma or wheeze [OR (95 % CI): 1.21 (1.07, 1.37)] in children aged 14 months to 16 years [35]. Within this meta-analysis, a distinction between allergic and non-allergic asthma phenotypes was not made. However, it is important to note that many individuals clinically diagnosed with asthma are non-atopic and cannot be said to have an allergic disease.

15.3.1 Intermediate and Modifying Factors in Observational Studies

Studies that examined whether or not impaired lung function underlies the association of maternal pre-pregnancy obesity with childhood wheezing and asthma are scarce [26, 28, 30]. In early life, associations of a higher maternal BMI with an increased risk of wheezing illnesses attenuated when lung function was taken into account, but not at an older age [28]. Maternal pre-pregnancy obesity was not associated with changes in spirometry parameters, measures of airway obstruction or levels of fractional exhaled nitric oxide (a measure of eosinophilic airway inflammation), at age 6 years, or bronchial hyperresponsiveness at age 8 years [26, 30]. Further studies are needed before any conclusions can be made about the relevance of impaired lung function with respect to the association found between maternal pre-pregnancy obesity and childhood asthma.

In addition to absolute measures of weight or adiposity, the influence of maternal gestational weight gain on childhood wheezing and asthma has also been explored [24, 32, 36, 37]. A meta-analysis of five individual studies observed that a 1 kg increase in gestational weight gain was associated with current asthma or wheezing [OR (95 % CI): 1.01 (1.01, 1.02)]. Women categorised as having high gestational weight gain, compared with normal gestational weight gain, had an increased risk of ever asthma or wheezing [OR (95 % CI): 1.16 (1.00, 1.34)] [35]. Where the effects of both gestational weight gain and pre-pregnancy BMI upon childhood wheezing have been investigated together, the two appear to be independent [24].

15.3.2 *Influence of Obstetric Complications*

Maternal obesity increases the risk of a number of complications in the pre- and perinatal periods [38–40]. A path analysis approach allows separate estimation of the indirect effects of BMI, mediated via pregnancy outcomes, and of the direct adjusted effect of BMI. Using this approach and data from the Norwegian Mother and Baby cohort (MoBa), it has been shown that the positive association between wheeze and maternal BMI is attenuated after adjusting for preterm birth, low birthweight, pre-eclampsia, hypertension, maternal diabetes, gestational diabetes, and caesarean section. However, the risk of wheeze is highest for children with mothers in the highest BMI category, even after adjustment for these complications [21].

It appears that at least part of the association between maternal pre-pregnancy obesity and allergic diseases in the offspring can be explained in terms of a higher incidence of obstetric complications. Certainly with respect to asthma, associations have been found with younger gestational age at birth and lower birthweight [41], pre-eclampsia, maternal diabetes [29] and caesarian delivery [42, 43]. Pre- and perinatal complications might also be associated with allergic diseases other than asthma. Gestational diabetes, for example, was positively associated with atopic dermatitis and allergen sensitisation in children aged 3 years in the Boston birth cohort, even after accounting for maternal pre-pregnancy BMI [44]. Moreover, meta-analysis has found caesarian delivery to be associated with increased risk of allergic rhinitis [45], and a recent study of over 4500 school children reported positive associations between caesarean delivery and atopic sensitisation in addition to those with ever wheeze and ever asthma diagnoses [42].

Other potential mechanisms underlying the association between maternal pre-pregnancy obesity and childhood asthma symptoms might include child's growth, current BMI and immune response to infections. Except for child's current BMI, effect estimates did not largely change when previous studies took such factors into account [21, 22, 24, 26, 28, 34]. Whether or not associations between pre-pregnancy obesity and wheezing or asthma tend to be stronger in children without [31, 35] than with [24, 30], a familial predisposition for asthma remains unclear.

For other allergic diseases, it has been reported that maternal pre-pregnancy obesity is not associated with allergic rhinitis, hay fever or atopic dermatitis from age 3 to 16 years [22, 26, 30, 32, 34]. Inconsistent results were observed for maternal pre-pregnancy obesity and inhalant and food allergen sensitisation measured by skin prick tests or IgE levels. This might partly be explained by the age, and related immune development, at which measurements were performed. Further studies with longitudinal measurements are therefore needed.

Although many potential confounders of the relationship between pre-pregnancy obesity and allergic diseases have been taken into account, residual confounding factors could still be present. Residual confounding may occur as a consequence of factors such as dietary patterns, supplement or vitamin use or maternal nutritional

status, for example free fatty acid blood levels [46]. Furthermore, the roles of genetics and epigenetics need to be further explored in observational and other studies [46, 47].

15.3.3 Observational Studies Supportive of an Intrauterine Effect of Maternal Obesity Upon Offspring Allergic Disease

Prospective cohort data demonstrate that maternal pre-pregnancy obesity is associated with an increased risk of some childhood allergic diseases. However, it is difficult to determine if these associations are explained by direct intrauterine effects or unknown socio-economic or lifestyle-related factors. To disentangle this, information on paternal obesity before or during the mother's pregnancy can be used [48]. If stronger associations of maternal pre-pregnancy obesity with childhood allergic diseases are observed than for paternal obesity, taking childhood obesity into account, this would support the hypothesis that intrauterine adaptive mechanisms underlie the observed associations. Similar associations for maternal and paternal obesity with allergic diseases would suggest either that paternal lifestyle influences are transmitted via epigenetic effects or that common and shared socio-economic or lifestyle-related factors within families might explain these associations. Such studies have not yet been conducted.

Mendelian randomisation studies could be used to examine the causal effects of maternal pre-pregnancy obesity and childhood allergic diseases [49]. The Mendelian randomisation approach examines associations of genetic variants with maternal pre-pregnancy obesity and asthma. This approach is considered to be unaffected by confounding or reverse causation because genetic variants are generally unrelated to confounding factors and do not change after conception. To date, the only Mendelian randomisation study that has assessed the relationship between obesity and asthma investigated the association between asthma and *childhood* rather than maternal obesity [50]. An increased BMI in childhood was associated with an increased risk of asthma [Relative Risk (RR) with 95 % Confidence Interval (95 % CI): 1.55 (1.16, 2.07) per kg/m^2]. A weighted allele score of 32 independent BMI-related single nucleotide polymorphisms (SNPs) was strongly associated with childhood asthma [RR (95 % CI): 2.56 (1.38, 4.76) per unit score], showing strong evidence that the association between childhood BMI and asthma was due to a causal effect. Mendelian randomisation studies for maternal pre-pregnancy obesity and childhood allergic diseases are still needed.

In summary, observational studies suggest that children born to mothers with pre-pregnancy obesity or a higher gestational weight gain are at greater risk of wheezing and asthma, but not of allergic rhinitis and atopic dermatitis, and inconsistently of inhalant and food allergy throughout childhood. There is no clear evidence that these associations are explained by children's lung function, growth

and mechanisms related to vulnerability to respiratory infection. Maternal obstetric complications or childhood obesity may mediate some of the effects of maternal obesity and familial asthma predisposition might, in turn, modify these effects.

15.4 Intervention Studies

Whilst the potential importance of maternal weight management during pregnancy is well recognised and a number of intervention studies have been conducted with the aim of avoiding excessive weight gain during pregnancy, no study to date has presented data assessing any pregnancy weight management intervention with respect to allergic diseases in children. A meta-analysis of 44 randomised controlled trials using any combination of dietary or lifestyle intervention found a 1.42 kg reduction in weight gain when compared to no intervention [51]. Interventions aimed at reducing weight gain were associated with a small reduction in the risk of pre-eclampsia. Maternal obstetric complications might have adverse developmental effects for the fetus. In addition, the risk of obesity faced by children of obese mothers is increased [52]. Whilst either of these consequences of maternal obesity might, in turn, influence the risk of allergic diseases in the offspring, no firm recommendations for maternal weight management can be made based upon the findings of intervention studies at present.

15.5 Mechanisms

There are a number of biologically plausible mechanisms which might explain the findings of observational studies concerning obesity in mothers and allergic disease in their children. Whilst inflammatory or immune consequences of obesity might adversely affect respiratory or immune development directly (Fig. 15.1), these associations might be mediated by obstetric complications during pregnancy or delivery, which occur with increased frequency in the context of maternal obesity (Fig. 15.2). Similarly, indirect effects may arise as a consequence of the influence of dietary behaviours leading to obesity upon the microbiome or epigenome (Fig. 15.2). It is also possible that these associations reflect common genetics or residual confounding by dietary patterns, or other behaviours, closely associated with obesity and strongly shared between parents and their children (Fig. 15.3).

15.5.1 Direct Consequences of Maternal Obesity

Adipocytes are the most abundant cells in white adipose tissue, whilst macrophages present in the stromavascular fraction constitute 10 % of all cells [53]. Together

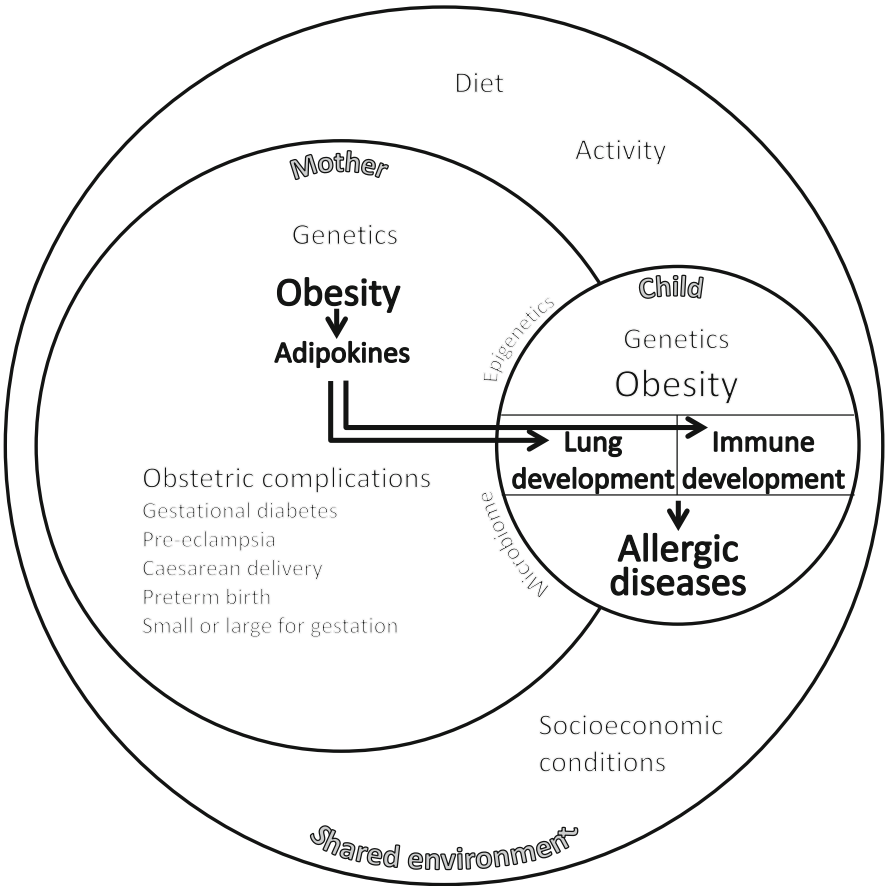

Fig. 15.1 Direct effects of maternal obesity mediated via adipokines. Factors such as adipokines which are increased in the context of maternal obesity might directly affect either the developing respiratory or immune system, thereby increasing the risk of allergic disease, for example allergic asthma

these cell types secrete a range of cytokines and chemokines, including tumour necrosis factor alpha (TNFa), interleukin 1b (IL1b), interleukin 6 (IL6) and interleukin 10 (IL10). Proteins secreted predominantly by adipocytes are termed adipokines, the principal two adipokines being leptin and adiponectin. Obese pregnant women are at risk of obstructive sleep apnoea, and this condition too is associated with a pro-inflammatory state, potentially attributable to intermittent hypoxia and frequent sleep arousals [54]. The serum concentration of leptin is markedly increased in obese compared to lean individuals [55, 56], whilst that of adiponectin is decreased. Elevated levels of leptin and IL-6 are associated with stimulation of a range of pro-inflammatory cytokines and downregulation of regulatory T-lymphocyte (Tregs) activity [57]. Low adiponectin levels, in contrast, are

Fig. 15.2 Indirect effects of maternal obesity and effects mediated by diet. Allergic diseases including allergic asthma might occur with increased frequency in the context of maternal obesity as a consequence of indirect effects mediated by the microbiome or epigenome. These mechanisms may reflect either the consequences of an obesogenic diet or those of pregnancy complications associated with obesity

associated with reduced mRNA expression of the anti-inflammatory cytokine IL-10 [58].

