Improving Mothers for a better PrenAral Care Trial BarCeloNa (IMPACT BCN)

Cover page:

The contents included in this document, contain:

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IMPACT Barcelona

Intervencions per a la Millora PrenAtal del Creixement feTal a Barcelona

Improving Mothers for a better PrenAtal Care Trial

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Date: 1st November 2016

Setting: BCNatal – Fetal iD+, Barcelona, Spain

Research line: Fetal cardiovascular programming

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1 Administrative information

This document provides information about an understanding of the background, rationale, objectives, and procedures for entering participants into the study, study population, interventions, methods, statistical analyses, ethical considerations, and administration of the study. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary.
The study will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), and the principles of Good Clinical Practice (GCP).

1.1 Structured study summary

Public title	IMPACT Bcn
Scientific title	IMPACT Barcelona: Improving Mothers for a better PrenAtal Care Trial
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Setting of recruitment	Hospital Clinic and Hospital San Joan de Deu, Barcelona, Spain
Problem studied	Pregnancies at risk to develop fetal growth restriction
Cohort	Pregnant women at high risk to develop fetal growth restriction
Study type	Randomized Controlled Study (3 arms)
Interventions	1) Reduction of maternal stress based on Mindfulness Program 2) Improvement of maternal nutritional status based on Mediterranean Diet
Target sample size	1218 patients: 406 patients for each arm of randomization (No intervention, , Nutritional Program , Mindfulness Program)
Key inclusion and exclusion criteria	Inclusion: • Maternal age at recruitment ≥18 years • Viable singleton non-malformed fetus • Speak Spanish fluently • High risk pregnancies • 19-23 weeks of gestation Exclusion: • Fetal anomalies including chromosomal abnormalities or

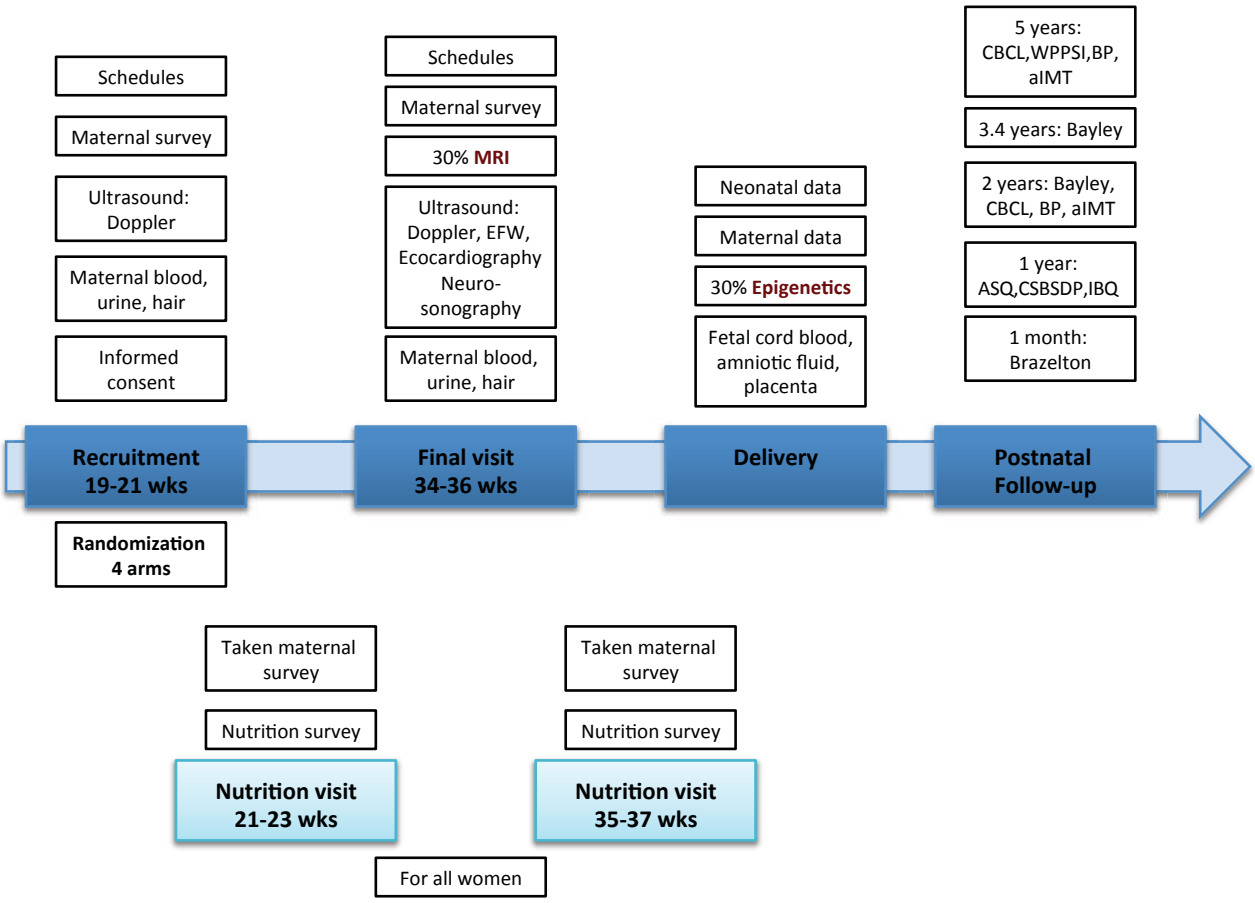
	structural malformations detected by ultrasound. • Maternal mental retardation or other mental or psychiatric disorders that impose doubts regarding the true patient´s willingness to participate in the study. • No possibility to come to additional visits.
Data of first enrolment	January 2017
Study aims	1) To demonstrate that an improvement on maternal well being, through intensive behavioral therapies based on stress reduction with mindfulness techniques and/or nutrition intervention with a Mediterranean diet, have an impact in the reduction of the prevalence of fetal growth restriction in pregnancies at high risk to develop this condition. 2) To demonstrate that an improvement on maternal well being, based on stress reduction with mindfulness techniques and/or nutrition intervention with a Mediterranean diet, could reduce the prevalence of adverse perinatal outcome of pregnancies at high risk to develop fetal growth restriction. 3) To demonstrate that an interventional program to the mother have a positive impact on fetal programming, in terms of fetal brain development, newborn neurodevelopment and cardiovascular profile later in life (at two years of age). 4) To identify epigenetic changes that may explain the improvement of fetal outcome and programming.

1.2 Roles and responsibilities

Name	Affiliation	Role
Prof Eduard Gratacos (EG)	BCNatal	Chief investigator, Director of Department of Obstetrics, Gynecology and Neonatology of Hospital Clinic Barcelona, Director of BCNatal Center
Dr Fatima Crispi (FCr)	BCNatal	Co-Chief investigator, Scientific coordinator of Fetal i+D Fetal Medicine Research Center, BCNatal
Dr Francesca Crovetto (FC)	BCNatal	Principal investigator, specialist in Obstetrics and Fetal Medicine
Dr Cristina Paules (CP)	BCNatal	Principal investigator, specialist in Obstetrics and Fetal Medicine
Estefania Callado (EC)	BCNatal	Project manager at Fetal i+D Fetal Medicine Research Center, BCNatal, project manager of the trial
Dr Maria Dolores Gomez (MDG)	BCNatal	Head of the Gynecology and Obstetrics Department at Hospital Sant Joan de Déu, BCNatal, co-supervisor of the recruitment at Hospital Sant Joan de Déu
Angela Arranz (AA)	BCNatal	Head of Nurses of the of Department of Obstetrics, Gynecology and Neonatology of Hospital Clinic Barcelona, responsible for nurses involved and postnatal follow-up
Dr Elisenda Eixarch (EE)	BCNatal	Coordinator of fetal neurology unit, specialist in Obstetrics and Fetal Medicine, responsible of the neuroimage acquisition and analysis
Dr Rui Simoes (RS)	BCNatal	Postdoctoral researcher at Fetal i+D Fetal edicine Research Center, Biochemist, responsible of the MRI spectroscopic analysis
Dr Merida Rodriguez (MR)	BCNatal	Specialist in Family Medicine and master in Epidemiology, involved in the statistical part of the project
Dr Irene Falgas (IF)	BCNatal	Consultant of Psychiatric, responsible of the Mindfulness-based Intervention
Dr Marta Garcia (MG)	BCNatal	Nurse and psychologist involved in the Mindfulness-based Intervention and postnatal follow-up
Marta de Lamo (MdL)	BCNatal	Nurse involved in the Mindfulness-based Intervention and postnatal follow-up
Marta Arnal (MA)	BCNatal	Nurse involved in the Mindfulness-based Intervention and postnatal follow-up
Noemi Hernandez (NH)	BCNatal	Nurse involved in the Mindfulness-based Intervention and postnatal follow-up
Prof Margarita Alegría (MA)	Harvard Medical School	Head of Disparities Research Unit from the Massachussets General Hospital-Harvard Medical School, reviewer and co-supervisor of the Mindfulness-based Intervention
Prof Zayda Vallejo (ZV)	University of	Certified Mindfulness instructor, trained and consultant, reviewer

	Massachusetts	and co-supervisor of the Mindfulness-based Intervention
Prof Eduard Vieta (EV)	H Clinic Bcn	Head of Psychiatric Service of Hospital Clinic Barcelona, reviewer and co-supervisor of the Mindfulness-based Intervention
Prof Ramon Estruch (RE)	H Clinic Bcn	Head of internal medicine of Hospital Clinic Barcelona, responsible of the Nutritional Intervention
Dr Rosas Casas (RC)	H Clinic Bcn	Biologist, co-responsible of the Nutritional Intervention
Dr Monica Domenech (MD)	H Clinic Bcn	Specialist in internal medicine of Hospital Clinic Barcelona, involved in the Nutrition Intervention
Conxa Viñas (CV)	H Clinic Bcn	Dietician involved in the Nutritional Intervention
Tania Freitas (TF)	H Clinic Bcn	Dietician involved in the Nutritional Intervention
Miriam Osorio (MO)	BCNatal	Technician coordinator of the BCNatal Biobank
Dr Oscar Pozo (OP)	IMIM	Senior Researcher at Bioanalysis and Analytical Services Research Group of IMIM-Hospital del Mar Research Institute, responsible for the cortisol axis analysis of biological samples
Prof Rosa Maria Lamuela (RML)	UB	Professor at the Department of Nutrition, Food Science and Gastronomy, School of Farmacy and Food Science, INSA – University of Barcelona, responsible for nutrimetabolites analysis of biological samples
Simon Heath (SH)	CNAG	Team leader of the Statistical Genomics team at Centre Nacional d'Anàlisi Genòmica, co-responsible of the epigenomic analysis.
Ivo Gut (IG)	CNAG	Director at Centre Nacional d'Anàlisi Genòmica, co-responsible of the epigenomic analysis.

1.3 Study diagram



2 Abbreviations

GCP	Good Clinical Practice
FGR	Fetal Growth Restriction
SGA	Small for Gestational Age
IUGR	Intrauterine Growth Restriction
BW	Birth Weight
SD	Standard Deviation
EFW	Estimated Fetal Weight
APO	Adverse Perinatal Outcome
HPA	Hypothalamic Pituitary Adrenocortical
CRH	Corticotropin Releasing Hormone
ACTH	Adenocorticotropic Hormone
NBAS	Neonatal Behavioral Assessment Scale
ADHD	Attention Deficit Hyperactivity Disorder
11β-HSD2	11β-Hydroxysteroid dehydrogenase type 2
GR	Glucocorticoids Receptors
MBSR	Mindfulness-Based Stress Reduction
RCT	Randomized Controlled Trial
MD	Mediterranean Diet
LC-PUFA	Long-Chain Polyunsaturated Fatty Acid
DHA	Docosahexaenoic Acid
IGF-1	Insuline Like Growth Factor 1
INMA	Infancia y Medio Ambiente
RHEA	Mother-Child Cohort
ACC	Anterior Cingulate Cortex
MRS	Magnetic Resonance Spectroscopy
GABA	Ɣ–Aaminobutryric Acid
fMRI	Functional Magnetic Resonance Imaging
MRI	Magnetic Resonance Imaging
DMN	Default Mode Network
NAA	N-Acetyl-Aspartate
NR3C1	Gene of the Glucocorticoid Receptor
NET	Noerpinephrine Transporter
IGF2	Insulin-Like Growth Factor 2

LTL	Leukocyte Telomere Length
OR	Odds Ratio
PTB	Preterm Birth
PE	Preeclampsia
BP	Blood Pressure
RCOG	Royal College of Obstetrics & Gynaecologists
PHQ-9	Patient Health Questionnaire
SD	Standard Deviation
PI	Principal Investigator
FFQ	Food Frequency Questionnaires
ASQ	Ages and Stages Questionnaires
CSBS DP	Communication and Symbolic Behavior Scales Developmental Profile Infant/Toddler Checklist
IBQ	Infant Behavior Questionnaire
CBC	Child Behavior Checklist
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition
aIMT	Aortic Intima Media Thickness
PSS	Perceived Stress Scale
STAI	State-trait Anxiety inventory
PCL-5	Post-Traumatic Stress Disorder
EPDS	Edindburgh Postnatal Depression Scale
LOT	Life Orientation Test
MAAS	Mindful Attention Awareness Scale
PSQI	Pittsburgh Sleep Quality Index
BPD	Biparietal Diameter
HC	Head Circumference
AC	Abdominal Circumference
FL	Femur Length
UA	Umbilical Artery
PI	Pulsatility Index
UtA	Uterine Arteries
AoI	Aortic Isthmus
MCA	Middle Cerebral Artery
CPR	Cerebroplacental Ratio

FMBV	Fractional Moving Blood Volume
TAPSE	Tricuspid Anular Plane Systolic Execution
MAPSE	Mitral Anular Plane Systolic Execution
MPI	Myocardial Performance Index
ICT	Isovolumetric Contraction Time
ET	Ejection Time
IRT	Isovolumetric Relaxation Time
NSG	Neurosonography
RF	Radio-Frequency
TR	Repetition Time
TE	Echo Time
FOV	Field Of View
DWI	Diffusion-weighted Images
PRESS	Point Resolved Spectroscopy
CHESS	Chemical Shift Selective module
IVIM	Intra-Voxel Incoherent Motion
PACE	Prospective Acquisition Correction
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MTX	Acquisition Matrix
NMR	Nuclear Magnetic Resonance
CDB	Biomedical Diagnostic Center
PCR	Polymerase Chain Reaction
PBMC	Peripheral Blood Mononuclear Cells
NET	Noerpinephrine Transporter
SLC6A4	Serotonin Transporter
ABCA1	Cholesterol Transporter
GLUT3	Glucose Transporter
LEP	Leptin
CNAG	National Centre of Genomic Analysis
BMI	Body Mass Index
ART	Assisted Reproductive Technologies

3 Introduction

3.1 Two main protagonists

3.1.1 The fetus

In the past, the only person considered to be involved in pregnancy was the woman. Forty years ago, the fetus virtually did not exist in medicine. All obstetric medicine was focused on the mother and which pregnancy complications could occur to her. In late 70s, the introduction of ultrasound and the possibility to visualize the fetus in utero brought about a true revolution in two respects. For doctors, it allowed to diagnose in fetal life problems that until then had been only known in the newborn. For parents, it facilitated the recognition of the fetus as a person (McCullough Am J Bioeth 2008). The combination of these two factors resulted in the development of a new concept, the fetus as a patient, and with it, the beginning of a subspecialty that we know today as fetal medicine. Fetal medicine is today a reality, and we can offer parents accurate assessment for many fetal problems. However, there are still many aspects to be clarified.