In obese pregnant women, not only are serum levels of proinflammatory cytokines known to be higher than those in pregnant women of normal weight [59], but levels of pro-inflammatory cytokines are also increased in the amniotic fluid, which surrounds and is breathed by the developing fetus [60]. If the immune and inflammatory changes associated with obesity are transferred to the fetus, this might programme an immune predisposition to allergic disease (Fig. 15.1). Increased IL-6, for example, is associated with IL-1, IL-4, TNFα and histamine release and Immunoglobulin E (IgE) modulation [61], whilst TNFa is an important mediator of IL-4 in allergen-induced T cells and of IL-5 from bronchial epithelial cells

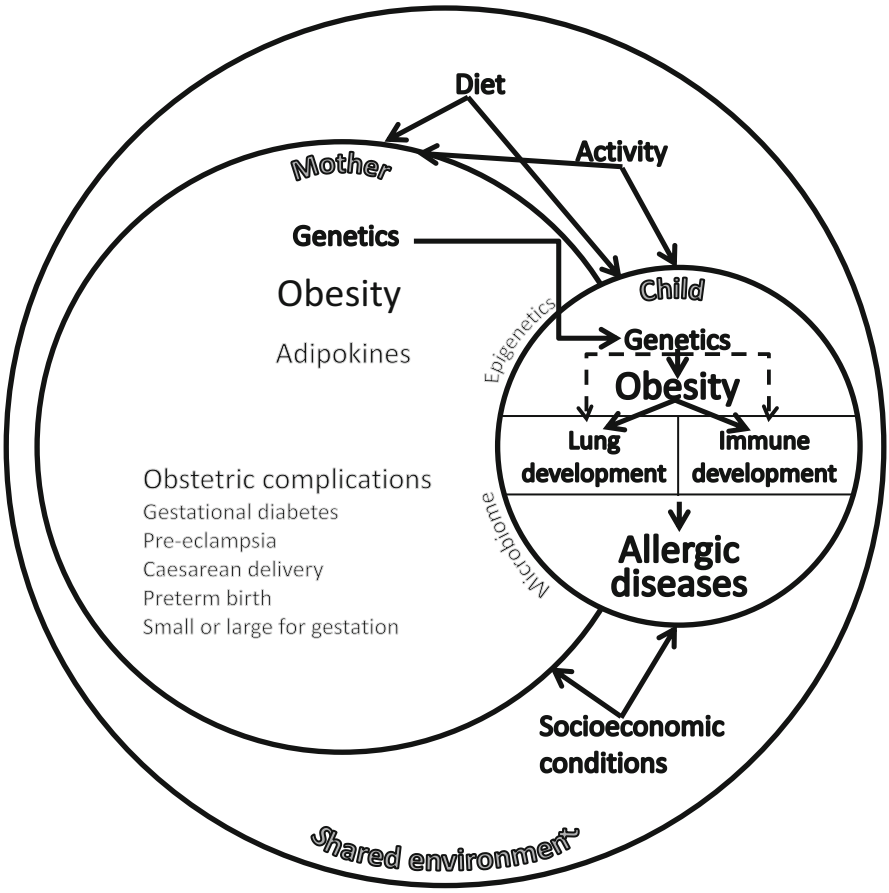

Fig. 15.3 Confounding factors of shared genetics and environment acting via childhood obesity or direct effects upon lung or immune development. Residual confounding either by shared genetics or environment, including diet and activity, might explain the perceived relationship between mother's body composition and allergic diseases in their children. The role of genetic influences is potentially complex; pleitrophic genetic effects might cause both obesity in the maternal generation and allergic disease in the child (*solid lines*), whilst obesity and associated direct consequences of increased adiposity upon immune and/or lung development might be also be inherited (*broken lines*)

[62]. Moreover, it has been hypothesised that the immunological changes associated with obesity result in decreased immunological tolerance to antigens and skewing of the immune system towards a Th2 cytokine profile [63]. This hypothesis has been proposed as a unifying explanation for the observations that older siblings appear to confer protection against allergic diseases, potentially because tolerance to fetal antigens has been induced.

Compared to infants born to non-obese mothers, those born to obese mothers have been shown to have fewer eosinophils and CD4 T helper cells, reduced

monocyte and dendritic cell responses to Toll-like receptor ligands and increased plasma levels of IFN-α2 and IL-6 in cord blood [64]. Moreover, data from the Tucson Infant Immune Study suggest not only that excess pregnancy weight gain appears to change the immune response of the fetus but that an immune marker, specifically persistently elevated LPS-induced TNF-α production, acts as a predictive biomarker for childhood asthma. Pregnancy weight gain was measured in over 250 mothers, and TNF-α, IL-6, IL-10 and IL-12 were measured in supernatants of LPS-stimulated peripheral blood mononuclear cells of their children at birth and 3 months alongside plasma TNF-α. Children of mothers in the highest tertile for pregnancy weight gain were at increased risk of developing asthma (OR, 3.4; CI, 1.7–6.9) and had persistently elevated TNF-α in early life (OR, 2.9; CI, 1.4–8.2) [33].

In addition to the immune and inflammatory properties of adipokines which potentially predispose to an allergic predisposition, adipokine effects upon the developing lung may predispose specifically to asthma (Fig. 15.1). Leptin and adiponectin receptors are expressed in the lung [65, 66], and leptin regulates the maturation of fetal lung cells [67]. In mice, administration of leptin enhances ozone-induced airway inflammation and responsiveness [68], whilst adiponectin administration attenuates allergen-induced airway hyperreactivity and inflammation [69]. Finally, leptin levels have been shown to be inversely related to spirometry measures of forced expiratory volume in children both with and without asthma [70, 71].

15.5.2 Mechanisms Mediating Effects of Obstetric Complications

Developmental mechanisms might explain the associations between the increased risk of obstetric complications faced by obese mothers and subsequent asthma or other allergic disease in their children (Fig. 15.2). Histologic studies suggest that preterm birth is associated with structural changes in the lung, including increased bronchial muscle, collagen and elastin [72], whilst animal studies suggest that structural and functional changes in the lung follow restricted prenatal growth [73, 74]. In pre-eclampsia disturbed regulation of vascular growth in the feto-maternal unit leads to overproduction of antiangiogenic factors in amniotic fluid [75, 76]; this too might adversely affect lung development [76]. In addition to effects upon lung development which might predispose to asthma, obstetric complications might be associated with allergic disease as a consequence of altered immune development. For example, altered cytokine profiles have been measured in mothers with gestational diabetes [77]; similarly, differences in the exposure to maternal vaginal or intestinal flora might mediate the effect of caesarian delivery upon neonatal cytokine response patterns [78], Th1/Th2 helper cells balance and the risk of developing atopy and allergic disease [79, 80].

15.5.3 Effects Mediated via Maternal and Offspring Microbiome

Maternal intestinal flora may be an important environmental influence upon early immune system development. Development of a diverse gut microbiota early in life has been demonstrated to be associated with a decreased risk of allergy [81–83]. Higher counts of maternal total aerobes and enterococci in third trimester stool samples were associated with increased risk of infant wheeze [OR 2.32 for 1 log increase in CFU/g stool (95 % CI 1.22, 4.42); OR 1.57 (95 % CI 1.06, 2.31), respectively). No organisms were associated with either eczema or allergic wheeze. Animal models have shown that maternal treatment with specific apathogenic bacteria during pregnancy can protect against allergic sensitisation in the offspring [84, 85]. The maternal gut microbiome has been shown to differ between overweight pregnant women and those of normal weight [86]. Moreover, distinctive changes to the maternal microbiome have been reported to be found in association with greater pregnancy weight gain [87]. Differences have been reported to exist in the gut microbiome of infants born to obese (BMI $\geq$30) versus non-obese mothers. During the first 6 months of life, fecal *Bacteroides* and *Staphylococcus* concentrations were significantly higher in infants of overweight mothers during the first 6 months. Prevalences of *Akkermansia muciniphila*, *Staphylococcus* and *Clostridium difficile* groups were lower in infants of normal-weight mothers and of mothers with normal weight gains during pregnancy [88]. Given the importance of the gut microbiota in shaping the developing immune system, perturbation of maternal flora in obese women, perhaps reflecting diet, might in turn affect the offspring's microbiota and risk of allergy (Fig. 15.2).

15.5.4 Epigenetic Effects

Epigenetic mechanisms present a further means by which maternal diet or adiposity during pregnancy might influence the in utero environment and impact upon fetal development (Fig. 15.2). Animal models have demonstrated that nutritional factors can invoke changes in the offspring epigenome which predispose to allergic asthma. Hypermethylation of the Runt-related transcription factor 3 (Runx3) under conditions of a high folate diet, for example, is associated with decreased expression of this gene. Runx3 negatively regulates airway inflammation; hypermethylation and decreased expression therefore increases inflammation and likely risk of allergic airway disease [89]. One study of the potential epigenetic consequences of maternal obesity has shown obesity in mothers to be associated with changes in methylation at differentially methylated regions (DMRs) of genes associated with early growth regulation. Moreover, this study found evidence that preconceptional exposures through the father might induce epigenetic shifts at DMRs of imprinted genes in the offspring. Newborns from obese fathers were

found to be hypomethylated at the mesoderm-specific transcript (MEST), paternally expressed gene 3 (PEG3) and neuronatin (NNAT) DMRs, independent of maternal obesity and other potential confounders [90]. Studies in mouse models suggest that demethylation of the MEST promoter may lead to overexpression of the gene, causing enhanced expression of genes related to metabolic conditions, such as diabetes [91]. Similar epigenetic mechanisms, potentially, could mediate transmission of obesity from either parent to effects upon immune development in the offspring, hence influencing allergic predisposition.