Even if the fetus is now considered a patient, we have to avoid the tendency to think of the fetus as separate from the pregnant woman (Lyerly-Mahowald Clinics in Perinatology 2003), obscuring the physical and psychological relationship between the pregnant woman and the fetus, the ways that maternal, fetal physiologies and welfare are linked, and perhaps most problematically, the woman herself with her background and psychological status (Lyerly Am J Bioeth 2009).

3.1.2 The mother

The physical care of pregnant women in the developed world has hugely improved over the past 100 years; however, the same has not been true of their emotional care (Glover 2014). Pregnancy is a complex and dynamic condition. Maternal psychological state changes produce a cascade of reactions, including changes in blood flow to the uterus as well as alterations to the intrauterine sensory environment experienced by the fetus. Given the intricate physiological relationship between the pregnant women and the fetus, it would be somewhat surprising if dynamic aspects of the maternal psychological environment did not serve to shape neurodevelopment of the fetus and ultimately that of the child (Di Pietro 2012). However, because there are no direct neural connections between the pregnant women and the fetus, the fetus requires transduction of a maternal physiological signal from a psychological state to experience it.

There is growing support for a central role of the prenatal period in the health and development of offspring throughout childhood and adult life. Considerable evidence from many prospective studies show that if the mother is depressed, anxious or stressed while she is pregnant, her child is more likely to experience a range of adverse neurodevelopmental outcomes such as an increased risk of behavioral, emotional and cognitive problems that do the children of other mothers (Van Den Bergh 2005, Talge 2007, Gover 2011). Although genetics and postnatal care clearly affect these outcomes, evidence for an additional prenatal causal component is substantial.

3.1.3 A unique bond

The relationship between the fetus and her future mother is unique: the fetus as a patient depends completely and entirely from her; he is physiologically enmeshes with another patient, his

"environment" is the body of an autonomous agent. On the other side, the mother is completely involved by the presence of the fetus. The maternal bond, that is the relationship between the mother and her child, typically starts during pregnancy, and both physical and emotional factors influence the mother-baby bonding process.

Central to obstetrical practice are efforts to prevent, diagnose and treat conditions that can affect the mother and the future baby before birth. However, we still considered the mother and the fetus as two different and separate entities, and the majority of therapies are for preventing/monitoring women complications of pregnancy or, on the other side, for preventing/monitoring fetus diseases.
Nevertheless, we should start considering the unique relationship they have and start thinking that probably any effort to improve the maternal status could also improve the fetal condition. It's seems very easy, but it is still one of the most neglected aspect of modern obstetrics.

3.2 Fetal Growth Restriction

Fetal growth restriction (FGR) is defined as the failure to achieve the endorsed growth potential and it affects 7-10% of all pregnancies (Figueras-Gardosi 2011, Figueras UOG 2014). Fetuses that are not fulfilling their growth potential have a 5- to 10- fold risk of dying in utero (Richardus 2003, Gardosi 2005). Moreover, growth restricted fetuses have a higher risk of perinatal morbidity and mortality (McIntire 99, Breeze 2007, von Beckerat 2013) and are also at higher risk of long-term disabilities and cognitive impairment (Larroque 2001, O'Keeffe 2003, Leitner 2007 Arcangeli 2012, von Beckerat 2013, Levine 2015).

Different terms have been used in this field of research: small-for-gestational-age (SGA) and intrauterine growth restriction (IUGR) for example, have often been used interchangeably, but not all small babies are growth restricted (Lee PA, Pediatrics 2003). Although the concept of abnormal fetal growth is basic to the modern practice of perinatal medicine, the threshold below which the fetus or newborn is considered growth restricted varies between studies. Pediatricians define SGA as a statistical grouping of infants whose birth weight (BW) and/or length is at least two standard deviations (SD) below the mean for gestational age, which is approximately the 3rd percentile (Lee PA, Pediatrics 2003). In obstetric practice SGA is normally defined as an estimated fetal weight (EFW) below the 10th percentile (ACOG Bollettin 2000), and IUGR when it shows signs of restriction (for example Doppler abnormalities). Since these fetuses have a higher risk of mortality and morbidity, it is fundamental to use not only an obstetrical definition, but also fetal charts to identify this population (Figueras 2008 Spain, INTERGROWHT-21). For the purpose of this project, we decided to use the term of FGR, for which we considered those fetuses born with a BW below the 10th percentile, in which some could have shown signs of restriction since the fetal life and some may not.

Several causes have been described to explain the origin of FGR including abnormal placenta development, tobacco use, maternal malnutrition, antiphospholipid syndrome, teratogen exposure, infections, and genetic/structural disorders. However, our knowledge of the spectrum of FGR continues to evolve and the hypothesis that FGR is a multi-phenotypic disease is the main objective of a project we're conducting in our research center (Phenomapping of Fetal Growth Restriction). Based on preliminary data, some cases of FGR seemed to be caused by higher levels of perceived stress and anxiety of the mothers, resulting in higher levels of cortisol in the amniotic fluid, and lower levels of fundamental micronutrients (such as iron) in fetal cord blood, reflecting a bad nutritional status of the mother (see paragraph below 3.9).

3.3 Fetal Programming

The question of the importance of prenatal environmental factors for development, behavior and health, has been scientifically studied from the 1940s onwards in humans. The seminal studies from David Barker have lead to the concept of “development plasticity”, describing a critical window during fetal development when the organs are sensitive to nutritional, hormonal and metabolic environment (Barker Lancet 1993). This is “fetal programming”, that is when the environment in uterus during specific critical periods for different outcomes can affect the development of the fetus and the child in the long-term (Barker Lancet 1986, Barker 1995). In the last years our research group gave a significant contribution in this field of research for growth restricted fetuses: these fetuses are at higher risk of adverse long-term consequences such as abnormal neural reorganization (Sanz-Cortes UOG 2010, Sanz-Cortes FDT 2013, Egana 2013, Egana UOG 2014, Sanz-Cortes AJOG 2014, Egana PD 2014) and metabolic changes (Sanz-Cortes UOG 2010, Sanz-Cortes AJOG 2015) in the brain, as well as poor neurological (Eixarch 2008, Figueras 2009, Larroque 2010, Sanz-Cortes 2014), cardiovascular (Crispi Circul 2010, Cruz FDT 2011, Crispi AJOG 2012), metabolic (Sanz-Cortes 2013) and endocrinological (Verkauskiene 2008) outcomes. Thus, the antenatal identification of a growth restricted fetus is essential not only to reduce adverse perinatal outcome (APO) (Linquist 2005), but also for the potential window of opportunity to reduce the consequences of an adverse intrauterine environment (Figueras-Gardosi 2011).
With physical outcomes, the phenomenon of fetal programming is well established. Fetal programming, however, seems to be equally important for the development of psychopathology (O’Connor 2002, Di Pietro 2006, Rice 2010); according to De Pietro *et al.*, early postnatal temperamental characteristics emerge during the prenatal period (Di Pietro Early Hum 2008).

3.4 Stress

Stress is a generic term linked to an organism's response to a wide range of different types of exposure, which can be acute and chronic. In humans, stress typically describes a negative or a positive condition that can have an impact on a person’s mental and physical well being. According to the stressful event, the body induces physiological changes that cause the sympathetic activation or hypothalamic-pituitary-adrenocortical (HPA) activation, and immunological function.
The HPA axis is a major neuro-endocrine pathway that modulates the stress response. The normal cascade starts with the release of corticotropin-releasing hormone (CRH) by cells in the paraventricular nuclei of the hypothalamus. CRH stimulates the anterior pituitary for the release of adenocorticotropic hormone (ACTH) into circulation. ACTH stimulates the biosynthesis and release of glucocorticoids from the adrenal cortex. Among glucocorticoids, cortisol, which is a steroid hormone, is the principal end product of the HPA axis; it plays a fundamental role in maintaining homeostasis and has several important metabolic, endocrine and immune effects on most cells. In addition, cortisol passes through the blood brain barrier with consequences for brain structure and function. Because of the damaging effects of chronic exposure to elevated levels of cortisol, the HPA axis is protected by a negative feedback loop whereby cortisol has a negative feedback binding to receptors in the pituitary and hypothalamus as well as the hippocampus and prefrontal cortex turning off the HPA axis response.

3.4.1 Stress in pregnancy and consequences

A wide range of different outcomes has been shown to be associated with prenatal stress in studies examining children from birth until adulthood. Stress has been associated with lower BW, prematurity (Wadhwa 1993, Rondò 2003, Sable 2000, Roy-Matten 2011, Class 2011, Ding 2014) and with a significantly smaller head circumference even if corrected by BW (Lou 1994).
In the last few decades, several studies on prenatal stress have looked at neurodevelopmental and psychopathological outcomes. Newborns from mothers who reported stress during pregnancy showed poorer performance on the Neonatal Behavioral Assessment Scale (NBAS) than newborns of mothers who did not report stress symptoms (Field 2002, Diego 2004). Studies of toddlers and infants whose mothers had higher stress during pregnancy had a more difficult temperament (Buitelaar 2002, Austin 2005), sleep problems (O'Connor 2007), lower cognitive performance and increased fearfulness (Bergam 2007). Evidence on children ages 3-16 years reported a link between maternal stress during pregnancy and an increased risk for child emotional problems such as anxiety, depression, symptoms of attention deficit hyperactivity disorder (ADHD) and conduct disorders (O' Connor 2002, O' Connor 2003, Van Den Bergh 2004, Van Den Bergh 2008, Rice 2010, Kleinhaus 2013).

A possible mechanism that may underlie fetus programming by prenatal stress is the increased exposure of the fetus to cortisol. Glucocorticoids are known to have a range of effects on the developing fetus, including on the brain (Herbert 2006). Although they are essential for fetal development and tissue maturation, overexposure can have effects that predispose to ill health in later life (Harris 2001). Fetal overexposure to glucocorticoids could occur for an increased maternal cortisol associated with anxiety that can cross the placenta. During pregnancy the CRH is released also from the placenta into both the maternal and fetal compartments. While cortisol has a negative feedback on hypothalamic CRH, during pregnancy it increases the production of CRH from the placenta. Because of this positive feedback between cortisol and placental CRH, pregnancy is characterized by elevated cortisol levels (2-4 times higher) and the effects of glucocorticoids on the fetus may be amplified. In addition to its effects on placental CRH, maternal cortisol passes through the placenta. However, the effects of maternal cortisol on the fetus is modulated by the presence of a placental enzyme, called *11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2)*, which oxidizes the cortisol into an inactive form, cortisone (Murphy 2006). In a sort of way this enzyme protects the fetus from maternal cortisol.
Indeed the placenta clearly plays a crucial role in moderating fetal exposure to maternal factors and presumably in preparing the fetus for the environment in which it is going to find itself (O'Donnell 2009). However, if there is less of this barrier enzyme then the fetus will be exposed to more maternal cortisol, independently of any change in the maternal level. Some evidence in rat models shows that prenatal stress can down-regulate placental 11β-HSD2 (Mairesse 2007). Recent studies in humans also found direct evidence that maternal prenatal anxiety and depression are associated with a down-regulation of 11β-HSD2 gene expression (Glover 2009, O'Donnell 2012). Reduction of placental 11β-HSD2 activity has been also found in FGR (Shams Hum Repr 2008, Dy J Placenta 2008). However, it is very difficult to see these differences in fetal cord blood or amniotic fluid (Evans 2008, Diego MA 2006, Sarkar-Glover 2006), probably because they are very crude index of fetal exposure to cortisol in specific tissues, although they are the best currently available. A study from McTernan and colleagues reported a 25% reduction in 11β-HSD2 expression in placentas from FGR pregnancies (McTernan 2001)

3.4.2 Fetal HPA axis

The human fetal HPA axis is developed and functioning at 22 weeks of gestation, although its plasticity is maintained during the first two years of life (Mesiano 1997, Tarullo 2006). Thus, maternal physical and psychological status during pregnancy has the potential to permanently alter the program of HPA axis functioning of their offspring.
Animal and human data strongly suggest that glucocorticoids program the fetal HPA axis and neurotransmitter systems with consequences for postnatal functioning (Kapoor 2008, Sandman 2010); they showed that changes in functioning of the HPA axis are associated with alterations of the glucocorticoids receptors (GR) number in regions including the prefrontal cortex, hippocampus, amygdala and pituitary gland that are important for activation and negative feedback regulation of the HPA axis (Liu 2001, Banjanin 2004, Diaz Heijtz 2010). However, in humans we know very little of the influence of stress hormones on the developing human fetal nervous system. It is clear that, although cortisol is essential for normal brain development, exposure to excessive amounts has long-lasting effects on neuroendocrine functioning and behavior (Rice 200). A recent review of studies suggested that HPA neurohormones exert programming influences on the nervous system and may impair emotional and cognitive development (Sandman 2011). Dysregulation of the HPA axis functioning during infancy put children at risk for physiological and behavioral problems throughout the lifespan (Gunnar 2003, Phillips 2007).
Human data in support of the role of glucocorticoids in mediating programming effects on neurodevelopment come from Finland, where the consumption of large amounts of licorice, that is an 11β-HSD2 inhibitor, is common. Maternal licorice consumption during pregnancy is associated with altered HPA axis activity in children, with those whose mothers consumed the most licorice during pregnancy having the highest circulating cortisol levels (Raikkonen 2009, Raikkonen 2010). The mechanism through prenatal glucocorticoid overexposure leads to a wide variety of changes in tissue structure and function is probably linked with epigenetics.

3.5 Mindfulness

In recent years there has been increasing interest in the concept of mindfulness, which is a practice that has its roots in Buddhist meditation. Its definition coined by Jon Kabat-Zinn described mindfulness as *"paying attention in a particular way: on purpose, in the present moment, and non-judgmentally"* (Kabat-Zinn 1990). In other words, mindfulness corresponds to the higher-level awareness of present-moment sensory, affective, and cognitive experiences.
Mindfulness training teaches participants meditation techniques that increase awareness of present-moment experiences, including thoughts, emotions, bodily sensations, and surrounding environment (Kabat-Zinn 1990, Bishop 2004). Mindfulness also involves acceptance, meaning that paying attention to thoughts and feelings without judging them—without believing, for instance, that there's a "right" or "wrong" way to think or feel in a given moment, rather than rehashing the past or imagining the future.
Practice of mindfulness has entered the American mainstream in recent years, in part through the work of Jon Kabat-Zinn and his Mindfulness-Based Stress Reduction (MBSR) program, which he launched in 1979 at the University of Massachusetts Medical School (Kabat-Zinn 1992, Kabat-Zinn 1994). Mindfulness-based interventions have been of increasing interest as a cost-effective, low-stigma, accessible treatment option for different psychological and medical symptoms, firstly with chronic pain, anxiety and depressive disorders (Kabat-Zinn 1992, Hofmann 2010, Hoge 2013) and then with other morbid conditions such as binge-eating in obesity (Ruffault, 2016) or reducing

cardiovascular risk (Louks 2015). Finally, a recent meta-analysis reported the use of MBSR reduces anxiety symptoms (Hofmann 2010).
The promising effect of this therapy comes also from psychological markers relevant to emotion such as stress hormones (cortisol) and immune markers (inflammatory cytokines), which affect the brain (Sternberg 2000).

3.5.1 Mindfulness in pregnancy

Despite mounting evidence that prenatal maternal stress and anxiety can influence perinatal and childhood outcomes, few stress-reduction programs have been tested in pregnancy and the majority of them where not methodologically correct: frequently there was a missing control group or random assignment, the use of nonspecific samples, failures to measure process variables…
The first randomized controlled trial (RCT) in pregnancy was planned as a pilot study and conducted to evaluate the effects of a mindfulness awareness practice based on 6-week series of 2-hour classes in a sample of 47 pregnant women who were recruited for elevated levels of stress during first or second trimester of pregnancy (Guardino 2014); the authors reported an improvement of anxiety in those pregnant women who experienced the mindfulness program, but the effects were not sustained through follow-up at six weeks post-intervention. However, a limit of this study, aside from its small sample, was that in both groups women have been conscious and motivated to reduce their high levels of prenatal stress and 30% of the control group took prenatal yoga classes during their pregnancies.
Another study also evaluated the effect of a mindfulness intervention during pregnancy on socio-emotional development and temperament in 10 months-old toddlers, reported that higher maternal mindfulness during pregnancy was associated with less infant self-regulation problems and less negative affectivity (Van Den Heuvel 2015).
Finally, a pilot RCT in a tertiary maternity hospital in Australia, even if only with 32 patients in total, provided evidence on the feasibility of an antenatal mindfulness intervention to reduce psychological distress during pregnancy (Woolhouse 2014).
Mindfulness seems to have potential during pregnancy, considering this special period of woman's life. However, all previous studies considered as outcome only the reduction of maternal stress ignoring its potential benefits to the fetus.

3.6 Nutrition

The traditional Mediterranean diet (MD) is the heritage of millennia of exchanges of people, cultures and foods of all countries around the Mediterranean basin. It is rich in plant food (cereals, fruit, vegetables, legumes, free nuts, seeds and olives), with olive oil as the principal source of added fat, with a high to moderate intakes of fish and seafood, moderate consumption of eggs, poultry and dairy products (cheese and yogurt), low consumption of red meat and a moderate intake of alcohol (mainly red wine during meals). This healthy, tradition MD has been popularized since 1995 using the world famous pyramid representation that graphically highlights the food groups to be consumed daily, weekly or less frequently (Willet 1995) (Figure 1). This pyramid is the result of an international consensus and it is based on the latest scientific evidence on nutrition and health published in hundreds of scientific articles in recent decades (Bach-Faig 2011). Today it is recognized as centuries-

old tradition that contributes to excellent health, provides a sense of pleasure and well-being and forms part of the world's collective cultural heritage. The diet, when consumed in sufficient amounts, provides the entire known essential micronutrients (vitamins and minerals), fiber ecc. Olive oil is the principal source of fat and it contains a large proportion of monounsaturated fat, a relatively low proportion of saturated fat and it is also the source of the antioxidant vitamin E.

Mediterranean diet with the supplementation of extra-virgin olive oil or nuts has been recently proposed among people at high cardiovascular risk, thanks to its power to reduce by 30% the incidence of major cardiovascular events (Estruch NEJM 2013). This was a great example of a behavioral intervention in a population at high risk, because the nutritional intervention was based on the idea to modify the food habits of people in a real-life context.

Figure 1. Mediterranean diet pyramid

3.6.1 Nutrition in pregnancy

Nutrition in pregnancy plays an important role in the well-being of the mother and the fetus, and may further influence the health of the children later in life (Godfrey Publ Health Nutr 2001, Harding In J Epidem 2001, Mason JB The first 500 days of life Glob health 2014). Dietary energy and nutrient requirements are generally increased to support increased maternal metabolism, blood volume and red cell mass expansion, and the delivery of nutrients to the fetus (Kaiser 2002). However, in a recent systematic review and meta-analysis of 90 dietary studies among pregnant women in developed countries (n=126,242), when compared to dietary recommendations in the specific countries, the intake of energy and fiber was generally lower, total fat and saturated fat higher, and carbohydrate intake was borderline or lower than recommendations (Blumfield 2012). Moreover, micronutrients intake during pregnancy, including folate, iron, zinc, calcium, vitamin D and essential fatty acid, was less than optimal (Blumfield 2013). A recent review based on evidence from epidemiological and RCT studies on the impact of dietary and supplemental intakes of omega-3 long-chain polyunsaturated fatty acid (LC-PUFA), zinc, folate, iron, calcium, and vitamin D reported that there is insufficient evidence for omega-3 fatty acid supplements' ability to reduce risk of low BW and more robust evidence from studies supplementing with zinc, calcium and/or vitamin D needs to be established (Grieger Nutrients 2015). On the contrary, iron supplementation appears to increase BW, particularly when there are increases in maternal hemoglobin concentrations in the third trimester.

This is of concern given the current consensus that maternal nutrition is relevant to both the short and long-term health of the infant. The available evidence suggest that undernutrition affects pregnancy

outcome, particularly for the higher rates of preterm delivery (Tompkins AJOG 1951, Venkatachalam WHO 1962), but also overweight and obesity exposes pregnancy to several complications (Callway Med J Austr 2006, Guelinckx Obes Rev 2008, Dodd Aus N Z J Obstet Gynaecol 2011).

Dietary patterns are related with specific foods. For fruits and vegetables for example, in developed countries they have been related with higher BW, especially vegetables (Mikkelsen Scand J Public Health 2006, Ramon R J Nutr 2009;139;561-7).

Optimal fetal neurodevelopment is also dependent on specific nutrients from dietary sources, including docosahexaenoic acid (DHA), an omega-3 essential fatty acid, of which seafood is a major source (Salem 1989). Fish is a rich source of nutrients such as LC-PUFA, protein, selenium, iodine, and vitamin D, which are considered to be beneficial for fetal growth and development (Philibert Am J Clin Nutr 2006;84:1299–307) but, in contrast, fish is also a well-known route of exposure to pollutants such as dioxins, polychlorinated biphenyls, methylmercury, and other heavy metals, which may adversely affect fetal growth and gestational length (Mahaffey Environ Res 2004;95:414–28, 5, Halldorsson Am J Epidemiol 2008;168:958–65). Findings from prospective birth cohort studies on the relation between fish intake during pregnancy and fetal growth have been discrepant, with reports of either positive or null (Brantsæter Br J Nutr 2012;107:436–44, Heppe Br J Nutr 2011;105:938–49) or negative effects (Halldorsson Am J Epidemiol 2008;168:958–65, Halldorsson Am J Epidemiol 2007;166:687–96, Mendez J Epidemiol Community Health 2010;64:216–22). A recent meta-analysis among 19 European birth cohort studies with more than 150,000 women, reported that fish's consumption, particularly for the blue fish, reduces the risk of preterm labor and increases BW (Leventakou Am J Clin Nutr 2014;99:506-16). Similarly, although in USA women are advised to limit the seafood intake during pregnancy up to 340 g per week to avoid fetal exposure to trace amounts of neurotoxins (US 2006), a study based on a large cohort (11,875 pregnant women, ALSPAC cohort) reported that maternal seafood intake during pregnancy of less than 340 g per week was associated with increased risk of their children being in the lowest quartile for verbal intelligence quotient, suboptimum outcomes for social behavior, fine motor, communication and social development scores; on the contrary, a consumption of more than 340 of seafood per week was beneficial for the child's neurodevelopment (Hibbekn 2007).

Milk has a high concentration of nutrients, including protein, calcium, phosphorus, potassium, iodine, vitamin B12 and riboflavin (Brantsaeter Food Nutr Res 2012). In addition, the consumption of cow's milk increases the concentration of insulin growth factor type 1 (IGF-1), determining growth during childhood (Hoppe Am J Clin Nutr 2004;80). A review (Brantsaeter Food Nutr Res 2012) showed suggestive but limited evidence that moderate consumption of milk during pregnancy, compared with nil or low food intake, was positively associated with fetal growth and BW of the baby in healthy West populations. In a prospective study (Olsen Am J Clin Nutr 2007;86:1104-10), it was noted that milk consumption was associated with a greater increase in BW and less risk of FGR.

There is also evidence that FGR is associated with a reduction in the ability of the placenta to transport amino acids (Lin Amino Acids 2014;46:1605-23). There are studies on the effect of certain amino acids related fetal growth, such as arginine, glutamine, the citrulline, branched amino acids, taurine and aromatics; the most positive results were observed in the growth and/or fetal development, although some studies showed contradictory conclusions (Lin Amino Acids 2014;46:1605-23). According to a Cochrane review (Ota Cochrane database syst rev 2012), nutritional advice to increase energy and protein intake seemed to have an effect on increasing protein intake of pregnant women and fetal growth, such as the cranial circumference, but long-term effects are not clear and the same Cochrane review concluded that there was no justification for the prescription of a diet rich of proteins.

Interventional studies with an understanding of optimal dietary patterns may provide promising results from both maternal and perinatal health. In this context it has been reported that a low adherence to the MD was related with lower BW (Timmermans Br J Nutrit 2012, Generation R study). The INMA (*Infancia y Medio Ambiente*) study in Spain and the RHEA (*Mother-Child Cohort*) study in Greece, have evaluated the adherence to the MD and fetal growth (Chatzi Br J Nutr 2012). In the INMA cohort, a stronger adherence to the MD was associated with a low rate of FGR (Rodriguez-Bernal Am J Clin Nutr 2010). Although a huge amount of observational study, few RCT studies were conducted to evaluate the effect of diet during pregnancy and their outcomes. One study from Norway randomized 290 pregnant women at 20 weeks' gestation to either continue their usual diet or to adopt a diet that promoted fish, low-fat meats and dairy products such as oil, grains, fruits, vegetables and legumes. The authors observed a modification in maternal lipid levels but not in cord blood or neonatal lipids, and a reduction of preterm delivery in those who were randomized for diet intervention (Khoury AJOG 2005).

3.7 Neuroimaging

Complex functions such as behavioral and emotional regulation are mediated through the prefrontal cortex. This region of the brain has many subdivisions and connections with all sensory systems, cortical and subcortical motor system structures, attention functions, and with limbic and midbrain structures involved in affect, memory and reward (Miller 2001 Annu Rev Neurosci). Behavioral functions are not localized in the prefrontal cortex, but the prefrontal cortex seems to be essential for the control of organized and integrated functioning (Spreen 1995 Oxford un). For example, besides the orbital frontal cortex, the anterior cingulate cortex (ACC) plays a role in the modulation of conditional fear responses and it is critically involved in performance monitoring and cognitive control (Bush 2000); it has also a key role in emotional and social behavior (Hermens 2012). The hippocampus is also one of the most important structures of the limbic system, being involved in memory functions, processing of emotional information and stress response through the HPA axis.

Proper timing and guidance of neurogenesis, neuronal differentiation, apoptosis, synaptogenesis and myelination, are critical for the appropriate organization and functioning of the neocortex (Nowakosky 2002). These processes can be altered with environmental factors, such as viruses, tobacco, drugs, cortisol…(Levitt 1998). Neuroplasticity is the main characteristics of the brain, which is the organ built to change in response to environment and experience. The interaction between genes and environment/experience is fundamental for the development of cortical plasticity (Grossman 2003).

Different neuroimaging techniques have been growing in the last few years, aimed at identify better some functions of the brain. Magnetic resonance spectroscopy (MRS) for example, analyzes and makes use of the intrinsic magnetic properties of atomic nuclei that, when they are placed in a static magnetic field, specific radiofrequencies are either absorbed or emitted, and this can be detected. Different signal peaks seen within the spectrum correspond to identifiable molecules. The potential usefulness of MRS is to understand pathophysiologic mechanisms of brain injury. In a recent study conducted on healthy people, higher levels of glutamate, combined glutamate and glutamine and -inositol in the ACC, had a predictive value for anxiety (Modi 2014). Changes in other brain metabolites, such as ɣ–aminobutryric acid (GABA) that is an inhibitor neurotransmitter, have found to

be lower in the ACC and in the medial prefrontal cortex in patients with a family history of panic disorders (Long 2013).

Functional magnetic resonance imaging MRI (fMRI) is a neuroimaging tool that employs magnetic resonance imaging (MRI) to image dynamic changes in brain tissue that are caused by changes in neural metabolism. Alterations of neural activity may be caused by asking the subject to perform a task designed to target a specific cognitive process, or can occur spontaneously while the subject is resting in the absence of conscious mentation (i.e., in the "resting state"). In the resting state, the implicit hypothesis is that there are distinct brain regions whose fluctuations are temporally synchronized, and thereby are connected as nodes of networks, such as the Default Mode Network (DMN) (Greicius 2003, Buckner 2008).