15.5.5 Genetic Links

Association between obesity and asthma might arise as a consequence of genetic pleiotropy, meaning shared genetic determinants might exist for these conditions. Data from same-sex twin pairs within the University of Washington Twin Registry indicate that obesity and asthma do appear to share genetic determinants [92, 93]. This study estimated that 8 % of the genetic risk for obesity is shared with asthma [92]. Genome-wide association scans for asthma have indicated linkage regions at 5q, 6p, 11q and 12q which contain candidate genes for obesity [94, 95]. Genes associated with obesity might be independently associated with asthma or these overlaps might occur by chance, merely mimicking pleiotropy, if obesity candidate genes co-segregate with closely linked genes which influence the risk of asthma. Either situation would lead to heritability of both conditions within families (solid lines Fig. 15.3). Alternatively, however, genes linked to obesity might encode protein products which directly increase the risk of asthma symptoms, offering opportunity for maternal (Fig. 15.1) or heritable childhood obesity to directly influence the risk of asthma in the next generation (broken lines Fig. 15.3).

A number of genes have been identified through association studies for which biologically plausible mechanisms can be proposed which explain shared inheritance of both obesity and asthma. For example, polymorphisms in the tumour necrosis factor-a (TNFa) gene have been found in association with obesity [96] and asthma and airway responsiveness [97, 98]. In a recent systematic genome-wide association study, an association was found between BMI and genetic variants in the Denn domain coding protein 1B (DENND1B) [99]. DENND1B is believed to exert pro-inflammatory effects through the TNF pathway and has also been found to associate with childhood asthma. The associations with BMI in asthmatic children were heterogeneous, however, and did not consistently replicate [100]. These heterogeneous genetic effects may arise as a consequence of gene–environment interactions.

15.5.6 Residual Confounding

Finally, factors which are independently associated with both maternal obesity and allergic diseases might be responsible for the apparent associations between these conditions in the absence of a true causal relationship (Fig. 15.3). Possible confounding factors include those shared by mothers and their children such as diet [101, 102] and activity levels [103] which might predispose to both maternal and childhood obesity. Allergy status might then reflect childhood rather than maternal body composition or diet. Given that the risk of allergy, particularly that of asthma, has been shown to be associated with childhood activity level [104, 105] and diet [106, 107]; 'in utero transmission' of any effect of a mother's body composition need not be invoked to explain the increased risk for her child. Equally, various environmental consequences of social class might confound the relationship if not appropriately recognised or accounted for [108].

15.6 Implications and Conclusions

Temporal and geographical gradients exist in obesity and allergic diseases which suggest a causal link explains the increases in prevalence of both these conditions observed over time as populations acquire a Westernised lifestyle. Previous observational studies have highlighted the importance of the early-life period for programming development, and plausible mechanisms exist whereby maternal obesity might be an important determinant of the in utero environment, thereby predisposing to allergic diseases. Whilst clinical and animal model data provide supporting evidence for intermediate steps linking maternal body composition with changes in adipokines and the maternal microbiome, or linking the neonatal microbiome to the risk of asthma, there are fewer examples linking maternal body composition or diet via a plausible intermediary mechanism to an allergic outcome in the offspring. An important exception to this is the association between increased pregnancy weight gain and elevated LPS-induced TNF-α production early in life, and between this elevation and childhood asthma in the offspring. Moreover, the epidemiological evidence, whilst supporting an association between maternal obesity and higher pregnancy weight gain and wheeze or asthma, provides little evidence of an association with specifically allergic asthma or indeed any other allergic disease.

Given the high prevalence and considerable impact of childhood allergic diseases upon morbidity and health-care costs, identifying causal pathways between maternal obesity and allergic diseases would be of great importance for public health. Further research is needed to identify the underlying mechanisms, effect modifiers and long-term consequences into adulthood. Further work to clarify whether risk varies most according to pre-pregnancy weight, pregnancy weight gain or aspects of maternal diet which promote weight gain is necessary to identify

at risk groups and to target interventions. Similarly, better understanding of the interaction with childhood obesity is needed. Potential interventions could include those targeting key mechanistic features such as altered maternal or infant microbiome in addition to those based upon dietary or other lifestyle change.

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Chapter 16
Epigenetic Mechanisms of Maternal Obesity Effects on the Descendants

Paul Cordero, Jiawei Li, Jonathan L. Temple, Vi Nguyen, and Jude A. Oben

Abstract Obesity has been described as a pandemic of the twenty-first century. Its prevalence among women of childbearing age continues to rise, increasing the risk of complications during pregnancy and the likelihood of their offspring developing obesity and its comorbidities in adult life. As our understanding of the developmental origins of health and disease has grown, the influence of maternal perinatal physiology has become more clear. Maternal programming appears to be shaped by epigenetic means. Diverse communities of epigenetic modifications determine the phenotypic characteristics of different cell types and are themselves adaptable to changes in cellular physiology and environment. It is now thought that such epigenetic programs are potentially heritable. Maternal body mass and other obesogenic cues have been widely associated with epigenetic alterations of offspring in human observational studies. Similarly, interventional studies in rodents demonstrate that obesogenic maternal diet, as well as maternal diabetes and obesity, manifests epigenetic and phenotypic alterations in different organs, often in association with genes related to appetite, glycaemic control and lipid biosynthesis. Whilst the dangers posed by obesity to the health of our society are undeniable, the impact of obesity upon the health of our children is only just beginning to emerge. Recent evidence suggests that, in addition to the effects of epigenetic programming upon first generation offspring, subsequent generations may also be affected. A greater understanding of the molecular phenomenology underlying

P. Cordero (✉) • J. Li • V. Nguyen
Institute for Liver and Digestive Health, University College London, Royal Free Hospital, NHS Foundation Trust, Rowland Hill Street, London NW3 2PF, UK
e-mail: paul.sanchez@ucl.ac.uk

J.L. Temple
Faculty of Life Sciences and Medicine, King's College London, London, UK

J.A. Oben
Institute for Liver and Digestive Health, University College London, Royal Free Hospital, NHS Foundation Trust, Rowland Hill Street, London NW3 2PF, UK

Department of Gastroenterology and Hepatology, Guy's and St Thomas' Hospital, NHS Foundation Trust, London, UK

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maternal epigenetic programming in obesity may well lead to the development of effective therapeutic interventions to combat this disease and its comorbidities.

Keywords Epigenetics • Obesity • DNA methylation • Histone • Developmental programming • Maternal obesity • Transgenerational • DOHaD • Epigenotype

List of Abbreviations

BMI	Body mass index
C/EBP-β	CCAAT/enhancer binding protein, beta
DOHaD	Developmental origins of health and disease
H19	H19, imprinted maternally expressed transcript
LINE-1	Long interspersed nuclear element 1
Mest	Mesoderm-specific transcript
NAFLD	Non-alcoholic fatty liver disease
NAFPD	Non-alcoholic fatty pancreas disease
NPY	Neuropeptide Y
Nr3c1	Nuclear receptor subfamily 3, group C, member 1
Peg3	Paternally expressed 3
POMC	Proopiomelanocortin
Ppargc1a	Peroxisome proliferator-activated receptor-gamma Co-activator 1-α
Ppar-α	Peroxisome proliferator activated receptor alpha
RXRA	Retinoid X receptor-α
TLR1	Toll-like receptor 1
TLR2	Toll-like receptor 2
Zfp423	Zinc finger protein 423

16.1 Introduction

Obesity is a chronic metabolic disease that arises from the complex interplay of numerous environmental, behavioural and genetic influences. It is characterised by an abnormally high proportion of adipose tissue constitutive of total body mass, and is strongly associated with an increased risk of cardiovascular disease, type 2 diabetes, various psychiatric disorders and cancers [1, 2]. As the global prevalence of obesity continues to rise at an alarming rate [3], there has yet to be any successful national or international effort to combat this disease, and its increasing economic burden upon health-care systems with limited resources [4].

Of particular concern is the rising prevalence of obesity among women of childbearing age, which has previously been demonstrated to increase the risk of complications during pregnancy and the likelihood that these children will, in turn, suffer from obesity and its associated comorbidities in adult life [5]. Prospective studies have repeatedly demonstrated strong links between maternal body mass index (BMI) in and around pregnancy and the incidence of obesity in adolescence and adulthood.

Barisione et al. reported that 22 % of children born to mothers suffering from obesity were, themselves, obese at 12 years of age [6]. However, the prevalence of obesity among their siblings, who were born following substantial surgically induced maternal weight loss, was just 3 % at the same age. This discrepancy extended even into adult life, where the mean weight and BMI for each group were 79.5 kg and 27.5 kg/m^2 and 66.7 kg and 23.4 kg/m^2, respectively [6]. Furthermore, animal models of maternal obesity induced by obesogenic perinatal diet have also described increased body mass and adiposity, hepatic steatosis, adverse metabolic lipid profiles and a greater response to obesogenic diet among the offspring [7–10].

The Developmental Origins of Health and Disease (DOHaD) theory suggests that maternal physiology and metabolism during the perinatal, fetal and even preconceptional phases of development are capable of modifying the metabolic profiles of their offspring by altering how different cell types express specific genes across different tissues and time [11–14]. Consequently, epigenetics has been proposed as the main molecular mechanism implicated in this perinatal programming [15].

16.2 Epigenetic Mechanisms

Epigenetics describes the translation and adaptation of genotype to phenotype, which is regulated by a complex and interacting network of covalent modifications of chromatin structure (Fig. 16.1). These epigenetic modifications determine the cellular state and the metabolism affecting gene expression patterns in a cell-specific manner whilst preserving the nucleotide sequence [16].

The cellular state is intrinsically related to the chromatin state, which describes the association of DNA molecules with specialised proteins, including histones, which package and configure the genetic code three-dimensionally within the confines of the nucleus. These histones, and in particular their N-terminal tails, are susceptible to a variety of post-translational modifications, including phosphorylation, ubiquitinylation, acetylation and methylation [17]. Commonly referred to as histone modification marks, each is believed to contribute to the regulation of gene expression by controlling the degree of condensation of the surrounding chromatin and hence the ease of access for the transcriptional machinery. The other main class of epigenetic modification is DNA methylation. This modification is mainly found at a cytosine with a guanine as next nucleotide (CpG site) and is commonly associated with transcriptional repression. These CpG sites are abundant within and around gene promoter regions, where specific transcription factors bind to DNA in order to recruit the transcriptional machinery and orchestrate the gene expression [18]. Thus, methylation of the promoter region at these sites represses transcription by means of steric impedance of transcription factor binding or via intermediary proteins that bind methylated DNA [19]. Finally, whilst not directly interacting with DNA and thus not strictly a class of epigenetic modification, short-chain RNA molecules, referred to as microRNAs, which are not themselves translated, appear to interact with mRNA sequences and regulate protein synthesis [20].

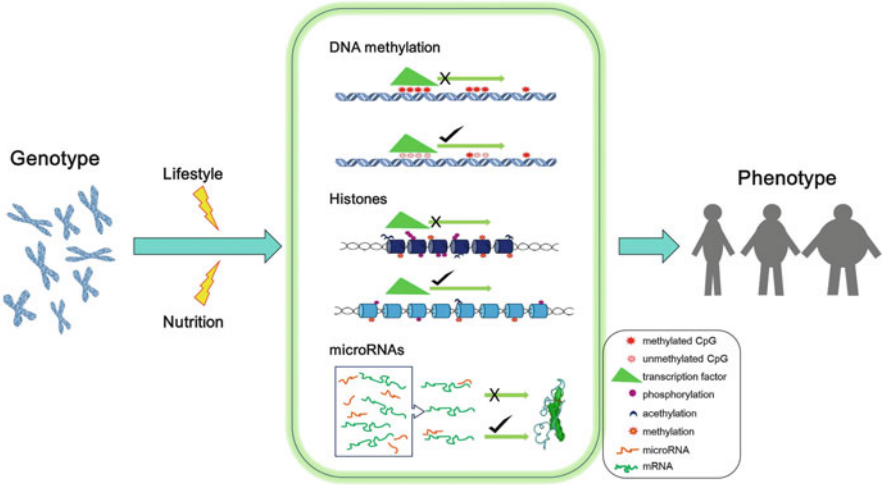

Fig. 16.1 External environment interacts with genotype altering epigenetic profile. Changes in DNA methylation at CpG sites and post-translational modifications on histone tails promote alterations in chromatin condensation profile, which regulates the join of the transcriptional machinery of the genes. Furthermore, the microRNAs interfere with post-transcriptional regulation of gene expression. The combination of these epigenetic changes defines phenotypic characteristics

There exist key time frames, during which a cell's epigenetic profile is more susceptible to change and adaptation, and hence more vulnerable to environmental insults, especially during pregnancy and breastfeeding. In this way, the maternal metabolism can affect or 'program' the epigenetic profile of their offspring and thereby alter that child's risk of developing obesity in adult life [21]. At such times, the rate of cellular division is dramatically increased and DNA more exposed to the chemical modification within the nucleus, which may represent an enticing opportunity for future clinical interventions [21].

Our growing understanding of the mechanisms of maternal programming in obesity may go on to explain how the features of this disease can be modified by lifestyle and nutrition, uncovering how the genetic information and environmental exposure interact at the molecular level [22, 23]. Differences in DNA methylation patterns between those who suffer from obesity and controls have been widely reported in the literature [24] and successfully used as a biomarker of dietary response in kilocalorie-restricted diets [25].

It is now apparent that epigenetic information can be passed on to the next generation. This has led some to observe that we are not only what we eat but also what our progenitors ate [26, 27]. Recent evidence suggesting that maternal programming can endure across successive generations is startling and hints at an epigenetic landscape in obesity of previously unimagined complexity and importance.

16.3 Epigenetic Programming in Maternal Obesity

Obesity induces an aggressive and degenerative physiological environment, increasing the levels of triglycerides, cholesterol, glucose and other metabolites in the plasma, raising blood pressure and causing systemic angio-dysgenesis and hypoxaemia [1, 2]. Ultimately, this can lead to multiple organ damage, including non-alcoholic fatty liver disease (NAFLD), non-alcoholic fatty pancreas disease (NAFPD) and various cancers [1, 2]. Maternal obesity at conception, during pregnancy and while breastfeeding exposes the offspring to this adverse environment, programming their physiology with a heightened susceptibility to developing metabolic diseases in their own lifetime (Fig. 16.2). These children are more likely to be born prematurely and often with abnormally high or low birthweight. This was demonstrated in a study of 319 such mother–child pairs, where global DNA methylation (quantified by Long Interspersed Nuclear Element 1, LINE-1) in samples of cord blood was found to be greater in premature and extreme birthweight newborns compared with controls, and associated with increased adiposity in later life [28].

In addition to affecting the development of obesity in their offspring, maternal obesity can also trigger the physiological dysregulation of other systems in offspring. For example, different patterns of DNA methylation were found in 57 genes related to the development of the central nervous system in samples of umbilical cord blood in infants from obese mothers versus normal-weight mothers, and may well indicate the onset of abnormal nervous system development in these children [29].

As technology has evolved, genome-related massive omic tools have arisen as the most useful initial approach in the search for biomarkers of maternal programming. Genome-wide interrogation of cord blood samples from more than a thousand mother–child pairs has also identified multiple CpG sites that are concordantly methylated in mother and child, and associated with high maternal weight and offspring adiposity [30]. Genome-wide methylation analyses have also found links between maternal BMI and patterns of cord blood DNA methylation in genes related to cardiovascular disease and several malignancies [31]. However, in order to identify the specific genetic loci affected by maternal obesogenic programming, a larger pool of data encompassing more diverse populations is still required. That said, numerous plausible candidates have already been identified. For example, in

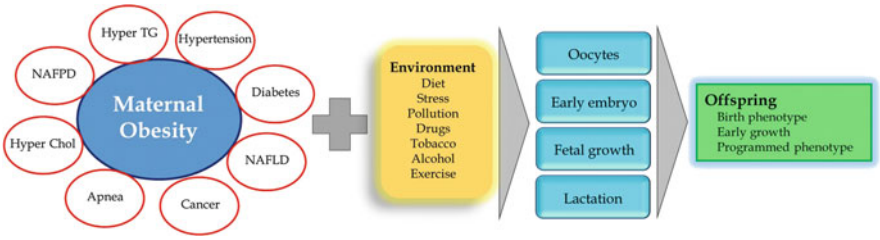

Fig. 16.2 Maternal obesity and other perinatal influences program offspring development (*NAFPD* non-alcoholic fatty pancreas disease, *NAFLD* non-alcoholic fatty liver disease, *Hyper TG* hypertriglyceridaemia, *Hyper Chol* hypercholesterolaemia)

cord blood, high levels of DNA methylation at the gene encoding Retinoid X Receptor-α (RXRA), a transcriptional regulator, have been associated with increased adiposity in children at 9 years of age [32]. Gemma et al. also describe a positive correlation between maternal BMI and methylation levels of the Peroxisome Proliferator-activated Receptor-gamma Co-activator 1-α (Ppargc1a) gene in cord blood, which encodes a transcriptional activator involved in regulating various metabolic processes, including energy homeostasis, hepatic gluconeogenesis and cholesterol levels [33]. Additionally, Lesseur and colleagues observed that levels of DNA methylation of the Leptin gene promoter were comparably lower in blood samples taken from obese mothers than normoweight mothers just before pregnancy, as well as cord blood samples from their children at birth, when compared to mothers of normal weight and their offspring. These neonatal methylation levels were shown to correlate with leptin concentration in maternal plasma [14]. Furthermore, differences in ambion microRNA expression profile between obese and normoweight women were associated with a downregulation of insulin and adipocytokines signalling pathways, among others [34].

The paternal epigenetic profile, carried by the sperm, may also have a role in predisposing subsequent generations to obesity. Paternal obesity prior to conception has recently been associated with higher levels of DNA methylation of several genes (MEST, PEG3, NNAT and Igf2) known to be affected by paternal imprinting in leukocytes extracted from cord blood [35, 36].

This same study found that methylation levels of different genes (PLAG1, MEG3, H19) were associated with maternal obesity prior to conception, which implies that paternal and maternal influences may affect their child's physiology differently [35, 36].

Besides the intrauterine environment, breastfeeding also represents a critical period of epigenetic reconfiguration for the neonate in response to maternal chemical influences. A study of 120 mother–child pairs, undertaken by Obermann-Borst et al., found a negative correlation between the duration of breastfeeding and leptin methylation in whole blood samples taken from offspring at 17 months post-partum [37].

Existing, as well as novel, interventions may be targeted during key developmental windows to ameliorate the risk of maternal obesity to the unborn [38]. For example, Guenard et al. describe a significant reduction in the cardiovascular risk profile of children born after substantial maternal weight loss induced by bariatric surgery when compared to their older siblings. Subsequent, transcriptomic and epigenetic analysis of whole blood samples identified 5698 genes that were differentially methylated between these sibling pairs, many of which were related to glycaemic control, inflammation and vascular disease [39]. The transcriptional patterns of five such genes linked to the innate immune system and inflammatory response were also shown to differ significantly between these two groups [40]. The critical importance of innate immunity and its regulation in the context of maternal obesity has received further support from animal studies of maternal obesogenic feeding that demonstrate innate immune dysfunction in offspring with developmentally programmed NAFLD [41].

However, it could also be argued that any of the comorbidities associated with maternal obesity may adversely condition the epigenetic profile and affect the

development of these children. Indeed, maternal diabetes mellitus and gestational diabetes have previously been described as 'conditioning factors' affecting development in utero. Global methylation levels of placental DNA appear to be decreased in gestational diabetes and pre-eclampsia but increased in maternal obesity [42]. This phenomenon has been associated with specific phenotypic characteristics, such as head circumference at birth and height in infancy. Reduced methylation at specific CpG sites within the leptin gene has also been demonstrated in cells derived from cord blood in the context of maternal hyperglycaemia [43]. El Hajj et al. further noted that the offspring of mothers with gestational diabetes display reduced DNA methylation of the genes Mest (mesodermic-specific transcript), Nr3c1 (nuclear receptor subfamily 3, group C, member 1) and Alu sequences in cord blood and placenta, when compared to mothers with adequate glycaemic control [44]. These results accord with prior evidence that Mest methylation is similarly reduced in blood samples taken from adults who were morbidly obese [44].

To date, research in this field is largely based upon observational studies in humans, but rodent experimental models of maternal obesity have now become the first choice for interventional studies seeking to elucidate the epigenetic mechanisms of maternal developmental programming in obesity [45]. Usually such interventions involve perinatal obesogenic feeding, enriched in simple sugars and fats, similar to the Western diet [46]. Expectant mothers are fed in this way during pregnancy and whilst breastfeeding. The simplicity and economy of rodent maintenance, their significant genetic, physiological and metabolic similarities with humans and their relatively short lifespans make them ideal for studying these phenomena over successive generations [45, 46].

The offspring of such high-Fat fed obese mice, for example, display decreased levels of methylation at the promoter of the gene encoding the zinc finger protein 423 (Zfp423), a transcription factor committing cells to the adipose lineage, in association with downregulation of histone marks H3K27me3, and higher levels of expression in fetal adipose tissue [47]. Maternal obesity during pregnancy has also been associated in rats with similarly reduced levels of DNA methylation at the genes encoding Zfp423 and C/EBP-β (CCAAT/enhancer binding protein, beta), another proadipogenic transcription factor, as well as increased levels of their respective mRNA transcripts in offspring adipose tissue [48]. Concordantly, the extent of hypothalamic DNA methylation of the genes encoding proopiomelanocortin (POMC) and neuropeptide Y (NPY), both involved in the regulation of appetite, was positively correlated with calorific intake [49, 50].

Obesogenic maternal diet prior to and after conception has also been shown to induce altered levels of microRNAs associated with cardiovascular disease within the myocardium of baboons [51]. Also, in the livers of similarly exposed neonatal rats, the expression of Cdkn1a, a gene associated with hepatocyte growth following liver damage and several cancers, was upregulated in tandem with lower levels of promoter methylation [52]. Maternal obesity appears to program a greater propensity for NAFLD in their offspring, exacerbated further by exposing them in turn to an obesogenic diet. In such circumstances, changes in the DNA methylation profile

of genes related to circadian rhythmicity in liver, Bmal1 and Per2, have since been reported [53].