3.7.2 Neuroimaging in growth restricted fetuses

The brain is one of the most complex organs to rapidly develop and who presents a fastest rate of expression change during the fetal period (Colantuoni 2011). Although early plasticity allows a proper adaptation to the environment that enables to survey, it might have negative effects later in life.
Neurodevelopmental impairment in FGR has been associated with specific neurostructural changes on MRI studies (Battalle 2012, Eikenes 2012). Several studies have demonstrated that growth restricted fetuses are associated with brain reorganization changes, showing alterations in brain microstructure and cortical development (Sanz-Cortes UOG 2010, Egaña AJOG 2014, Egaña UOG 2014, Egaña PD 2014, Sanz-cortes AJOG 2014) as well as in brain metabolism (Sanz-cortes FDT 2013, sanz-cortes Plose 2013, Sanz-cortes AJOG 2015). Additionally, these changes were also seen in neonates (Battalle 2012) and one-year toddlers (Simoes AJOG 2015).
Going more in details, we have just demonstrated that FGR fetuses are characterized by a decrease in brain volume, even after adjusting for EFW percentile, a physiological brain asymmetry, a decrease gray matter, deeper measurements in some cortical sulcations (insula fissure, left cingulate fissure) (Egaña AJOG 2014), differences in insular cortical thickness, (Egaña UOG 2014), in cerebellar and brain stem morphometries, that are associated with a suboptimal neonatal neurobehavior (Sanz-Cortes AJOG 2014). Both insula and cingulated fissures are areas that play an important role in the limbic system (Allen J Comp Neurol 1991), which is responsible for interoceptive awareness and higher cognitive functions; these areas are particularly vulnerable to sustained undernutrition and/or hypoxia (Hernandez-Andrade UOG 2008).

Differences in metabolites, such as decreased N-acetyl-aspartate (NAA) to choline, considered a surrogate marker of neuronal activity, have been associated with corpus callosum development in growth restricted fetuses (Sanz-Cortes AJOG 2015). Interestingly, differences in MRS have been also reported in FGR newborns at one-year of age (Simoes AJOG 2015). In toddlers that were restricted during fetal life, we also shown alterations in fMRI revealing an hyper-connected but sub-optimally organized functional brain networks (battalle 2016) and also altered connectivity in motor and cortico-striatal-thalamic networks (Eixarch AJOG 2016).

3.7.3 Neuroimaging and Mindfulness

While mindfulness-based interventions are increasingly applied in the therapeutic context (Baer 2003, Grossman 2004), the investigation of neurobiology underlying the beneficial effects is still in its infancy (Davidson 2003, Holzel 2010, Gard 2012, Goldin 2013). The most promising studies were conducted

on patients with anxiety disorders, generally associated with abnormalities in prefrontal activation, and altered relationship between activity of prefrontal regions and amygdala (Kim 2011, Kim 2012). For example, lower activation of the ventrolateral prefrontal cortex and higher activation of the amygdala have been reported in patients with generalized anxiety disorders (Monk 2006, Monk 2008). The ventrolateral prefrontal cortex is involved in inhibitory control (Cohen 2013) and its activation typically increases in people who can down-regulate their emotions (Ochsner 2004, Phan 2005, Wager 2008); in addition, it also modulates amygdala responses during emotion regulation processes (Ochsner-Gross 2005). In a study with fMRI based on tasking evoking, Creswell *et al.* found that higher trait mindfulness were related with a greater activation of prefrontal areas, including ventrolateral and medial regions of the cortex, lower amygdala activation, and greater amygdala-prefrontal connectivity (Creswell 2007), suggesting a potential neural mechanism of mindfulness training. More recently, in a RCT of 26 patients affected by generalized anxiety disorders, in half of them who attended a MBSR program, the fMRI after 8 weeks of intervention revealed a decrease activation of the amygdala and grater activation in the ventrolateral prefrontal regions, suggesting that mindfulness practice leads to changes in fronto-limbic areas crucial for the regulation of emotion, and these changes corresponded also with reported symptoms improvements (Holzel 2013). Therefore, there is increasing evidence that mindfulness training is associated with enhance activation and connectivity between several brain regions that are known to be crucial to successful emotion regulation, both for healthy and anxiety disorder populations, such as increased brain activation in sensory areas (insula, thalamus, ACC and secondary somatosensory cortex) and decreased activation in an area that mediates executive control (the lateral prefrontal cortex) or in emotion-related areas (amygdala, hippocampus) (Kim 2011, Gard 2012).

A recent study on resting state in fMRI (Doll 2015) investigated the association between mindfulness and functional connectivity of intrinsic brain activity among three central neurocognitive networks: the connections between DMN and silence network, and between silence and central executive networks, were significantly associated with mindfulness scores. This might be one possible pathway for beneficial effects of mindfulness therapy.

Regarding MRS, a recent study reported different changes in some metabolites, such as an increasing myo-inositol in the posterior cingulate cortex and a decreasing glutamate and NAA in the left thalamus in a group of zen meditators compared to controls (Fayed-Campayo 2013).

3.8 Epigenetics

Epigenetic is defined as the study of changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic processes, including DNA methylation, histone modification and non-coding RNAs, play a central role in regulating this gene expression. Besides, these marks, though potentially reversible, are stable and could be long lasting.

There is growing evidence that the epigenome is particularly susceptible to a number of environmental factors, such as maternal diet and behavior during prenatal and early postnatal life, known as “environmental programming”. They are key players in the development underling many of the processes of fetal programming, in addition to genetic factors and leading to long-term phenotypic alterations. Thus, improvement in maternal behavior and diet during pregnancy should be considered interventions that could impact the epigenome and consequently mitigate long-term phenotypic alterations.

3.8.1 Epigenetics and maternal behavior

Variations in maternal stress and behavior have been extensively correlated with stable alterations of DNA methylation and chromatine structure (Field T The Int J Neurosci 2008, Diego M Early Hum Dev 2009, Grote NK Arch Gen Psychiat 2010).

Maternal depression/anxiety in the third trimester was found to be associated with increased methylation of the gene which encoded the glucocorticoid receptor (NR3C1) in the cord blood of neonates and with increased salivary cortisol response to a visual new stimulus at 3 months of age (Oberlander Epigenetics 2008). The same methylation of the NR3C1 gene has been reported in children (10-19 years) whose mothers experienced intimate partner violence during pregnancy (Radtke 2011). Besides, different animal studies reported that offspring of mothers that showed high levels of pup licking and grooming were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter. These findings provide evidence that maternal care influences hippocampal GR expression and thus the HPA functions in the offspring (Weaver I nature neuroscience 2004, Meaney, trends in neurosciencs 2005, Turecki Biol Psychiatry 2016).

Epigenetic variation in the placenta is emerging as a candidate mediator of environmental influence on placental functioning and a key regulator of pregnancy outcome. Some placental genes such as noerpinephrine transporter (NET), 11B-HSD2 and NR3C1 have been implicated in changes of HPA axis. (Lester Clin Obst Gynecol.2014) Regarding the placental 11β-HSD2 enzyme, we have reported above a recent study where prenatal maternal anxiety was correlated with a decreased 11β-HSD2 mRNA expression (O'Donnell 2012); this decrease is thought to be mediated, at least partially, by epigenetic mechanisms such as DNA methylation (Jensen Peña 2012). In contrast, prenatal exposure to maternal depression was associated with decreased methylation of the placenta SLC6A4 gene encoding the transmembrane serotonin transporter (Devlin PlosOne 2010). Besides, this decreased methylation for SLC6A4 in peripheral tissues, such as blood and saliva has been associated with early life adversity and depression.

3.8.2 Epigenetics and nutrition

Prenatal and early postnatal nutrition has been described as other critical environmental factor that could alter the epigenetic programming and could increase the susceptibility to chronic diseases (Lucas J Nutr 1998). Environmental exposures during early life may lead to an increased risk of obesity and metabolic syndrome later in life via alterations in DNA methylation. For example, in the Dutch Famine cohort, 60-year-old adults who were prenatally exposed to famine showed hypomethylation of whole blood of IGF2 gene, and hypermethylation of two obesity-related non-imprinted genes (tumor necrosis factor, leptin) as compared with those whose mothers were not unexposed to the famine during pregnancy (Tobi Hum Mol Genet 2009). Recently, parental obesity and specifically paternal obesity was associated with IGF2 hypomethylation in umbilical cord blood leukocytes of newborns (Soubry Int J Obes 2013, Soubry BMC Med 2013). Furthermore, specific maternal characteristics, including gestational weight gain and gestational diabetes, have also been associated with signature DNA methylation in cord blood and increased placental leptin gene methylation, respectively. (Morales BMC Res Notes 2014)

Regarding FGR, changes in the expression and the activity of the placental nutrient transporters have been associated to pregnancy pathologies such as this condition. It has been reported that maternal metabolic status determines adaptations of placental nutrient transporters thought the DNA metylation;

the methylation of the cholesterol transporter ABCA1 appeared to be sensitive to in utero environment and may have long-term impact on lipid profile in cord blood (Houde Epigenetics 2016).
In addition, a recent review has summarized the animal models demonstrating how dietary manipulation impacts perinatal programming (Breton J Endocrin 2013).

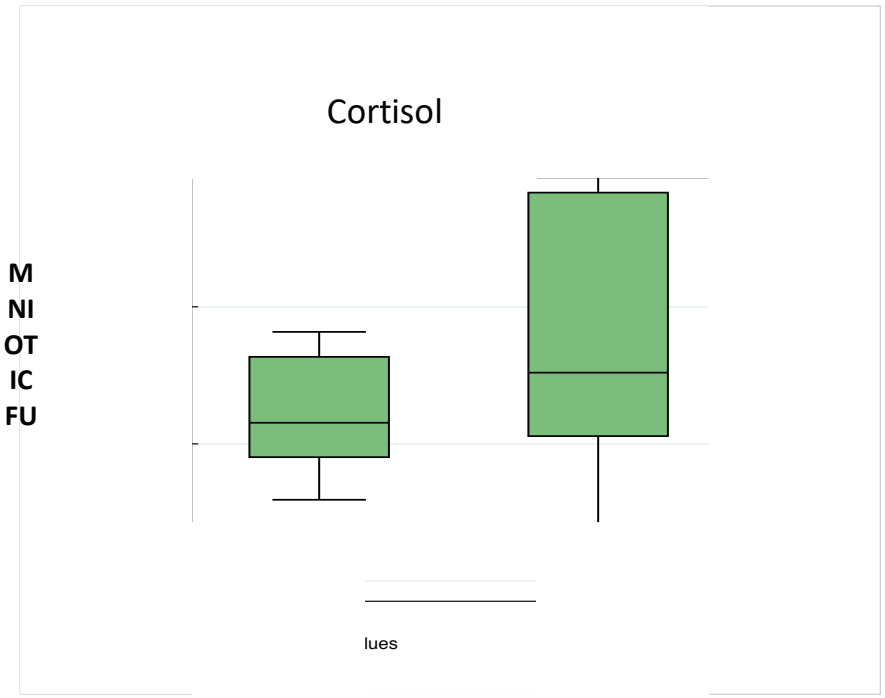
3.8.3 Ageing

Telomeres are nucleoprotein structures consisting of 5-15 kilo base pairs of repetitive DNA sequences, located at the termini of the chromosomes. They are essential for chromosome stability and for cell survival ((Moyzis 1988, Blasco Genes Dev 1999). Telomeres are progressively shortened with each cell division and also by environmental factors. Shortened telomeres promote cell cycle arrest, apoptosis, and genomic instability. The enzyme telomerase adds telomeric repeats to the ends of the chromosomes (Harley Nature 1990) and the human telomerase reverse transcriptase is the catalytic component of telomerase and it is considered to be the rate limiting factor in telomerase activity.
In recent years, ageing has emerged as a particularly attractive candidate among the molecular mechanisms underlying the link between stress and disease risk (Sahin 2010, Sahin 2011, Jaskelioff 2011). Telomere maintenance has relevance for long-term health. Shorten leukocyte telomere length (LTL) in humans has been associated with earlier mortality and morbidity (Samani 2001, Cawthon 2003, Epel 2009). It has been recently demonstrated a linking between maternal psychological stress exposure during prenatal life and shorter LTL in young adulthood (Entringer 2011). More interestingly, this link has been reported to be evident as early as the time of birth: in a prospective cohort of 27 stressed-pregnant women, the analysis of cord blood peripheral blood mononuclear cells for LTL measurement reported a linear effect of pregnancy-stress on newborns LTL (Entringer 2013). This is the first evidence in human being that maternal psychological stress may put forth a programming effect on the newborn telomere biology system, reflecting cellular aging.

3.9 Previous preliminary results

As previously described (paragraph 3.2), there are still a lot of doubts regarding possible causes of FGR. In the last years our group has given an important contribution to this, in particular with a big cohort in humans and with an animal model.

Regarding the first one, the hypothesis that FGR is a multi-phenotypic disease is the main objective of a project we are conducting in our research center (Phenomapping of Fetal Growth Restriction). Based on preliminary data, some cases of FGR seemed to be caused by higher levels of perceived stress and anxiety of the mothers, resulting in higher levels of cortisol in the amniotic fluid, that is the urine of the fetus, as reported in the figure 2A. Additionally, the levels of the gene expression of 11β-HSD2 were lower in FGR cases compared to controls (Figure 2B), as also reported by others (Glover 2009, O'Donnell 2012).



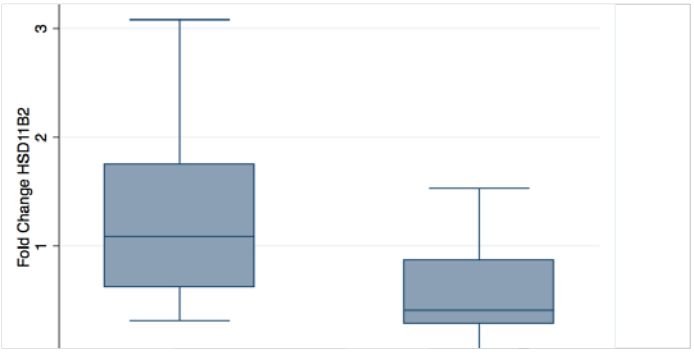


Figure 2A. Expression of cortisol in the amniotic fluid of FGR and controls.

Figure 2B. Expression of 11β-HSD2 gene in the placentas *of FGR and controls.*

In the same cohort, the nutrition patterns of the mothers were often suboptimal (Figure 3A) and showed clear signs of fetal malnutrition, such as low levels of ferritin in cord blood (Figure 3B).

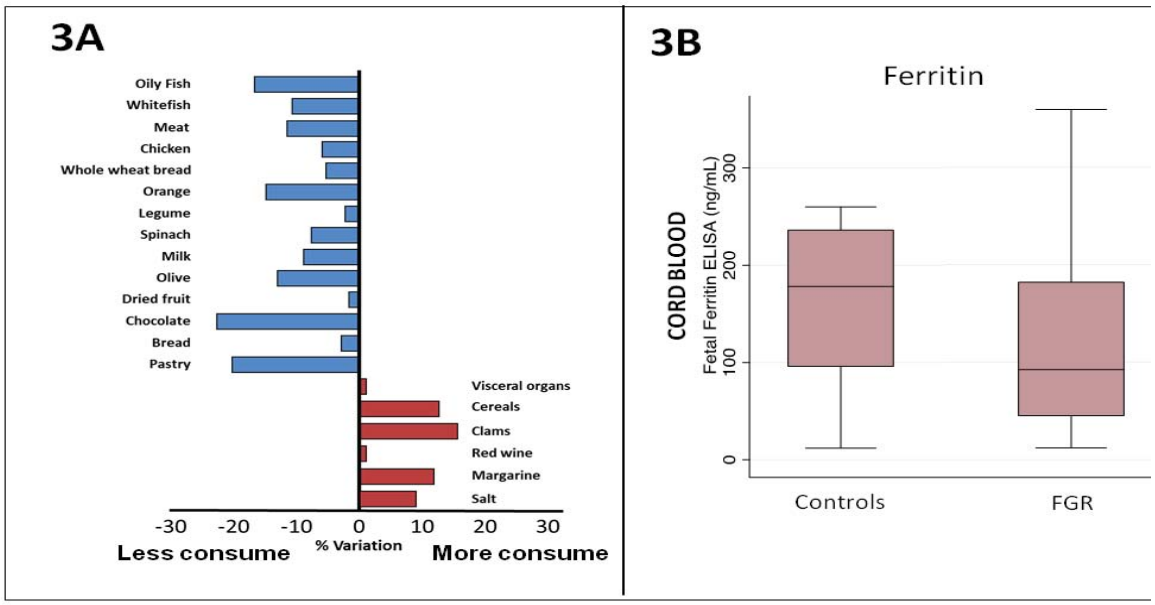


Figure 3B. Levels of ferritin in the cord blood of FGR and controls

In parallel, data from our group from an experimental rabbit model of FGR indicated that dietary supplementation with LC-PUFA (DHA) and lactoferrin can improve birth weight, mortality and neurodevelopment of offspring (Figure 4)

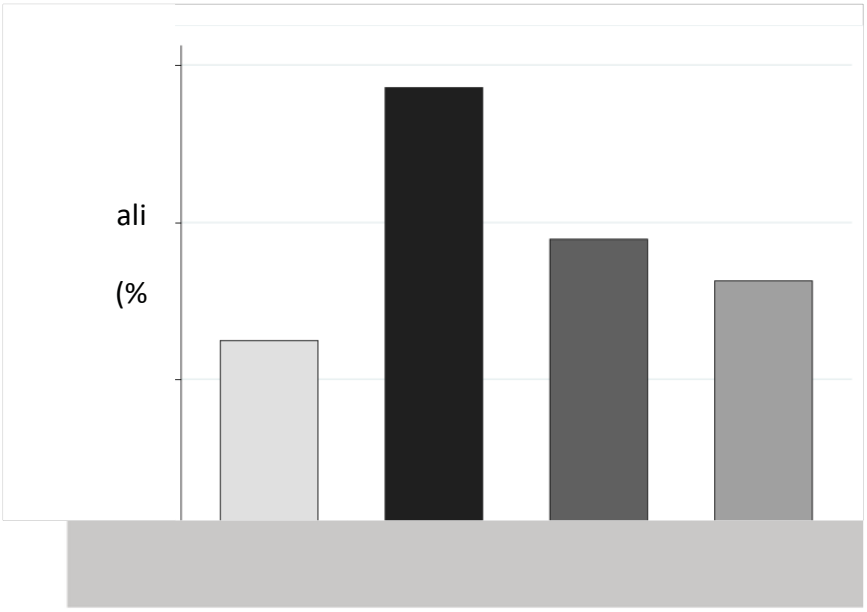


Figure 4. M *HA or Lactoferrin)*

3.10 Rational of the study

From a clinical prospective, given the high prevalence of FGR (7-10%) and its impact in future life, the critical goal is now to apply some strategies that could reduce the incidence of this disorder during prenatal life. Maternal psychological state as well as her nutrition profile seem to be fundamental for pregnancy environment and it probably shapes the neurodevelopment of the fetus and consequently of the child. We wonder if improving the maternal well being with specific strategies we could see an improvement on fetal outcome.

4 Study aims

4.1 Hypothesis

Our **primary hypothesis** is that specific treatments improving maternal well-being have a positive impact on pregnancy outcomes as well as on fetal growth and development.

Our ***secondary hypothesis*** is that interventions to improve maternal well-being have a positive impact on offspring's outcome later in life, in terms of neurodevelopment and cardiovascular profile, mediated by epigenetic changes.

4.2 Aims

1) To demonstrate that an improvement on maternal well-being, through intensive behavioral therapies based on stress reduction with mindfulness techniques and nutrition intervention with a MD, reduces the prevalence of FGR in pregnancies at high risk to develop this condition.

2) To demonstrate that an improvement on maternal well-being, through intensive behavioral therapies based on stress reduction with mindfulness techniques and nutrition intervention with a MD, reduces the APO of pregnancies at high risk to develop FGR.

3) To demonstrate that an interventional program to the mother improves fetal brain development and infant cardiovascular profile and neurodevelopment.

4) To identify offspring's epigenetic changes associated to an interventional program to improve maternal well-being.

4.3 Specific objectives

4.3.1 Principal objectives

For each of these principal objectives a sample size has been calculated (see below specific section 5.5.1).

1) The ***primary objective*** of this study is to evaluate whether an interventional program aiming at improving the maternal well-being in pregnancies at high risk to develop FGR (Odds Ratio, OR >2, 30% of risk for this category of women) could improve the fetal growth and subsequently reduces the prevalence of newborns with a BW below the 10th percentile (attend improvement: reduction of 30% of the prevalence of babies born with a BW <10th percentile: reduction from 30% to 20%).

   The ***secondary objectives*** are to:

2) Demonstrate a reduction of 50% (from 15% to 8%) of APO, defined according to the presence of any of the following neonatal measures:

   a. Preterm birth (PTB): delivery <37 weeks' gestation
   b. Preeclampsia (PE): defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy (Brown 2001), that requires two recordings of systolic blood pressure (BP) ≥ 140mmHg or diastolic BP ≥ 90mmHg at least 4 h apart after 20 weeks of gestation and proteinuria of ≥300 mg in 24 h.
   c. Perinatal mortality: fetal or neonatal mortality (within 28 days of life).
   d. Severe FGR: birth weight <3rd percentile
   e. Metabolic acidosis: an umbilical artery pH below 7.10 and/or base excess >12 mEq/L in the newborn and/or an Apgar score at 5-minute below 7.0 assigned by the attending neonatologist or midwife.
   f. Major neonatal morbidity: presence of intraventricular hemorrhage grade III/IV, necrotizing enterocolitis, periventricular leucomalacia, sepsis, broncopulmonary dysplasia, hypoxic ischemic encephalopathy.

3) Demonstrate an improvement on offspring's neurodevelopment after maternal intervention, defined with Bayley test at two years of corrected postnatal age.

4) Demonstrate an improvement of offspring's cardiovascular profile after maternal intervention, defined with arterial BP assessed at two years of corrected postnatal age.

4.3.2 Other objectives

5) Demonstrate an improvement on offspring's fetal/neonatal neurodevelopment from mothers who attend the interventional program, revealed by different techniques of fetal MRI (cortical development, brain volumes, spectroscopy assessment) and with NBAS at one month of corrected age.

6) Determine different epigenetic changes in fetal cord blood in pregnancies affected by FGR and in those who attend the interventional program as compared to non-complicated pregnancies with no intervention.

7) Demonstrate an improvement in placental perfusion by MRI and a decrease placental cell death in pregnancies who attend the interventional program as compared to pregnancies with no intervention.

8) Describe an improvement on maternal psychological status during pregnancy thanks to an interventional program based on mindfulness practice, evaluated by different questionnaires, maternal brain metabolites and cerebral circuits assessed by maternal MRI.

9) Describe an improvement on maternal physical status during pregnancy thanks to a healthier nutrition based on MD, evaluated by different questionnaires and maternal metabolic profile and its impact on placental nutrient transport and fetal metabolic profile.

5 Methods

5.1 Study design

The project will be a RCT among women at higher risk to have a growth restricted fetus (30%) according to the Royal College of Obstetrics & Gynaecologists (RCOG) Green-top Guidelines No. 31 (January 2014) (see Appendix 1). These high-risk women will be randomized in order to evaluate an improvement in several outcomes thanks to different strategies apply to the mothers: a stress reduction program based on mindfulness techniques and/or a nutrition interventional program based on Mediterranean diet.

The study design of the RCT adheres to the CONSORT quality standard criteria for randomized trials (Antes BMJ 2010), and it has been registered in the *Clinical Trials Gov* (https://clinicaltrials.gov/).

5.2 Study Setting

The study will take place in two obstetric referral institutions (Hospital Clinic and Hospital Sant Joan de Déu -BCNatal) in Barcelona (Spain).

5.3 Study population

Women at high risk to have a growth restricted fetus according to the RCOG Green-top Guidelines No. 31 (January 2014) (Appendix 1).

5.4 Participants

5.4.1 Participant selection

Eligible participants will be pregnant women resulted at high risk to develop FGR during pregnancy (OR >2) according to the criteria of the RCOG (Guidelines No. 31). Women will be selected at the moment of the routine second trimester ultrasound scan (19-23 weeks' gestation) and will be randomized to four equally sized groups:

1) Stress-reduction program based on Mindfulness techniques
2) Nutrition program based on Mediterranean diet
3) Control group with no specific intervention (usual care)

Doctors who manage these patients will be blinded for each group of randomization the patient is belonged to. Additionally, the subjects are asked not to reveal their group of randomization to any of the other subjects.

5.4.2 Participant inclusion criteria

- Maternal age at recruitment ≥18 years
- Speak Spanish fluently
- Viable singleton non-malformed fetus
- High risk pregnancy
- 19-23 weeks of gestation

5.4.3 Participant exclusion criteria

- Fetal anomalies including chromosomal abnormalities or structural malformations detected by ultrasound.
- Mental retardation or other mental or psychiatric disorders that impose doubts regarding the true patient´s willingness to participate in the study.
- No possibility to come to additional visits.

5.5 Sample size estimation

5.5.1 Sample size rationale

Specific sample size estimation has been calculated for each of the principal objectives of the study explained above. We used for calculation the program "Power and Size Program version 3.1.2, 2014 (Department of Biostatistics, Vanderbilt University).

For fetal growth and neonatal growth percentiles we use the charts of the INTERGROWTH-21 study (Papagorghorgiu Lancet 2014), which takes into account the gestational age at delivery and gender.

1) Reduction of 30% of rate of neonates born with a BW <10th centile:
   Based on the criteria of the RCOG (Guidelines No. 31), 30% of pregnant women belonged to this high risk category will have a growth restricted fetus, defined with a BW<10th percentile. The main outcome of this RCT is to reduce this prevalence by 30% (from 30% to 20%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 293 subject for each arm of randomization. Considering a loss of 20%, the final sample size calculated is 367 participants for each group (total=1101 subjects).

2) Reduction of 50% of rate of APO:
   Based on several evidence in the literature (Poon-Nicolaides) the 15% of this population considered at high risk will develop an APO during pregnancy. The second outcome of this RCT is to reduce this prevalence by 50% (from 15% to 8%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 325 subject for each arm of randomization. Considering a loss of 20%, the final sample size calculated is 406 participants for each group (total=1218 subjects).

3) Improvement of Bayley score at two years of age:
   Based on previous data from our group (Savchev UOG 2013), the difference of the mean score at Bayley test at two years of age between controls and FGR was 6 (standard deviation, SD 14). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 87 subject for each arm of randomization. Considering a loss of 30%, the final sample size calculated is 124 participants for each group (total=372 subjects).

4) Reduction of BP at two years of age:
Based data from the guidelines for cardiovascular health and risk reduction in children and adolescents (Pediatrics 2011;128 Suppl 5:S213-56, table 8.3), the 90th percentile for blood pressure at two years of age is 102/57 mmHg. Based on a recent paper from our group (Cruz-Lemini AJOG 2014), the prevalence of a mean BP >95th centile in FGR fetuses at 6 months of age was 41% compared to 5% of controls. In this study we expect a prevalence of 20% of BP >95th centile at 2 years of age in the controls group, and the objective is to reduce this prevalence by 50% (from 20% to 10%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 199 subject for each arm of randomization. Considering a loss of 30%, the final sample size calculated is 259 participants for each group (total=777subjects).

5.6 Recruitment

At the moment of the routine second trimester ultrasound scan (19-23 weeks' gestation), all women are invited to check a list of risk factors based on the criteria establish by the RCOG (Guidelines No. 31, Appendix 1). These criteria set up a subgroup of women (approximately 30% of all pregnant women, 800 for year in each of our hospitals) that are at higher risk to have a FGR. These guidelines establish that women are considered at risk if they meet a major risk factor (OR >2) or three or more minor risk factors (OR >1 for each) (see Appendix 1): the RCOG guideline establishes that these women should be referred for serial ultrasound measurements of fetal size and assessment of well-being from 26-28 weeks' gestation (Guidelines N31 RCOG). In our hospitals, these women are scheduled for extra ultrasound assessments at 28 and 32 weeks' gestation, while women who do not fulfill these criteria are just submit to third trimester ultrasound at 37 weeks.

5.6.1 Enrolment visit

Participants at high risk to develop FGR are considered eligible for this clinical trial and the same day of the routine second trimester ultrasound scan they will be identified by one of the two principal investigators (PIs) (FC & CP) for a formal screening visit.

The visit serves to identify inclusion/exclusion criteria in a more comprehensive manner and to address all eligible patients about the purpose of the study and the voluntary nature of the participation. This 30-45 minutes visit includes:

1) A face-to-face administration of questionnaires to inquire about psychosis, major depression and bipolar disorder (see document in the Appendix 2). In case of positive answer to any psychosis or bipolar disorder, the patient will be considered not eligible from the study and referred to the Psychiatric Unit of our Hospital (responsible: Prof. Vieta). In case of a positive answer to the question number 9 of the PHQ-9, the patient will be considered not eligible from the study and the specific questionnaire for suicide (Palkel, Appendix 2) will be given to her and she will be referred to the Emergency Psychiatric Unit.

2) If the candidate meets all the requirements, an informed consent form will be given to her to be signed after a detailed explanation of all study's procedures. The informed consent comprised

two parts, one for study participation and another for biochemical analyses and DNA collection for genetic analyses (Biobank informed consent).

3) Demographic data, clinical history, anthropometric measures and biological samples will be taken (maternal hair, peripheral blood and urine) (Case report form, Appendix 3)

4) A brief feto-placental ultrasound with Doppler parameters will be done.

5) Several questionnaires will be given and explained to the patient for a self-report to be completed at home and/or provided thought a specific web site (see Appendix 4-13).

6) To a random 30% of women, a fit-bit bracelet will be given to the patient and it will be asked to use it for the next week, in order to register their basal activity.