The key question then becomes how we might effectively intervene and attenuate the risk of maternal obesity to the next generation. Animal studies of maternal physical exercise have, for example, demonstrated successful prevention of Pparg1a hypermethylation in the offspring of mothers fed an obesogenic diet, normalising its expression and that of its target genes in skeletal muscle [54]. Maternal weight loss in sheep prior to conception has also been shown to affect hepatic insulin signalling and microRNA expression profiles in their offspring [55]. It is perhaps not surprising, then, that physical maternal exercise appears to protect the offspring from the physiological changes mediated by maternal obesity. Maternal micronutrient supplementation in rats while breastfeeding has also been found to prevent maternal obesity-induced homocysteinaemia in offspring, in association with changes in the activity of DNA methyltransferases and global levels of hepatic DNA methylation [56].

Given that the epigenetic profile of each cell defines its identity and its role within the organism, it is conceivable that the maternal nutritional condition affects different cell types in different ways [11]. When occurring in germ cells, these alterations gain the potential to endure through successive generations, extending the implications of maternal programming in obesity.

16.4 Transgenerational Epigenetic Programming in Maternal Obesity

Whilst the majority of research has sought to elucidate the mechanisms and manifestations of maternal epigenetic programming in obesity by focusing on the first generation of offspring, recent evidence suggests that these maternal programs can endure across successive generations [57]. The implications of transgenerational programming in maternal obesity are startling, hinting at an extremely complex and multidimensional epigenetic landscape that is yet to be fully understood.

The time constraints implicit in such experimental models of transgenerational obesity inevitably render rodents preferable subjects to humans. High-fat perinatal maternal feeding in mice has recently been demonstrated to increase the birthweight, adiposity and macrophage infiltration of adipose tissue across three generations of their descendants. This immunomodulation was accompanied by a decrease in the levels of promoter methylation and increased expression of Toll-like receptor 1 and 2 (TLR1 and TLR2), both involved in the activation of T cells [58]. A similar study of maternal obesogenic diet in mice prior to conception induced traits of the metabolic syndrome in five subsequent generations of their offspring, as well as altering patterns of histone marks at the genes encoding leptin and adiponectin and their expression in white adipose tissue [59]. When offspring of these animals returned to a control diet, these alterations were completely

abolished after three generations, implying that effective intervention of this kind is possible.

When epigenetic modifications are induced in germ cells by the perinatal maternal environment, they gain potential transmissibility across subsequent generations [57] (Fig. 16.3). Ge et al. described significant alteration of Peg3 (paternally expressed 3) and H19 (H19, imprinted maternally expressed transcript) promoter methylation in the spermatozoa of offspring in a mouse model of maternal obesity and diabetes mellitus [12]. Oocytes harvested from obese females displayed increased methylation of the leptin promoter and reduced peroxisome proliferator-activated receptor alpha (Ppar-α) promoter methylation. Whilst similar transcriptional and epigenetic profiles were observed for Ppara-α in the livers of their female offspring, oocytes harvested from these same offspring displayed increased levels of Ppar-α promoter methylation [13]. Hence, different cell types appear to be differentially affected by maternal programming in obesity, perhaps even at different stages of development. Interestingly, maternal weight loss prior to conception appeared to reprogram patterns of DNA methylation in the liver and normalise the expression of genes related to lipid metabolism in their offspring [60]. This emphasises how changes in maternal physiology, even prior to conception, hold the potential to influence the metabolism of their offspring by affecting the epigenetic processes that regulate gene expression.

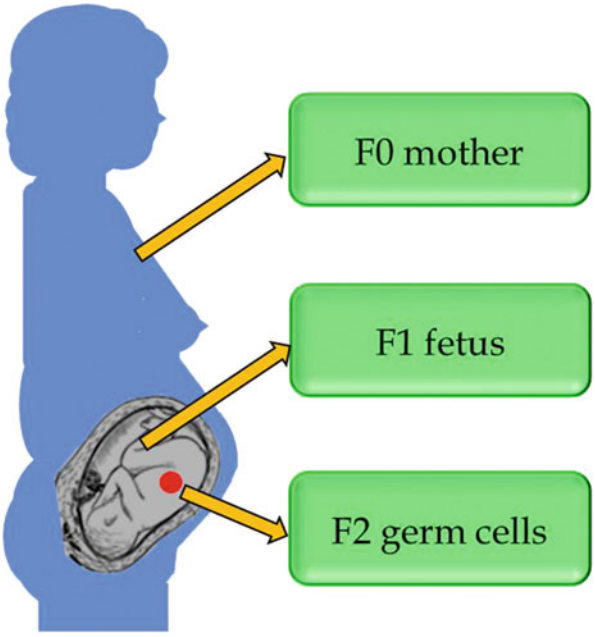

Fig. 16.3 Transgenerational transmission by maternal environment during pregnancy

Whilst most of the transgenerational animal models were originally designed for the study of potentially teratogenic agents [61] and may require further adaptation, they have already shed considerable light upon the nature of transgenerational epigenetic programming in maternal obesity. However, it must also be acknowledged that our mechanistic comprehension of this phenomenon remains, itself, in its infancy.

16.5 Conclusion

Maternal obesity during pregnancy, through breastfeeding and mechanisms preceding conception, can program their offspring with a physiological predisposition towards developing obesity and its associated comorbidities in adult life. Maternal programming in obesity engenders changes in the epigenetic profiles of diverse cell types, affecting how certain genes associated with obesity are expressed at different stages of a child's development. The epigenetic programs that mediate the phenotypic characteristics of this disease appear to be transmissible and can endure across successive generations. However, they remain amenable to appropriately targeted intervention. A deeper understanding of the molecular phenomenology underlying maternal epigenetic programming in obesity is desperately needed in order to develop more effective therapeutic approaches in the management of this burgeoning global epidemic.

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Chapter 17
Early Microbe Contact in Defining Child Metabolic Health and Obesity Risk

Erika Isolauri, Samuli Rautava, Maria Carmen Collado, and Seppo Salminen

Abstract The background to the increase in nutrition-related chronic conditions such as overweight and obesity is more complex than is generally anticipated. Recent scientific data suggest that metabolic disturbances can arise from aberrant gut microbiota, with or without alterations in dietary composition. In particular, early-life dysbiosis induces lasting alterations in the immune and metabolic phenotype. The compositional development of the indigenous intestinal microbiota, co-evolving with the key regulatory systems of the body, is highly sensitive to the mode of delivery and early feeding, antibiotic use and maternal immune and nutritional state during pregnancy. All these elements interact with the microbiota. Consequently, considerable research interest is currently focusing on the microbial inoculum provided by the feto-maternal interface, along with microbe contact during delivery and through lactation. The early colonisers provide a framework conceptualising the way early-life (pre-, peri- and postnatal) exposures are linked to disease processes and even the pathogenesis of disease. To quote Hippocrates: "All disease begins in the gut". This holds especially true for nutrition-related diseases, polarised in the detrimental consequences of undernutrition or overnutrition. The impact of the gut microbiota culminates in early infancy, when the immune responsiveness and metabolic phenotype are consolidated. The gut microbiota contributes to nutrition, immunity and metabolism by processing nutrients and regulating their access to and storage in the body, producing chemicals of hormonal nature and controlling the secretion of pro-inflammatory mediators locally and

E. Isolauri, MD, PhD (✉) • S. Rautava, MD, PhD
Department of Paediatrics and Adolescent Medicine, Turku University Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland

Department of Clinical Sciences, Faculty of Medicine, University of Turku, Turku, Finland
e-mail: eriiso@utu.fi

M.C. Collado, PhD
Institute of Agrochemistry and Food Technology-National Research Council (IATA-CSIC), Burjassot, Valencia, Spain

S. Salminen, PhD
Functional Foods Forum, Faculty of Medicine, University of Turku, Turku, Finland

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systemically. Another quotation from Hippocrates states that “Natural forces within us are the true healers of disease”. Recent experimental and clinical studies have attracted scientific interest in reprogramming deviations in the gut microbiota. Promoting the predominance of specific non-pathogenic microbes and thereby modifying the intestinal milieu may be taken as an alternative means of attaining prophylactic or therapeutic effects in metabolic and inflammatory conditions. As the critical time window for these to exert their programming effects falls around birth, early initiation of preventive measures is of the essence; influencing the feto-maternal microbe contact may promote the health of the next generation.

Keywords Allergic disease • Atopy • Child • Growth • Gut microbiota • Microbiome • Mode of delivery • Obesity • Overweight • Pregnancy • Probiotics

17.1 One Child in Four Is Overweight or Obese: Research on the Gut Microbiota Has Opened Up New Angles on Regulation of the Host Metabolism

Overweight and obesity currently constitute a global threat to human well-being, not limited to the heightened risk of morbidity from cardiovascular diseases, diabetes and asthma. Devising preventive measures is challenged by the increasing incidence of obesity in children, as it is likely to persist into adulthood [1]. In affected females, gestational diabetes is one sequela, carrying a risk of complications during pregnancy and delivery and hampering breastfeeding [2]. A vicious circle ensues: maternal obesity is associated with neonatal adiposity and high birthweight and an increased risk of childhood obesity. An escalation of the obesity problem and comorbidities may thus be envisioned in the future, since the velocity of propagation is high in the population at reproductive age. Indeed, according to the current understanding, pregnancy and the perinatal period constitute the most critical stage and by the same token an optimal target for interventions aiming to reduce the risk of non-communicable diseases.

One review of systematic reviews [3] has sought to detect early-life determinants of obesity. Altogether 22 eligible reviews from a database of 12021 publications were evaluated. The studies in question showed later overweight and obesity to be associated with maternal diabetes and smoking, rapid infant growth, no or short breastfeeding, obesity in infancy, short sleep duration, less than 30 min daily physical activity and consumption of sugar-sweetened beverages. However, the authors [3] called for intervention studies into the problem, concealed as it is by a complex web of associations and reciprocal influences.

The theory of obesity development appears simple: more calories are consumed than expended. In the past decades, each of the energy nutrients has been taken individually as the source of the problem, in terms of either quantity or quality. Replacing energy nutrients, however, for example caloric sugars by non-caloric artificial sweeteners with an eye to reducing energy intake may in fact have contributed to the obesity epidemic instead of fighting the problem [4]. Moreover,

energy nutrients and their metabolites have been the focus of research aiming to identify bioactive compounds regulating our digestive, metabolic and immune systems. To take one example, whey proteins may exert anti-obesity effects [5].

Importantly, metabolites, nutrient components and nutrients contributing to or potentially ameliorating the development of obesity have been studied separately for decades, implementing the traditional reductionist approach in nutrition research. The recognition, however, that the whole may be more than the sum of its parts also in respect of the diet [6], without ignoring the individual host per se, would seem to imply that the development of the obese state depends on more than host genes and diet.