7) An individual visit with a nutritionist for the compilation of two food questionnaires (Food frequency questionnaires, FFQ and 15-points) and a physical activity questionnaire will be scheduled for the all patients for the next 7-10 days: during this visit questionnaires and fit-bit bracelets will be collected from each patient (see Appendix 14-16).

8) Randomization of the patient trough a specific website accessible only for researchers: depending on the arms in which she will be, a brief general explanation of the study and following appointments will be given each woman.

9) The patient will be informed about the web site of the study, with a specific access depending on her arm of randomization.

10) If extra-appointments are necessary (depending of the group of randomization), they will be given to the patient.

5.6.2 Randomization

After obtaining the informed consent, the patient will be randomized in one of the four arms of this trial. Using an online service (http://www.randomization.com), randomization sequences will be generated to assure balanced distribution within study arms. The randomization assignment will be registered in the database and also in the project storage.

5.7 Intervention treatments

The idea behind both interventions treatments are focused on pregnant women's well being: if women could do a significant change in her life, this could lead to a benefit not only for themselves and their pregnancies, but might also reach their fetuses and consequently improve their growth and development. Both interventions are not based on medical treatment, but on behavioral counseling and group therapies, derived from robust scientific evidence and already validated in several important studies.

5.7.1 Mindfulness intervention

The mindfulness-based intervention program is a psychotherapeutic intervention of 8 weeks of group classes and daily home practice. Weekly group classes of 2 hours will be conducted and coordinated by a psychiatrist and expert in mindfulness with years of experience (IF) and training nurses (MG, MdL, MA, NE). Before the implementation of the protocol, training of the nurses involved (from 2 to 4) consists of approximately 20 hours of initial theoretical and practical group discussion with the expert of the mindfulness program (IF) and then a participation of a pilot group and of the first groups with a later supervision done by the expert of mindfulness herself (IF).

The intervention is based on different exercises to cultivate awareness of internal present-moment experiences with an accepting, non-judgmental stance, management of stress techniques and other pleasurable activities focused on enhancing the adherence to the program. The program has been supervised by the Disparities Research Unit from the Massachussets General Hospital-Harvard Medical School (MA), by the head of Psychiatry Service of Hospital Clinic de Barcelona (EV), and by a certified mindfulness-based stress reduction teacher and professional trainer at the University of Massachusetts (ZV). A specific manual based on this purpose has been created.

After the randomization, before starting with the group of intervention, an individual interview with the one of the mindfulness trained nurses will be done to any woman eventually with her husband, in order to evaluate better each participant and to reinforce the importance of this treatment.
All classes will be audio-taped, and 20% of them will be randomly reviewed by an expert supervisor, using a classes' checklist to assess fidelity.
Women will be also instructed for formal and informal meditation as home practices (e.g. present-focused awareness during eating, bathing, cleaning…) with the reminder of a personal workbook and a CD or USB with meditation guides. Meditation guides will be also available on the website of this arm of intervention. Before starting with a new section, the frequency of home-practice will be recorded in order to assess the adherence to the program.

Adherence and effectiveness:
Adherence to the intervention will be assessed by class attendance and time spent engaged in mindfulness meditation outside of class sessions. The treatment will be considered complete if at least 10 hours of meditation are done (both during class and at home). Each group of intervention is composed of a maximum 9 women. At the end of the treatment, biomarkers related to stress (cortisol axis) will be evaluated in maternal hair and fetal amniotic fluid (fetal urine) in a random sample of 30% at the Bioanalysis and Analytical Services Research Group - IMIM (OP).
If a woman has completed the entire interventional program (8 weeks), she can attend some extra session every week until delivery, in order to keep in mind this intervention.

Ideal timeline for intervention: from 24-26 to 32-34 weeks' gestation (group classes every week to a total of 8 sessions)

5.7.2 Nutritional intervention

The nutritional intervention program is based on the results derived from the PREDIMED study (Predimed, NEJM 2013), in which a MD supplemented with extra-virgin olive oil or mixed nuts improved men's quality of life with a significantly reduction of major cardiovascular events.
One main focus of this intervention strategy is to change the dietary pattern in general instead focusing on changes in single food or macronutrients. Diet pattern is adapted to pregnant women and will be also adapted during the first individual assessment with the dietician to participant's weight, culture and nutrition questionnaire. The intervention program is done by two expert dieticians (CV and TF) and supervised by the Department of Obesity and Nutrition of the Hospital Clinic of Barcelona (RC, MD, and RE).

The intervention is based on individual visit of 30 minutes assess every two weeks and on monthly group classes of 1 hour, where theoretical information and discussion about MD will be done in order to identify problems and solution in diet implementation. Participants will receive extra-virgin olive oil (1 liter every four weeks) and 30 g of mixed nuts per day at no cost.
Each individual visit includes three steps: assessment, intervention and future directions. Specific materials (recipes, a quantitative 1-week shopping list of food items according to the season of the year, a weekly plan of meals with detailed menus...) will be given and will be also available on the web site. During each visit the 17-item dietary screener to assess baseline adherence to the MD will be checked. Every month the 7-days register diet will be also evaluated.

Adherence and effectiveness:
Adherence to the intervention will be assessed by an improvement adherence to the MD, based on an improvement of ≥2 points of their total final score of the 17-item dietary screener compared to their total initial score. At the end of the treatment, biomarkers of compliance will be evaluated in a random sample of 30% of participants, including urinary hydrohytyrosol levels (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid levels (to confirm compliance of mixed nuts) at the School of Pharmacy and Food Science at the University of Barcelona (RML).

Ideal timeline for intervention: from 24 to 36 weeks' gestation (individual visits every 15 days + group class every month)

5.8 Participant timeline

5.8.1 Promotion of adherence

Efforts to promote adherence begins at the earliest stages of the study. During the first visit at enrollment, participants are repeatedly provided with information about key features of the study. After randomization, for intervention groups an individual interview with the specific trainer (mindfulness expert o dietician) will be done in order to focus more individual goals and to do the treatment as more efficient as possible.
In both intervention strategies is fundamental to do home practice: classes are useful to get some theoretical notions, but the majority of the treatment needs to be done in every-day life.
Nurses involved in the project will do phone calls at least twice during the treatment. In the website with a different access depending on the arm of randomization, several information and material will be also available.

For mindfulness intervention, the personal workbook and meditation audio-types will be given free to ensure a high adherence. For nutrition intervention, the free distribution and supply of key food items ensure a high adherence to. For women in the control group, usual care will be given.

5.8.2 Assessment of compliance

Home-practice is essential for both interventions, and people involved will reinforce this concept. To obtain also an objective evaluation, data from fit-bit bracelets will be analyzed in a random sample of 30% of participants.
The personal workbook with mindfulness meditation will be revised weekly during class in order to evaluate any problems and to reinforce the practice. Daily practice of mindfulness will be taken into account and used for subsequent analysis (adherence: 10 hours meditation).
For nutrition intervention, the 17-item dietary screener will provide information about compliance and attainment by participants. Additionally, if it is possible in random sample of 30% of participants, a blood sample and urine aliquots will be used to blindly ascertain the markers of compliance of mixed nuts and extra-virgin oil, respectively. To relate these measurements to the time of intake, participants are asked the time spent since the last consumed the specific food when blood and urine samples are taken.
At the last visit during pregnancy a present for the newborn will be given to each mother. Similarly, during postnatal follow-up, in every visit a small gift for the baby will be given (a different book according to different ages).

5.9 Measurements

All participants will receive an intervention divided in several phases:

1) Recruitment and randomization: 19-23 weeks
2) First nutritionist interview: 21-23 weeks
3) Final assessment, almost at the end of interventions: 34-36 weeks
4) Final nutritionist interview: 36-37 weeks
5) A random subgroup of women (30% of each arm) will receive a fit-bit at the beginning and at the end of the study, will do a maternal and fetal MRI at 37 weeks' gestation, and delivery samples will be used for epigenetic assessments.
6) Delivery
7) Postnatal follow-up

All patients will be part of each phase including the following interventions:

1. ***Recruitment and randomization: 19-23 weeks*** *(FC, CP)*
   a) Screening for depression and psychosis (PHQ-9) before enrollment
   b) Signature of informed consent form
   c) Collection of socio-demographic data
   d) Feto-placental ultrasonographic assessment (EFW, Doppler)
   e) Maternal bio-sampling collection (blood, urine, hair)
   f) Maternal lifestyle (nutrition/sleep/stress) assessment will be given
   g) Fit-bit assessment will be given randomly to a 30% of participants from each group

h) Randomization

i) Scheduled for: nutritionist (for all women), trained mindfulness nurse (for mindfulness intervention)

2. ***First nutritionist interview: 21-23 weeks***
   a) Collection of maternal lifestyle assessment (questionnaires)
   b) Collection of fit-bit from those participants who had it
   c) Interview for the assessment of FFQ, 17-items scale and physical activity
   d) Only for nutrition arms: schedule for next appointments

3. ***Final assessment: 34-36 weeks***
   a) Feto-placental ultrasonographic assessment (EFW, Doppler, Ecocardiography, Neurosonography).
   b) Maternal bio-sampling collection (blood, urine, hair)
   c) Maternal lifestyle (nutrition/sleep/stress) assessment (the same of the beginning) will be given
   d) Fit-bit assessment will be given randomly to a 30% of participants from each group (the same of the beginning)
   e) Scheduled for: nutritionist (for all women), MRI (for 30% of women randomly from each group)

4. ***Final nutritionist interview: 36-37 weeks***
   a) Collection of maternal lifestyle assessment (questionnaires)
   b) Collection of fit-bit from those participants who had it
   c) Interview for the assessment of FFQ, 17-items scale and physical activity

5. ***MRI: 30% of women from each group, randomly identified at the beginning: 37-38 weeks***
   a) Maternal MRI (structural, functional, spectroscopy)
   b) Fetal MRI (structural, spectroscopy)
   c) Placental MRI (spectroscopy, perfusion)

6. ***Delivery***
   a) Maternal bio-sampling (urine, blood)
   b) Fetal bio-sampling (amniotic fluid, cord blood)
   c) Placenta bio-sampling and tissue samples
   d) Placenta pathology
   e) Anthropometric evaluation of the neonate
   f) Obstetrical outcomes

7. ***Postnatal follow-up:***
   During every postnatal follow-up a bio-sampling of the mother and the newborn (hair) will be taken.
   a) Neonatal morbidity and mortality
   b) 1 month: Brazelton tests (NBAS)
   c) 1 year:
      - Ages and Stages Questionnaires (ASQ): general questionnaire

- Communication and Symbolic Behavior Scales Developmental Profile Infant/Toddler Checklist (CSBS DP): autism screening
- Infant Behavior Questionnaire (IBQ)
- Nutrition assessment

d) 2 years:
- Bayley III test
- Child Behavior Checklist (CBCL): temperament questionnaire
- Arterial BP and echocardiography measurements (carotid intima-media thickness)
- Nutrition assessment

e) 3.5 years:
- Bayley III test
- Nutrition assessment

f) 5 years:
- CBCL
- Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition (WPPSI-IV)
- Arterial BP and echocardiography measurements (aortico intima-media thickness, aIMT)
- Nutrition assessment

5.9.1 Maternal lifestyle assessment

At the moment of recruitment, participants will receive a pack containing one of each of the questionnaires below (Appendix 4-13). The same questionnaires will be given during the final visit. All questionnaires are self-reported, a part from some diet quality assessments that will be assessed by the nutritionist itself.

Anxiety and stress: The *Perceived Stress Scale* (PSS) and the *State-trait Anxiety inventory* (STAI) are the best currently available instruments to evaluate the presence of anxiety and depression during pregnancy (Nast 2013) (Appendix 4 and 5). Patients will be evaluated using the Spanish validated version of the STAI (Garcia-Esteve 2013). Also *Post-Traumatic Stress Disorder* will be evaluated by the questionnaire PCL-5, Spanish version (PTSD CheckList for DSM-5) (Appendix 6). In the post-partum period (at the Brazelton test for the newborn), the *Edindburgh Postnatal Depression Scale* (EPDS) will be given to the mother (Appendix 11).

Quality of life and psychological assessment: The *Life Orientation Test* (LOT), in its Spanish version, evaluates the optimism view of life (Ferrando-Tous 2002) (Appendix 7).The *Mindful Attention Awareness Scale* (MAAS) evaluates the individual predisposition and capacity to pay attention in a particular way. This scale is validated in Spanish (Soler et al. 2012) (Appendix 9). We will also assess psychosocial aspects of the pregnancy linked with the mother-baby attachment using the *Cuestionario de Evaluación Prenatal* (Armengol 2007) (Appendix 10).

Sleep quality: The *Pittsburgh Sleep Quality Index* (PSQI) is based on eighteen self-reported questions about the person own sleep quality (Appendix 8). The scale evaluate seven rated components, including, sleep subjective, quality, duration, disturbances, and latency, habitual sleep efficiency, use of sleeping medication, as well as daytime function. The score from each category is added to achieve a global score that range from 0 – 21. A cutoff score of 5 or above is indicative of a sleep disturbance. This scale has been recently validated in the obstetric population (Okun 2011, Mindell 2015), in which an abnormal result was associated with an increase risk of preterm birth (Okun 2011), gestational diabetes (Bisson 2014), and abnormal labor progression (Naghi 2011).

Diet quality: A questionnaire about physical activity is given to any woman (Appendix 12) with also 7 days diary of their own diet (Appendix 13).

Diet quality (assessed by the nutritionist): FFQ are the preferred dietary assessment method in most epidemiological studies mainly due to their low cost and easy administration, and they have been validated in many different populations. We plan to use a semi-quantitative FFQ of 146 food items to assess the usual daily intake of foods and nutrients (PREDIMED plus and Appendix 14). In addition, the 17-items scale (Appendix 15) and a questionnaire about physical activity will be registered for each woman (Appendix 16).

5.9.2 Feto-placental ultrasonographic examination

Fetal biometry: includes evaluation of fetal growth using the Hadlock formula based in a composite sonographic measurement of fetal head (BPD, HC), abdominal circumference (AC) and femur (F) (Hadlock). Fetal measurements will be performed following previously published techniques.

Fetoplacental hemodynamics: Umbilical artery (UA) pulsatility index (PI) will be performed from a free-floating cord loop. Normal UA will be considered as a PI below the 95th percentile (Arduini 1990). Doppler PI for UA and mean PI from both uterine arteries (UtA) will be performed according to previously reported techniques (Vergani 2002, Severi 2002, Gosh 2009, Cruz-Martinez 2014). The Aortic isthmus (AoI) PI will be measured either in a sagittal view of the fetal thorax with clear visualization of the aortic arch, placing the gate a few millimetres beyond the origin of the left subclavian artery; or in a cross sectional view of the fetal thorax, at level of the three vessel and traquea view, placing the gate just the converge of the AoI and the arterial duct (Fouron 2004, Del Rio 2006, Rizzo 2008). Prenatal Doppler ultrasound examinations will be performed in the absence of fetal movements. Pulse Doppler parameters will be performed automatically from three or more consecutive waveforms, with the angle of insonation as close to zero as possible.

Cerebral flow evaluation: The middle cerebral artery (MCA) PI will be obtained in a transversal view of the fetal head, at the level of its origin from the circle of Willis (Eixarch 2008, Cruz-Martinez 2011, Hershkovitz 2000, Crimmins 2014). Three consecutive high-quality images with no artefacts will be recorded using previously reported parameters (Bashat 2003, Cruz-Martinez 2011). The cerebroplacental ratio (CPR) will be calculated as a ratio of MCA PI to UA PI (Arbeille 1987, Wladimiroff 1987, Gramellini 1992). The MCA PI and CPR values below the 5th percentile will be considered indicative of cerebral blood flow redistribution (Arbeille 1987, Wladimiroff 1987, Gramellini 1992, Basjat 2003). Cerebral blood perfusion will be evaluated by *fractional moving blood volume* (FMBV) using power Doppler ultrasound. The cerebral blood perfusion will be evaluated in the frontal area, basal ganglia and posterior brain. Five consecutive high-quality images with no artefacts will be

recorded using fixed settings, as previously described (Cruz-Martinez 2010, Flood PORTO study) and all images will be examined offline and FMBV will be estimated and expressed as a percentage.

Fetal cardiovascular assessment: Fetal cardiovascular remodeling will be assessed by measuring cardio-thoracic ratio, ventricular sphericity and wall thickness, tricuspid and mitral anular plane systolic execution (TAPSE and MAPSE) and myocardial performance index (MPI). Cardiac area will delineate in end-diastole from a 4-chamber view and divided by thoracic area in order to calculate cardio-thoracic ratio (Awadh 2006). Left and right sphericity indices will calculate by dividing the end-diastolic base to apex ventricular length by the transverse ventricular diameter measured in 2D in an apical or basal 4-chamber view (Crispi 2010). Left, right and septal wall thicknesses will be measured from an apical or basal 4-chamber view at end-diastole. TAPSE and MAPSE will be calculated using M-mode real time from an apical or basal 4-chamber view, measuring the maximum displacement of the valvular rings between end systole and end-diastole (Gardiner 2006). The MPI will be measured in a cross sectional view of the fetal thorax, in an apical projection and at the level of the four-chamber view of the heart (Hernandez-Andrage 2012). Briefly, the Doppler volume sample will be placed to include both the lateral wall of the ascending aorta and the mitral valve where the click corresponding to the opening and closing of the two valves can be clearly visualized. The isovolumetric contraction time (ICT), ejection time (ET), and isovolumetric relaxation time (IRT) will be calculated using the beginning of the mitral and aortic valves clicks as landmarks and the MPI will be calculated as follow: (ICT+IRT)/ET.

Fetal neurological assessment:

Systematic evaluation of cortical development has been described by Alonso *et al.* (Alonso) and Pistorius *et al.* (Pistorius), allowing quantification of brain sulcation in normal fetuses by ultrasound examination. Following this evidence, Egaña *et al.* (Egaña AJOG) applied the same methodology to MRI technique in a population of FGR and controls.

A detailed **neurosonography (NSG)** will be performed at the moment of enrolment in the study population by one expert examinator (FC, CP or EE) using a two-dimensional transabdominal and transvaginal approach. Fetal brain exam will be perform and standardize based on the ISUOG guidelines for fetal brain assessment (ISUOG 2007, Youssef UOG 2013), which include the following parameters:

- ✓ Axial planes:
  - *Transventricular plane*: lateral ventricle, parieto-occipital fissure, central sulcus, frontal and parietal area
  - *Transthalamic plane*: Biparietal diameter (BPD), head circumference (HC), Sylvian fissure, superior temporal fissure, frontal area, temporal area
  - *Transcerebellar plane*: Transverse cerebellar diameter, cerebellum, cisterna magna
- ✓ Sagittal planes:
  - *Midsagittal plane*: Corpus callosum, vermis
  - *Parasagittal plane*: Central sulcus, frontal area, parietal area
- ✓ Coronal planes:
  - *Transcaudate plane*: Anterior horns, cinculate sulcus, mesial area
  - *Transcerebellar plane*: Calcarine sulcus, occipital area

Neurosonographic images will be obtained prospectively and later will be analyze offline. Imaging post-processing and measurements will be performed using the semiautomatic software by expert examiners blinded to group randomization.

5.10 Magnetic Resonance Imaging

The final MRI protocol will be adjusted so that it does not exceed 1.30h, including fetal brain, adult brain and placenta. This should include patient repositioning, between abdominal MRI (fetal brain and placenta) and head MRI (mother's brain). The decision of which one is the first will be depending of fetal movements and position.

5.10.1 Fetal neurodevelopmental examination by MRI

Estimation time: 30-45'

Fetal MRI will be performed at 37 weeks of gestation in all study population, in a clinical MR system operating at 3 Tesla, using a body array radio-frequency (RF) coil. This will include several sequences for the evaluation of fetal brain (micro-) structures and metabolic profile by MRS. First, single-shot fast spin-echo T2 weighted sequences (repetition time, TR 2010ms, echo time, TE, 137ms, slice thickness 3.5mm, field of view (FOV) 260 mm, voxel 1.3X1.3X2.6, acquisition time 46 sec) in three orthogonal planes oriented along the axis of the fetal brainstem obtaining 4-loops of transverse, 2-loops of coronal and 2-loops of sagittal single shoot slices with 1.3mm slice separation will be obtained. Then, diffusion-weighted images (DWI) sequence (TR 3400ms, TE 94ms, FOV 250, voxel size 2X2X5mm, b values 0s/mm^2 and 1000s/mm^2 on the three orthogonal planes) will be obtained in the axial plane.
3D reconstruction of fetal brain will be performed following previously described methodology (Jiang 2007). Using fetal MRI data we are going to perform several measurements:

- ✓ Structural MRI:
  - *2D measurements* including grading and measurements of the cortical development (Egaña AJOG)
  - *Reconstruction of the 3D volumes* by a semiautomatic process for the automatically measure of:
    - Brain tissue volume
    - Cortical gray matter
    - White matter volume
    - Cortical surface sulcation
    - Cortical thickness

- ✓ Spectroscopy MRI:

  Additionally, we will perform localized single-voxel ^{1}H-MR spectroscopy of fetal brain during the same MRI session/exam. MRS data acquire will be acquired from the frontal lobe based on T2-w reference images and with *Point Resolved Spectroscopy* (PRESS) localization, essentially as previously reported (Sanz-Cortes et al. FDT 2014): 2 x 2 x 4 cm voxel size, TR 2000 ms, TE 144 ms, 98 transients, and partial water suppression with a *Chemical Shift Selective module* (CHESS); a reference spectrum will also be acquired with 16 transients and

no water suppression. To improve the performance of the technique at 3T, we will implement the dynamic acquisition mode previously reported at 1.5 T (Simoes et al. AJOG letter 2015). Additional, we will test the feasibility of (i) using short echo-time (TE = 30 ms) for the frontal lobe detection of relevant metabolites with coupled spins, such as the glutamate-glutamine pool (Sanz-Cortes et al. FDT 2015), and (ii) spectral editing for the detection of glutamate and GABA (MEGA-PRESS sequence: O'Gorman et al. JMRI 2011, Mullins et al. Neuroimage 2014). The MRS data obtained will be processed by linear fitting in the frequency domain (LC Model) based on metabolite basis-sets available, essentially as reported before (Sanz-Cortes et al. FDT 2014); each metabolite will be quantified based on a reference water spectrum, as well as using metabolite ratios. Depending of these results obtained, we will also consider pattern-recognition analysis based on the entire spectral vectors, to identify potential additional features of interest for classification (Simoes et al. PlosOne 2015).

5.10.2 Placental examination by MRI

Besides the fetal brain, we will also image the placenta during the same MRI session. Specifically, we will acquire reference T2-weighted and DWI over a larger field of view that includes all the entire placenta, DWI will be acquired with 10 b-values (0, 10, 20, 40, 60, 150, 300, 500, 750, 1000 s/mm^2) to fit an *Intra-Voxel Incoherent Motion* (IVIM) model and determine several biophysical parameters of the placenta, including the diffusion coefficient, perfusion fraction, and the pseudo-diffusion coefficient (Barbieri et al. Radiology 2016, Siauve et al. AJOG 2015). Additionally, we will acquire MRS data form a region closest to the umbilical insertion, as reported by others (Denison et al. PlosOne 2012, Macnaught et al. NMR Biomed 2014), using the PRESS protocol described before (2 x 2 x 4 cm voxel size, TR 2000 ms, echo time TE 144 ms, 98 transients, and CHESS partial water suppression; and a reference spectrum with 16 transients, no water suppression), and processed by linear fitting (LC Model).

5.10.3 Maternal neurological examination by MRI

Estimation time: 30'

In the context of fetal MRI, we are also planning to do an MRI for maternal brain. Maternal MRI will be performed at 37 weeks of gestation in all study population, in a clinical MR system operating at 3 Tesla, with Quantum gradients (30 mT/m) and an 8-channel neurovascular head coil. This will include several sequences on fetal for the evaluation of maternal brain (micro-) structures and metabolic profile by MRS.
With the data of MRI of maternal brain we are going to perform several measurements:

- ✓ Structural brain analysis: A structural T1-weighted magnetization prepared rapid gradient echo sequence will be used with: repetition time = 7.1 ms, echo time = 3.45 ms, intensity time = 1000 ms, 128 sagittal partitions, 1.33-mm slice thickness, square field of view of 256 mm, and acquisition duration of 8.5 min. The FreeSurfer 5.1.0 software package (Harved University, Boston, Massachusetts) will be used to create a 3-dimensional model of the cortical surface for measurement of cerebral and cerebellar gray and white matter volume, cortical thickness, and surface area.

- ✓ Resting state Functional brain analysis: Resting functional MRI scans will be lasted 6 minutes during which participants will be instructed to "keep your eyes close and think of nothing in particular." Resting scans consisted of 67 interleaved oblique, 2 mm thick axial slices, covering the entire brain (repetition time = 6.0 s, echo time = 30 ms, flip angle = 90°, FOV 128 x 128, resulting in 2 mm isotropic voxels). *Prospective acquisition correction* (PACE) will be used to mitigate artifacts due to head motion.

- ✓ Spectroscopy MRI:
  Localized brain 1H-MRS will additionally be performed on the mothers, in this case using a head matrix RF coil. This will be carried out in the same 3T MR system and during the same exam, based on the protocol reported for 1-year-old infants (Simoes et al. AJOG 2015). Since the adult brain is well myelinated, high resolution T1-weighted anatomical images will be acquired instead of T2-weighted, as we did before for the infant brain (Simoes et al. AJOG 2015): *Magnetization Prepared Rapid Acquisition Gradient Echo* (MPRAGE) sequence, TR 2050 ms; TE 2.41 ms; inversion time 1050 ms; 192 sagittal slices with 0.9 mm thickness, without interslice gap; in-plane acquisition matrix (MTX) 256×256; FOV 22×22 cm; voxel size, 0.86×0.86×0.9 mm. Then, proton spectra (^{1}H-MRS) will be obtained from the frontal lobe region using single voxel PRESS: voxel size, 4 x 2 x 2 cm, TR 2000 ms, TE 30 ms; 98 transients 98, and partial water suppression with the CHESS module. A reference spectrum will be additionally acquired, with 16 transients and no water suppression. In this case, we will also acquire the data dynamically (Simoes et al. AJOG letter 2015) and additional acquire MEGA-PRESS to quantify glutamate and GABA. The data generated will be processed for individual metabolite quantification, by linear fitting with LC Model (Simoes et al. AJOG 2015), and we will also consider pattern-recognition analysis based on the entire spectral vectors, to identify potential additional features of interest for classification (Simoes et al. PlosOne 2015).

5.11 Biological samples

5.11.1 Sample collection and storage

Maternal blood and urine: Blood (30mL) will be drawn from an indwelling cannula in the brachial vein and kept in serum and EDTA treated tubes. Serum, plasma and buffy coat will be separated by centrifugation at 3000 rpm for 10 minutes at 4°C and stored immediately at -80°C. Besides urine (10mL) will be collected and store at -80°. These collections of urine and blood samples are carried at the time of enrolment and final assesment.

Cord blood: 30mL of blood will be obtained from the umbilical vein after cord clamping at delivery and kept in serum and EDTA treated tubes. All samples will be processed within 1 hour. Serum, plasma and buffy coat will be separated by centrifugation at 3000 rpm for 10 minutes at 4°C and stored immediately at -80°C.

Amniotic fluid: 5-10 mL of amniotic fluid will be retrieved during delivery when is possible and stored immediately at -80°C.

Maternal hair: A lock of hair approximately 3 mm thick will be cut from the vertex posterior region of the head as close to the scalp as possible and will be stored.

Placenta tissue samples: Upon delivery, placentas will be transported to the laboratory immediately after delivery, measured, weighed and sampled according to protocols previously described (includes a minimum of four sections of the placental disc, two sections of the umbilical cord, and two membrane rolls). Tissue sections will be formalin-fixed and paraffin-embedded, followed by H&E staining. Samples will thoroughly rinse in saline (NaCl 0.9%) to remove maternal blood. Besides, one sections will be obtained to be stored at -80°C until DNA a protein extraction and other was collected in RNA later and stored at -80ºC for RNA extraction. Experienced placental pathologists blinded to the clinical diagnosis will evaluate all placentas to ascertain the presence of placental lesions.

Biological samples storage
Samples of plasma, serum, urine, and hair, will be collected in Clinic Hospital and HSJDD from all patients at the moment of enrollment and final assessment. The total blood volume for banking of plasma, serum and DNA will not exceed 40 mL and will be collected according to the procedures outlined above. Cord blood, amniotic fluid and placental samples will be derived from one-time collection. Samples will be processed after collection and stored in -80°C freezers at Hospital Clinic-IDIBAPS and HSJDD biobank. All samples for specimen banking are stored in coded tubes without any other attached information that would allow identification of the individual from whom the sample is collected. All specimens are stored in secured, monitored and alarmed freezers at -80°C. Before inclusion in the clinical trial, each patient will sign two written consent. One of them regarding to research project and other related to future uses of the bio-specimens collected. Participants could voluntarily retire whenever they decide without any repercussion.