A myriad of experimental studies demonstrate that the ensuing immune and metabolic changes are caused not by direct effects of dietary intake but rather by consequent changes in the gut microbiota (reviewed in: [7, 8]). In fact, the impact of energy nutrients such as a high-fat diet on weight gain depends notably on the gut microbiota and the immunological status of the host. To take one example of host–microbe interaction, gut barrier dysfunction, activation of immune genes and pro-inflammatory cytokines in response to a high-energy diet precede the development of obesity (reviewed in: [9]). In human studies, aberrant compositional development of the gut microbiota is documented during breastfeeding in infants in whom overweight development ensued [10]. Equally, aberrancies in the gut microbiota tend to define the obese state. Adiposity, insulin resistance and dyslipidaemia are reportedly associated with low bacterial richness and higher abundance of faecal *Bacteroidetes* and *Proteobacteria* accompanied with limited production of organic acids, such as lactate, propionate and butyrate, and an inflammatory immune state [11]. Within the group of subjects with low bacterial richness, obese individuals also gained more weight over time [11]. The significance of the richness of *Bacteroides* species, however, may be difficult to verify due to different continuously developing methods and their varying accuracy. Different studies may therefore not be easily comparable and the significance of these differences awaits further studies to uncover the role of both *Bacteroidetes* and organic acids on energy extraction from food and energy storage in fat [12]. Organic acids such as butyric acid are additionally thought to be involved in glucose regulation and insulin resistance by controlling energy homeostasis and modulating adipose tissue, sustaining the propensity to excessive weight gain [13].

According to the hypothesis of Developmental Origins of Health and Disease [14], our health is particularly endangered if the environment after birth differs from the situation during pregnancy, for example restricted in utero nutrition followed by the abundant nutrition characteristic of the Western lifestyle increases susceptibility to metabolic disorders [15–17]. Adaptation processes during the critical stages of fetal development might permanently affect the activity of human genes by epigenetic mechanisms to the anticipated extrauterine environment, while the experienced postnatal environment would necessitate regulation to the opposite direction. Clinical evidence of early programming in obesity has been provided for both maternal undernutrition and overnutrition (reviewed in: [18]). The Western lifestyle, again, is a relatively recent development consequent upon

the introduction of agriculture, and one explanation for immune and metabolic morbidity would indicate that our genome has not had time to adapt to such an environmental change. Viewed through this lens, the process of adaptation of the complex collection of genes in the gut microbiota (the microbiome), exceeding 150-fold the number of our genome [19], to our modern nutrition is far from complete. Indeed, food is the major determinant of the gut microbiota composition and activity. In view of the fundamental direct impact of the gut microbiota on the processing of nutrients and the regulation of their access to and storage in the body [20] and the inflammatory responses causally related to insulin sensitivity [21] and indirect effects on the hypothalamic–pituitary–adrenal axis [22], it would clearly be simplistic to assume that the complex collection of the microbiome is a bystander in the process of metabolic programming.

17.2 Origins of Healthy Microbiota and Early Developmental Impacts on the Gut Microbiota

17.2.1 Prenatal Microbe Exposure

Recent findings that the placenta has been shown to be colonised with bacteria provide the basis for a counterargument against the paradigm of sterile fetal life [23]. Consequently, the colonisation of the fetus is initiated already in utero (Fig. 17.1) and further reinforced by the exposure of the newborn to the mother's microbiota during delivery and subsequently through breastfeeding (reviewed in:

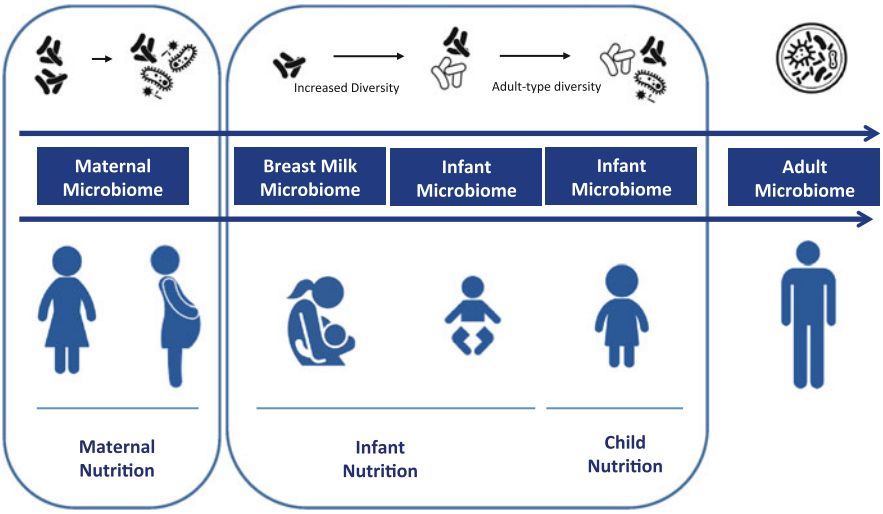

Fig. 17.1 Critical stages in gut microbiota development

[24]). In fact, the foundation of the step-wise compositional development of the offspring gut microbiota may be laid at conception, when the male seminal microbiota is in contact with the vaginal microbiome. In an Estonian study [25], the bacterial diversity of vaginal and semen samples was determined by sequencing the V6 region of 16S rRNA genes. Seminal and vaginal bacterial communities shared a high number of phylotypes, the most common being *Lactobacillus*, *Veillonella*, *Streptococcus* and *Atopobium*. Vaginal samples harboured more species from the genera *Lactobacillus*, *Streptococcus* and *Gardnerella* compared to semen samples, and in most vaginal samples lactobacilli were the dominant microorganisms with *Lactobacillus crispatus*, *Lb jensenii* and *Lb gasseri* as the most abundant [25].

The microbial environment encountered by the fetus in both the placenta and amniotic fluid is much more extensive than was formerly understood [26–29]. Specific genera reported to be present in the amniotic fluid and placenta include *Propionibacterium*, *Enterococcus*, *Staphylococcus*, *Citrobacter* and *Lactobacillus*, some of which, e.g. *Propionibacterium*, *Staphylococcus* and *Lactobacillus*, are also often present among skin microbiota [30]. Genera shared between amniotic fluid and meconium, the first faecal specimen passed by the neonate, include *Bacteroides*, *Lactobacillus*, *Prevotella* and *Peptostreptococcus* as assessed by DNA-based methods. Culture of specimens from amniotic fluid and placenta resulted in the identification of mainly lactobacilli, *Streptococcus agalactiae* and *Fusobacteria* [28–30]. Possibly the fact that the fetus constantly ingests amniotic fluid explains the likeness of amniotic fluid and placenta microbiota to that of meconium [28, 31, 32]. Some studies in vaginally delivered neonates report only 2–5 genera present in the meconium, these comprising *Bifidobacterium*, *Enterobacteriacease*, *Enterococcaceae* and *Bacteroides-Prevotella*, confirming microbial exposure in utero [33].

17.2.2 Impact of Delivery

The initial microbe contact is complemented perinatally by implantation of the mother's intestinal and vaginal microbes during vaginal delivery, and such microbe transfer will obviously vary from one mother to another, fluctuating between the main genera *Lactobacillus*, *Streptococcus* and *Gardnerella*. Bäckhed and associates [34] reported that 72 % of early colonisers of vaginally delivered newborns matched the species in their faecal samples with those observed in their mother. Thus, the gut colonisation of the neonate originates specifically from microbes in the amniotic fluid and placenta, and these are complemented by the first bacteria from environmental contact depending on the mode of delivery.

17.2.3 Role of Breast Milk in Microbial Colonisation

Several reports suggest that mother–infant transfer of microbes is mediated partly by the microbiota of human milk (Fig. 17.1). The breast milk microbiota consists of a large number of organisms, some of which appear to originate from the mother's gastrointestinal tract and some from her skin [35, 36]. Furthermore, the biodiversity of the microbiota in breast milk includes a wide spectrum of bacteria, including *Staphylococcus*, *Streptococcus* and some lactic acid bacteria as the most common groups [36–39]. There are reports that skin microbes may also be present in human milk and these include specifically *Staphylococcus* and *Propionibacterium* of skin origin. These may be partly contaminants from the skin area but also natural inhabitants of the breast tissue.

It is important to recognise that the breast tissue itself also contains bacteria even in cases without any signs of pathogenic processes. A Canadian–Irish study analysed breast tissue samples from 81 women with and without cancer [40]. A wide range of bacteria was identified within all sites around the breast tissue in women, none of whom had a history of lactation. The principal phylum was *Proteobacteria*. The most abundant taxa in the Canadian samples were *Bacillus*, *Acinetobacter*, *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus*, *Propionibacterium*, *Comamonadaceae*, *Gammaproteobacteria* and *Prevotella*. In the samples from Irish women, more than 30 % contained *Enterobacteriaceae* and additionally *Staphylococcus*, *Listeria*, *Propionibacterium*, and *Pseudomonas*. No symptoms of infection were observed, and the viability of some of the detected bacteria was verified by culturing [40].

It is important to note that breast milk composition, including the microbiota, is associated with the mother's diet and nutritional state, the environment and the use of pharmaceuticals [37–42]. Furthermore, Hunt and co-workers demonstrated that human milk bacterial communities are complex, with several genera representing more than 5 % of the relative community abundance, for example *Streptococcus*, *Staphylococcus*, *Propionibacterium* and *Actinomyces* [38].

The *Bifidobacterium longum* group bacteria are the most common amongst the bifidobacterial species in human breast milk samples [36] and continuously reflected in faecal samples of breast-fed infants after a few days of breastfeeding. *Bifidobacterium longum*-type bacteria continue to be present later, while other species of bifidobacteria also appear and the species composition changes gradually following weaning. Reports from Northern Europe, Malawi and Brazil [41, 42] demonstrate that all breast-fed infants are generally colonised by bifidobacteria and specifically *Bifidobacterium longum*-type bacteria, most likely of breast milk origin.

17.2.4 Weaning Period and Beyond

Gradual weaning induces an increase in *Bacteroides*, *Clostridium* and *Ruminococcus* in the infant gut microbiota, while at the same time *Bifidobacterium* and *Enterobacteriaceae* decrease, rendering the *Bifidobacterium* and *Lactobacillus* dominant environment more diverse. Bäckhed and co-workers [34] report that during the first year of life the microbiota of an infant evolves from a relatively simple composition towards a complexity resembling the adult-type microbiota (Fig. 17.1); α-diversity is increased while β-diversity is decreased, suggesting a more complex and less heterogeneous microbial community. By the end of the first year of life, infants possess an individually distinct microbial profile, converging towards the characteristic microbiota of an adult (Fig. 17.1), such that by 2–5 years of age, the microbiota fully resembles that of an adult in terms of composition and diversity [43–45].

Few reports have been published on longer term monitoring studies, but one study from Finland monitoring microbiota during the first 13 years of life demonstrates that bifidobacteria continue to form a significant part of the infant and child microbiota composition. It has also been reported that the gut microbiota composition alters considerably between 6 months and 12 months of life, and at the age of 12 months is already slowly being converted towards a profile characteristic of an adult microbiota in healthy Finnish breast-fed children, who also remained healthy in a long-term follow-up study [46]. In the same manner, a recent study following the microbiota composition in children with a healthy growth pattern residing in Dhaka, Bangladesh [47], revealed that the gut microbiota composition is defined by age more strongly than by individual variation.

The adult type gut microbiota is reported to be relatively stable (Fig. 17.1). However, it may also be influenced by diet and bacteria in our food supply. Recent studies from the USA have suggested that a diet recommended by the United States Department of Agriculture provided the largest number of live food microbes to the gastrointestinal tract (1.3×10^9 CFU/day), while the typical American diet based on more processed convenience foods (such as TV meals and fast foods) provided the least exposure to microbes, i.e. 6×10^6 CFU/day, on a daily basis [48]. The exposure ensured by the recommended diet would provide more stimuli to impact the gut microbiota composition profile compared to processed convenience foods.

17.3 Gut Microbiota and Metabolic Health

Microbiota disruption during the step-wise developmental process described above may manifest as the emergence of non-communicable diseases, including obesity. The early colonisers coevolving during the immunological and metabolic maturation process during the critical stages of pregnancy, delivery and breastfeeding (Fig. 17.2) confer propensity for health and disease. There is a growing awareness

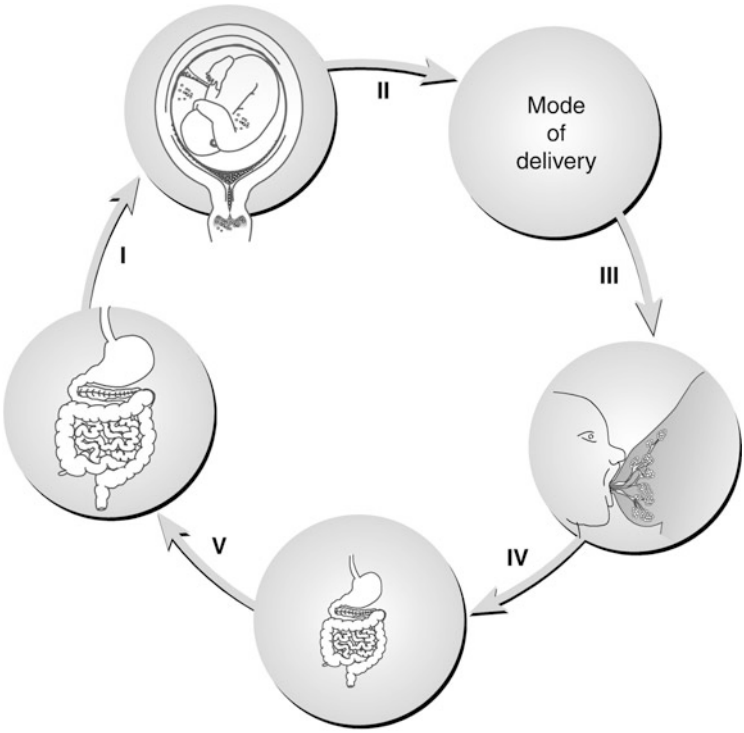

Fig. 17.2 Perturbing factors for microbiota composition patterns during critical stages of development. (I) Pregnant state. Gut microbiota becomes more pro-inflammatory and less diverse during the third trimester of pregnancy. Placenta microbes colonise and influence the feto-maternal interphase during pregnancy. Amniotic fluid microbiota provides the first ingested microbes to the fetus. (II) Mode of delivery. Normal microbiota from mothers' birth canal in vaginally delivered infants. Deviated microbiota, derived from oral and skin microbiota and the immediate environment, typifies the gut microbiota composition of caesarean section-delivered neonates and persists beyond infancy. (III) Breastfeeding. Deviations in breast milk microbiota composition in overweight mothers and those with excessive weight gain during pregnancy. (IV) Infant microbiota composition. Step-wise compositional development of microbiota in healthy infants who remain healthy long term. Impact of weaning and the type of weaning foods; deviations in gut microbiota development due to formula feeding, if not supplemented with specific probiotics. Microbiota of weaning foods and other foods. (V) Stable healthy adult microbiota. Environmental disturbances (toxins, microbes, food, living environment) cause deviations. Use of pharmaceutical products and impact of specific diseases or conditions may permanently alter gut microbiota composition

that dietary and other environmental exposures impact on gut microbiota development. Moreover, the age-appropriate composition appears to be fundamental to health. Undernourished Bangladeshi children exhibited a younger gut microbiota profile than expected for their chronological age (reviewed in: [47]), indicating immaturity. In contrast, precocious maturation of the microbiota during early infancy has been linked to overweight development in a Singaporean birth cohort

[49], supporting reports from Finland of lower numbers of bifidobacteria, but higher numbers of *Staphylococcus* in breast-fed children with overweight development later in life [10].

The involvement of the microbiota in metabolic programming has been corroborated in subsequent studies. A recent birth cohort study linked early *Bacteroides fragilis* group colonisation at 1 month of age with an elevated body mass index (BMI) z-score and excessive weight gain by the age of 10 years [50]. In the same vein, a birth cohort study of vaginally delivered full-term infants reported that higher *B. fragilis* group numbers at 3 and 26 weeks of age were related to higher body mass indices (BMI) during follow-up from 12 to 36 months of age, while higher levels of *Staphylococcus* ssp. at 3 and 52 weeks of age were related to lower BMI z-scores in preschool children [51]. A lower *Staphylococcus* to *Bacteroides* ratio was associated with a higher BMI standard deviation score during the first three years of life [51]. On the other hand, it has been reported that higher *Lactobacillus* spp. and lower *Bacteroides* spp. in the infant gut during the first 3 months of life may be linked to the risk of childhood overweight [52], underlining the difficulty to directly compare studies applying different methods of varying accuracy. Moreover, the rate of acquisition of a certain microbiota pattern, with for example lower abundances of *Bifidobacterium* and *Collinsella* spp., may predict increased adiposity at 18 months of age [49].

Maternal obesity is associated with differences in the infant gut microbiome during the first 18–27 months of life, particularly amongst those of higher family socio-economic status [53]. The study in question showed significant differences among *Faecalibacterium* spp., *Eubacterium* spp., *Oscillibacter* spp. and *Blautia* spp. numbers in infants born to obese compared to non-obese mothers. Obesity has been reported to be associated with changes in the gut microbiota already at 4–5 years of life [54]. In particular, obese children tend to harbour a higher abundance of *Enterobacteriaceae* and a lower abundance of *Desulfovibrio* and *Akkermansia*-like bacteria as compared to normal-weight infants.

17.4 Perturbations of Early Microbial Contact and the Development of Obesity

The microbiota appears to be particularly susceptible to perturbations during early life, and this is of great significance for later health and disease risk, due to its fundamental role in the induction, education and function of the host immune system and metabolic programming [55]. Indeed, a more profound understanding of the complex nature of host–microbe interaction is called for: by eating, we modify the gut microbiota composition, and reciprocally, the gut microbiota modulates appetite and satiety as well as host metabolism and immunity. Consequently, the microbiota may control the nutritional value of food and the fate of the nutrients

in our body [20, 21, 56]. Importantly, the increase in fat mass in response to high-energy intake necessitates the presence of gut microbiota [57] and the importance of its composition culminates in critical stages of development when the colonisation process is differently exposed to nutritional and environmental challenges [58]. The problem appears to culminate in the perinatal period due to intergenerational programming: aberrancies in the mother's microbiota composition and activity are transferred to the offspring by different routes: during pregnancy, at delivery via microbes in the mother's birth canal, and close contact between the mother and the newborn after delivery and through breast milk.

17.4.1 During Pregnancy and Breastfeeding

Gut-specific immune and metabolic changes typify the progress of pregnancy (Fig. 17.2), and these in turn may affect the microbiota composition and activity, or vice versa. Pregnancy induces a shift in gut microbiota composition by increasing the number of pro-inflammatory bacteria, including *Proteobacteria* [59]. In addition, changes in the vaginal [60] and subgingival oral microbiota have been documented [61, 62]. The net result at the end of pregnancy is a gut microbiota profile of elevated *Proteobacteria* and *Actinobacteria* and reduced bacterial richness [59]. Lower *Bifidobacterium* and *Bacteroides* numbers may be paralleled by increased numbers of *Staphylococcus*, *Enterobacteriaceae* and *Escherichia coli* in overweight compared with lean pregnant women [63].

Excessive weight gain during pregnancy exaggerates the microbiota deviation associated with pregnancy, including alterations in the placenta microbiota and its metabolic profile [63–65]. Maternal weight, BMI and weight gain during pregnancy extend their effects to the composition of breast milk microbiota (Fig. 17.2) and other bioactive compounds therein, for example anti-inflammatory transforming growth factor-β and CD14 mediating host–microbe communication [66]. The accompanying alterations are reflected in a lower presence of *Bifidobacterium* spp. in breast milk as compared to that in metabolically healthy mothers.

17.4.2 Mode of Delivery

Delivery involves the most massive exposure to the microbial environment of the individual; the neonate receives a decisive inoculum for the step-wise colonisation from the mother during vaginal delivery. In the immediate neonatal period, vaginally delivered neonates are colonised by microbes from the maternal birth canal, including *Lactobacillus*, *Prevotella* and *Sneathia* species, while the gut microbiota of neonates born by caesarean section (CS) is characterised by species belonging to the genera *Staphylococcus*, *Propionibacterium* and *Corynebacterium*, which

originate from the maternal skin [67]. The transmission of microbiota in CS-delivered neonates has been reported to be compromised, and only 41 % of the species found in their gut match those found in the stool of their mother—a significant difference from vaginally born infants [44]. Thus, bacteria in the CS neonate originate from oral or skin microbiota or the microbiota present in the delivery room and environment as first colonisers. The deviant gut colonisation pattern in infants born by CS (Fig. 17.2) has been reported to extend beyond the neonatal period, underlining the instrumental impact of early microbe contact. According to a prospective follow-up study of 24 neonates from Sweden [68], infants born by CS displayed lower gut microbiota diversity during the first 2 years of life as assessed by pyrosequencing of the 16S RNA gene. In particular, CS infants harboured a lower abundance and lower diversity of bacteria belonging to the phylum *Bacteroidetes*. In a report based on analyses by fluorescent in situ hybridisation of faecal samples of 60 children from Finland, in contrast, subjects born by CS harboured more clostridia as compared to vaginally born children at the age of 7 years [69].

The mode of birth also affects infant immune and metabolic development [70–72]. While this may in part be explained by the lack in CS of stress signals which are induced by vaginal delivery or which induce the delivery, it is likely that disturbances in gut colonisation are also an issue, particularly as intestinal permeability increases concomitantly. Augmented humoral immune responses have been observed in CS infants throughout the first year of life when compared to vaginally born infants, and these differences coincide with differences in gut colonisation patterns [71], furnishing one explanation for the heightened risk of allergic and inflammatory conditions in those delivered by CS [72]. Furthermore, a systematic review and meta-analysis of 28 studies on this question estimated the risk of obesity as 1.34-fold (CI 1.18–1.51) in children born by CS as compared to those delivered vaginally [73]. These data demonstrate that the effects of CS on host metabolism extend into childhood. It is important to note, however, that obesity is markedly heritable through both genetic and lifestyle-associated mechanisms and that overweight or obese mothers exhibit an increased risk of CS as a mode of delivery, also delaying breastfeeding. However, when only studies in which the results were adjusted for maternal pre-pregnancy weight as a potentially confounding factor were included in the meta-analysis, the risk of obesity in children born by CS as compared to vaginally born children remained 1.29-fold (95 % CI 1.16–1.44) [73]. In addition to maternal weight, the association between CS, aberrant gut colonisation patterns and obesity risk may be confounded by prenatal exposure to antibiotics, which are often prophylactically administered to mothers undergoing CS [74]. Using a mixed multivariable model adjusted for birth weight, gender, parental body mass, family socio-demographics, gestational factors and infant feeding patterns, CS was linked to high adiposity in infants from 6 weeks to 15 years of age [75], and affected children had 1.83 times the odds of becoming overweight or obese by the age of 11 years (95 % CI 1.24–2.70; $p = 0.002$).

17.4.3 Antibiotic Exposure in Early Life

Exposure to antibiotics is known to cause drastic disturbances in gut microbiota composition and may lead to the antibiotic-associated diarrhoea caused by organisms such as *Clostridium difficile*. The intestinal perturbations after antibiotic use are usually temporary in adults and the gut microbiota is relatively rapidly restored to its steady state [76]. In contrast, infants exposed to ampicillin and gentamicin during the first days of life have been reported to harbour reduced proportions of bifidobacteria and lactobacilli and significantly higher proportions of *Proteobacteria* as compared to non-exposed infants at the age of 4 weeks, and even at the age of 8 weeks the gut microbiota in exposed infants had not yet fully recovered [77]. In line with these observations, neonatal antibiotic exposure has been associated with increased numbers of faecal *Enterobacteriaceae* up to the age of 2 months [78], but longer-term data on the impact of neonatal antibiotic exposure on gut microbiota development are not currently available. Single-dose maternal *intra partum* ampicillin prophylaxis, on the other hand, has been reported to cause drastic changes in the infant microbiota at least up to the age of 3 months in a cohort comprising 13 infants [79].

The critical stage of development may be important in determining the consequences of antibiotics long term. While elimination of the microbiota by antibiotics or its modification by specific prebiotics or probiotics in experimental models improves insulin sensitivity and weight control [80], perinatal antibiotic exposure amplifies the obesogenic effect of a high-fat diet [55]. Cox and colleagues demonstrated the importance of age in a sophisticated series of experiments in a murine model [55]. Mice exposed to penicillin prenatally and in the neonatal period displayed aberrant gut microbiota composition and gained significantly more weight and fat mass compared to non-exposed mice. The weight gain was particularly prominent in males. It is also of note that perinatal antibiotic exposure particularly potentiated the fat mass accumulation induced by the introduction of a high-fat diet after weaning. In another series of experiments, gut microbiota adaptation to a high-fat diet was shown to be delayed which resulted in increased weight gain in mice subjected to pulsed antibiotic treatment [81]. Interestingly, the gut microbiota disturbances were transient and recovered after antibiotic exposure was discontinued, but the obesity-prone phenotype persisted. This observation is consistent with the notion of metabolic programming by microbial contact during a susceptible period in very early life. The role of gut microbes in mediating the development of obesity was further demonstrated in the study by transferring the obese phenotype to non-antibiotic-exposed mouse pups by colonising them with the gut microbiota from exposed mice.

It is alarming that, according to one recent report, more than 40 % of neonates in North America are exposed to antibiotics either directly or through the mother [82]. Little is currently known as to the long-term health impact of perinatal antibiotic exposure in term neonates, but accumulating evidence suggests a link between antibiotic use later in infancy and the development of overweight and

obesity. The risk of childhood overweight was recently reported in an epidemiological study of 12,000 children from Finland to be significantly increased in infants exposed to antibiotics during the first 6 months of life as compared to non-exposed infants [83]. The risk of overweight associated with early-life antibiotic exposure was more pronounced in boys (adjusted OR 1.34 with 95 % CI 1.06–1.66) than in girls (adjusted OR 1.16 with 95 % CI 0.87–1.56). The risk of childhood obesity, on the other hand, has been associated with repeated antibiotic exposure during the first 2 years of life in a cohort of more than 64,000 children from the USA [84]. It is further of note that a dose–response pattern between the number of antibiotic courses and obesity risk is evident in the study.

17.5 Breast Milk: Source of Natural Forces

Among breast-fed infants, transmission of specific intestinal *Bifidobacterium* strains from mothers to infants has recently been reported [85, 86], supporting the maternal microbial transfer hypothesis and suggesting that each mother–infant pair has unique family-specific strains. Additionally, human milk glycans such as oligosaccharides, glycoproteins and glycolipids have also been recognised as modulators and drivers of infant microbiota development which promote the growth and activity of specific bacterial populations, in particular *Bifidobacterium* and *Bacteroides* spp. [39, 87]. An exceptional composition is reflected in the stepwise compositional development of the infant gut microbiota and regulation of the inflammatory environment in the infant gut. Indeed, accumulating evidence suggests that breastfeeding may aid in reprogramming the non-communicable disease risk, including protection against overweight and obesity in childhood. Several differences have been observed in the gut and also upper respiratory tract microbiota between breast-fed and formula-fed infants [88–91], and the distinction is associated with more frequent *Bifidobacterium*-dominated microbiota in breast-fed than in formula-fed infants. Furthermore, experimental findings have demonstrated that high numbers of bifidobacteria correlate positively with normalisation of the inflammatory status and improved glucose tolerance and glucose-induced insulin secretion [92]. Clinically, it is of note that lower bifidobacteria numbers in early infancy distinguish children who develop allergic disease or excessive weight gain later in life (reviewed in: [7]) and that current formulas have been supplemented with specific probiotics or prebiotics in order to alleviate this limitation.

17.6 Reprogramming Early Gut Microbiota

An attractive prospect arising from recent experimental studies on microbial manipulation is to target disease risk by bringing the gut microbiota into balance. Reprogramming the regulatory biological features in at-risk populations by promoting healthy microbe contact may aid in reducing the risk of disease in mother and child. The few intervention studies available corroborate these findings; most evidence thus far of an association between clinical conditions and increased intestinal permeability, inflammatory response and aberrant gut microbiota composition is indirect. In the relevant studies, specific probiotics have been shown to control gut barrier functions and systemic and local inflammation and to avert deviant properties in the gut and breast milk microbiota (reviewed in: [7, 24]).

The definition of probiotic has developed and the widely accepted FAO/WHO (2001) working group report has recently been grammatically corrected to the present form as follows: "Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [93]. Probiotics have to be assessed for efficacy in strain-specific studies, and extrapolation of data from one strain to another is virtually impossible even if a detailed knowledge of the strain genomes and a practical knowledge of efficacy obtained in human intervention studies are available.

Novel tools to halt the vicious circle of microbiota disruption around birth are indeed called for. The probiotics approach necessitates, firstly, a more profound understanding of the complex interaction between nutrition and the gut microbiome, the total genetic pool of the microbiota, and, secondly, clinical intervention studies in humans as proof of causality.

17.7 Gut Microbiota and the Development of Obesity: Establishing Causality and Uncovering Mechanisms

The link between intestinal microbes and obesity has been demonstrated by studies reporting aberrant gut microbiota composition in obese compared to lean individuals [94, 95]. In these classical studies, obesity is associated with reduced microbial diversity and a shift in the relative abundance of *Bacteroidetes* and *Firmicutes*, as well as altered bacterial gene expression patterns. Although no direct evidence on specific phyla or species responsible can be argued, due to different and continuously developing methods and their varying accuracy, a causal relationship between gut microbiota composition and obesity risk may be hypothesised. This conception is based, firstly, on observations suggesting a chronological sequence of gut microbiota perturbations in infancy preceding the development of obesity in later life [10, 49–51] and, secondly, on recently discovered potential mechanisms underlying the observed associations. Thirdly, the epidemiological data linking early gut microbiota composition, or factors known to disrupt the gut microbiota

in early life, including CS and antibiotic exposure, to the subsequent development of overweight and obesity have been discussed in detail above. Finally, sophisticated experimental studies have provided more reductionist but direct evidence of causality and mechanisms.

The presence of the microbiota appears to be mandatory for the development of obesity even in the context of excessive dietary intake. Animals devoid of intestinal microbiota ('germ-free' mice) did not develop the obese phenotype observed in conventional animals when fed a Western-type high-fat and sugar-rich diet [58]. The energy-rich diet not only resulted in obesity development in conventional mice but also induced significant changes in gut microbiota composition, with a particular increase in *Mollicutes* within the phylum *Firmicutes* [96]. Notably, the gut microbiota perturbations were reversible after a change to a less energy-rich diet. In a similar fashion, the unbalanced microbiota composition reported in the gut microbiota of obese humans gradually normalises to resemble that observed in lean individuals during a low-energy diet [97].

Interestingly, it has become evident that an aberrant microbiota may also induce the development of obesity. Colonising germ-free mice with gut microbiota from mice suffering from diet-induced obesity reportedly leads to greater weight gain as compared to mice colonised with microbiota from lean mice fed a conventional diet [96]. The potential of obesity-related microbiota to trigger weight accumulation has been corroborated by a report demonstrating that mice colonised with gut microbiota obtained from obese human individuals display significantly greater weight gain and adiposity as compared to mice whose gut microbiota originated from a lean person [98]. The fact that the trait is transmissible points strongly to a causative role of specific microbe contact in non-communicable diseases. Furthermore, the mechanisms of the obesity-inducing potential of aberrant gut microbiota are gradually being uncovered.

Intestinal microbes have been reported to affect the efficiency of energy harvest from the diet and to directly modulate host physiology. Germ-free mice colonised with gut microbiota from obese mice display significantly greater weight gain than mice colonised with microbiota from lean mice despite similar dietary energy content. This may be explained by altered fermentation of dietary complex carbohydrates, since germ-free rats excrete considerably more calories in their faeces and exhibit decreased intestinal short-chain fatty acid (SCFA) concentrations compared to conventional rats (reviewed in: [99]). SCFAs have recently been suggested to increase intestinal energy harvest, but the role of SCFAs in the development of obesity is not clear since there are data indicating that SCFAs may exert beneficial metabolic effects in adipose tissue and the liver and improve insulin sensitivity (reviewed in: [13]). The reduced adiposity in germ-free rats is rapidly restored after colonising them with conventional microbiota [99]. The increase in body weight and adiposity in germ-free mice colonised with gut microbiota from obese humans, on the other hand, is accompanied by enhanced metabolism of branch-chain amino acids and increased microbial transformation of bile acids but decreased fermentation of SCFAs. It is also well established that obesity and metabolic disease are associated with a moderate systemic inflammatory response [57, 100], which may

in part be induced by intestinal microbes or microbe-associated molecular patterns (reviewed in: [99]).

Taken together, these data suggests a vicious circle in obesity development. An inappropriate high-energy diet may lead to aberrant gut microbiota composition, which in turn further increases energy harvest from the diet already abundant in energy. The altered microbiota may also contribute to the inflammatory immune milieu, which perpetuates the detrimental effects of weight gain. However, there are encouraging data which suggest that the vicious circle may be broken by interventions such as gastric bypass surgery, which are capable of restoring healthy gut microbiota composition and normalising energy metabolism [101]. Whether such reprogramming is possible by using less invasive means at an early age remains to be established.

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