5.11.2 Biological analysis

5.11.2.1 Hormonal profiling

Maternal blood, urine and hair will be collected twice: after the enrollment visit and at the final visit according to a common standard operating procedure and sent to the laboratory for measurement.
Single measurement will be performed for adiponectin, leptin, estriol, copeptin, and other hormones arising significant from preliminary results of the Phenomapping study (see specific protocol), using fully automated ELISA systems according to the manufacture instructions.
Additionally, the cortisol axis will be evaluated in several sampling (maternal hair and fetal amniotic fluid) collaborating with IMIM (Hospital del Mar Medical Research Institute).

5.11.2.2 Nutrimetabolomics in maternal and cord blood

Cord blood will be obtained immediately after delivery. Maternal venous blood will be also collected at the final visit for each mother-neonate pair. Samples will be collected in 10-mL EDTA tubes. Plasma will be separated immediately by centrifugation (5000X g, 10 min, 4°C) and stored a -80°C. Nuclear magnetic resonance (NMR)-based metabolite profiling is a quantitative nondestructive, noninvasive, non-equilibrium perturbing technique that provides detailed information on solution-state molecular structures, based on atom centered nuclear interactions and properties (Beckonert 2007), it will be

performed according to previously describe technique (Sanz-Cortes PLOS1 2013). Metabolomics will be carried out at The Andalusian Centre for Nanomedicine and Biotechnology (BIONAND).
Additionally, we are planning to measure at the Biomedical Diagnostic Center (CDB) several vitamins both in maternal and fetal blood: vitamin A, vitamin B with the most important isoforms, vitamin D (25-hydroxyvitamin D) and vitamin E. We will also measure hemoglobin, mean corpuscolate volume, iron stores (serum ferritin), serum iron, transferrin and folates.
Adherence to nutritional intervention will be assessed by an improvement adherence to the MD, and biomarkers of compliance will be evaluated, including urinary hydrohytyrosol levels (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid levels (to confirm compliance of mixed nuts) by the Faculty of Pharmacy of the University of Barcelona.

5.11.2.3 Placenta pathology

Histopahological examinations of the placenta will be carried out on all for purposes of disease verification. An experienced and qualified pathologist will perform placental histological findings, which is essential for addressing all specific aims of the protocol. Initial evaluation of placenta histology is made under the light microscope in haematoxylin and eosin stained sections. Placental analyses will be performed at Hospital Clinic-IDIBAPS.

Placental patterns of placental maldevelopment and injury: Placental tissue sample collected according to our protocol. Samples will be used for studying the placental vascular assessment and the placental inflammatory profile by Redline classification (Redline 2015), immunohistochemical analysis and Western blotting.

Placental ageing: Samples collected in the same way will be used for analyzing apoptosis, autophagy and senescence. Apoptosis will be measured through expression of different proteins involved in the p53 pathway as Caspase, p21, p53 or Bax by polymerase chain reaction (PCR). Senescence will be studied by determining the activity of telomerase, the telomere length and the cell senescence markers (p21, p16, SIRTs) by PCR. Autophagy will be determined thought inmunohistochemical analysis and Western blotting.

Maternal-fetal nutrient transport: amino acids, glucose and lipid transport will be studied through immunohistochemical analysis, PCR and Western blotting. Placental samples are going to be immunostained with antibodies to SLC38A1 to detect System A transporter, SLC2A1 for glucose transporter, ABCA1 for cholesterol transporter and FABP1 for fatty acids transporter.

5.11.2.4 Epigenetics

Methylation profiling: cord blood and placenta samples will be used for studying the methylation profiling using Methilome analysis. It will be provide quantitative methylation measurement of 850,000 methylation sites per sample at the single-CPG-site level. Cord blood DNA will be extracted from *peripheral blood mononuclear cells* (PBMC) that were obtained from buffy coat. Placental DNA will be extracted from the samples using the DNA commercial Kit. After that, DNA was bisulfite modified, amplified, fragmented and hybridized to perform the Methilome analysis.

Bisulfite pyrosequencing DNA methylation analysis: We will use the gold-standard pyrosequencing technology, an accurate and quantitative sequencing assay, to determine base-specific cytosine methylation levels at different loci within the CpG island of gene promoters. Pyrosequencing assays combine sodium bisulfite DNA conversion chemistry, PCR, amplification, and sequencing by synthesis assay of the target sequence. Single analysis will be performed in placental or cord blood samples for different genes resulted significant at the first general analysis, and for several genes already known to be important, such as noerpinephrine transporter (NET), 11B-HSD2, glucocorticois receptor (NR3C1), serotonin transporter (SLC6A4), cholesterol transporter (ABCA1), glucose transporter (GLUT3), IGF-2 and leptin (LEP).

Epigenetic changes will be evaluated in several sampling (cord blood and placenta) collaborating with CNAG (National Centre of Genomic Analysis).

5.12 Neonatal and infant postnatal follow-up

Anthropometric measurements: BW and head circumference will be extracted from maternity hospital records. At 1, 12 and 24 months of age, weight and height as well as head circumference will be measured at planned follow-up visits. Obesity will be defined as BMI above 90th percentile. Weight catch-up will be defined when changes in the Z score of the child weight compared to the Z score at birth are higher than 0.67 (Skilton 2013).

Neurobehavioral assessment:

1. *The Neonatal Behavioral Assessment Scale (NBAS)* will be prospectively evaluated in all newborns at 40 weeks (+/- 1) corrected age by observers accredited by The Brazelton Institute (Harvard Medical School, Boston, USA). The observers will be blinded to the study group. The examination consists of six behavioral areas rated on a 1 to 9 scale where nine is the best performance for some areas and five for others (Nugent 2000). In order to compare the evaluations of the infants' behavior, the items will be grouped into six clusters as follows: habituation (range 1-6), social anime organization (range 1-5), organization of the state, regulation of the state, autonomous nervous system, and motor area. The behavioral items will be converted in percentiles according to normal curves references for our population (Costas Moragas 2007), and each area will be considered abnormal at a score below 5th percentile.

2. *Ages & Stages Questionnaires, 3rd Edition (ASQ-3),* will be evaluated at a corrected age of 12 months. It's the most widely used developmental screener across the globe, designed for the use by early educators and health care professionals. It relies on parents as experts and it evaluates the progress in children between the ages of one month to 5 ½ years. It takes just 10–15 minutes for parents to complete and 2–3 minutes for professionals to score.

3. *The Communication and Symbolic Behavior Scales Developmental Profile Infant/Toddler Checklist (CSBS DP)* will be evaluated at a corrected age of 12 months. It's the first step in routine screening to see if a developmental evaluation is needed. It has been demonstrated that is a screening tool at children's 1-year to identify those in need of further evaluation for autism and other developmental delays (Pierce, Detecting, Studying, and Treating Autism Early: The One-Year Well-Baby Check-Up Approach, J Pediatrics 2011). In our study we will use this test at a corrected age of 12 months.

4. *The Infant Behavior Questionnaire (IBQ)* will be evaluated at a corrected age of 12 months. It assesses 6 domains of infant temperament (activity level, social ability, fear, distress to limitations, smiling and laughter, and duration of orienting). The items on the IBQ ask parents to rate the frequency of specific temperament-related behaviors observed over the past week (or sometimes 2 weeks).

5. The *Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III)*, which is a revision of the Bayley Scales of Infant and Toddler Development, 2nd Edition (Bayley III) will be evaluated twice, at a corrected age of 24 months and then at 3.5 years. The Bayley-III is an individually administered instrument that assesses infant development across five domains, including cognitive, language and motor competencies. Parent reported questionnaires are incorporated into the Bayley-III to assess social-emotional and adaptive behaviors.

6. *The Child Behavior Checklist (CBCL)* will be evaluated at a corrected age of 24 months and then at 5 years. It is a widely the most used method of identifying problem behavior in children (Achenbach, T.M., & Rescorla, L.A. 2000). There are two versions of the checklist for caregivers, depending on the age of the youth. The preschool checklist (CBCL/1½-5) is intended for use with children aged 18 months to 5 years and it contains 100 problem behavior questions. There are two "broad band" scales that combine several of the syndrome scales: *Internalizing* problems sums the Anxious/depressed, Withdrawn-depressed, and Somatic complaints scores; *externalizing* problems combines Rule-breaking and Aggressive behavior. There also is a total problems score. The standard scores are scaled so that 50 is average for the youth's age and gender, with a standard deviation of 10 points. Higher scores indicate greater problems. For each syndrome, Internalizing and Externalizing problem scales, and the total score, scores can be interpreted as falling in the normal, borderline, or clinical behavior.

7. The Wechsler Preschool and Primary Scale of Intelligence – 4th Edition (WPPSI-IV) is an innovative measure of cognitive development for preschoolers and young children, testing measures intellectual abilities in young children.

Cardiovascular assessment:

1. *Blood Pressure:* systolic and diastolic BP will be obtained at two years of age by a trained physician from the brachial artery using a validated ambulatory automated Omron 5 Series device, while the infant is resting. Blood pressure percentiles were calculated according to published reference values (Pediatrics 2011;128 Suppl 5:S213-56).

2. *Aortic intima media thickness (aIMT)*: at two years of age it will be measured using Vivid Q (General Electric Healthcare, Horten, Norway), with a 12L-RS linear-array 6.0-13.0 MHz transducer. Infants will be studied when resting quietly. aIMT measurement involves obtaining longitudinal clips of the far wall of the proximal abdominal aorta in the upper abdomen76, 77. aIMT measurements will be performed offline according to a standardized protocol based on a trace method with the assistance of a commercially available software (GE EchoPAC PC 108.1.x, General Electric Healthcare). To obtain aIMT, three end-diastolic frames will be selected across a length of 10 mm and analyzed for mean and maximum aIMT, and the average reading from these three frames will be calculated.

3. Infant e*cocardiography*: Cardiovascular child evaluation will be performed at two years of age using Vivid Q (General Electric Healthcare, Horten, Norway). Children will be studied when resting quietly. A

complete two-dimensional M-mode and Doppler echocardiographic examination, with a 10S-RS phased-array 4.5-11.5 MHz transducer, will be performed to assess structural heart integrity and morphometry.

- *Cardiovascular morphometric parameters*: left atrial area, left sphericity index and wall thicknesses.

- *Systolic function parameters*: stroke volumes, heart rate, cardiac output, shortening fraction, ejection fraction, mitral and tricuspid annular plane systolic excursion (MAPSE, TAPSE) and systolic annular peak velocities (S').

- *Diastolic function parameters*: IRT, peak early (A) and late (A) transvalvular filling velocities, E/A ratio, E deceleration time, A wave duration time, early-diastolic (E') and atrial contraction (A') annular peak velocities, E/E' ratio, E'/A' ratio, isovolumetric relaxation time by TDI (IRT').

5.13 Co-variables

Epidemiological data: Parental demographics, both parents education and professional status, maternal age, ethnicity, pre-gestational body mass index (BMI), smoking status, and socioeconomic level.

Maternal and obstetrical history: past medical history (chronic hypertension, diabetes, renal disease, autoimmune disease, coagulation disorders, etc.), previous preeclampsia, FGR, or fetal death, therapies before or during pregnancy.

Actual pregnancy: Assisted reproductive technologies (ART), any treatment, any complication, blood analysis (Hemoglobin…), any practice of yoga o meditation, any diet or nutrition restriction done during pregnancy.

Maternal sleep quality: Pittsburgh sleep questionnaire.

Maternal stress exposure: The State-trait Anxiety inventory, the Perceived Stress Scale, the Post Traumatic Stress Disorder scale

Dietary patterns: Survey of dietary and toxic exposure (tobacco, drugs in urines).

Maternal Physical characteristics: blood pressure at the time of diagnosis, weight gain, BMI.

Maternal organ function: proteins in urine 24 hours (at enrollment and at 37 weeks), protein-to-creatinine ratio, hepatic enzymes, hemoglobin, and hematocrit at the time of enrollment and delivery.

Antenatal ultrasound findings: Estimated fetal weight and Doppler ultrasound measurements (UA PI, MCA PI, AoI, means PI of UtAs, and CPR).

Perinatal data: pregnancy complications (gestational hypertension, preeclampsia, eclampsia or HELLP syndrome), exposure to corticoids, perinatal death, gestational age at delivery, route of delivery, induction to labor, and emergency cesarean section.

Neonatal data: Gender, BW, weight percentile, Apgar score at 1 and 5 minutes, cord arterial and venous birth pH, base excess, pO_2, head circumference and percentile.

6 Statistical Analysis

We estimate that a sample of 1200 women would be recruited to provide statistical power of 80% to detect a relative risk reduction of BW <10th percentile of 30% in each intervention group *versus* the control group with usual care. Interim analysis will be conducted after half of patients involved.
The characteristics will be summarized for each treatment group. Missing data will be analyzed and if missing at random, multiple imputations to treat data.
The primary analysis will be based on the intention-to-treat population, which is defined as all randomized participants regardless of compliance with the protocol. A secondary analysis will be performed according to effective treatment. One interim analysis of the primary outcome data will be conducted and the P value will be adjusted by O'Brien-Fleming (p= 0.005); this analysis will be performed once the half of the sample size is obtained. Single and multiple models will be tested. Logistic regression for qualitative and linear regression for quantitative outcome (BAyleys).

6.1 Data analysis

Data from this study will be analyzed at two intervals: one interim analysis and the final analysis.
Early termination of the study should be considered only if the intervention effect is really great after the first interim analysis (>50% of reduction of BW<10th percentile in the three interventions groups).

7 Management

7.1 Data management and quality control of data

The participant has to agree on the handling of her personal information and their newborn within the informed consent form. Principal investigators will monitor data safety and confidentiality of patient information at all times. Subjects participating in the study will not be identified by name in any publically available written or oral reports. Paper records and case report forms will be maintained in locked cabinets, rooms, or in computer files protected through the use of computer passwords. Only authorized personnel will have access to this data. Human specimens are stored under codes in locked freezers and laboratories. Only PIs and listed co-investigators can make the linkage between coded specimens and human data.

Quality assurance and quality control measurements: To reduce the "learning curve effect", several pilot scans will be performed, to standardize the technique and image views and strict scanning protocols will be established. A single high-level, high-resolution machine will be used to collect all the ultrasound and Doppler measurements. Ultrasound data collection will be monitored by a random selection of ultrasound scans by the supervisor of the study (FCr).

Monitoring: To assess the reliability of data included in the questionnaires and database, an external monitor will review medical records and material of the study of a random sample of the participants. Data of around 25% of the participants included will be evaluated.

7.2 Ethical aspects

Standards from 1947 Nuremberg Code and 1964 Helsinki Declaration will be considered. This study will be conducted according to globally accepted standards of GCP. Data will be used only for scientific proposes, including publications or scientific-academic meeting. Confidentiality and anonymity will be ensured. Name of participant will not appear in any cases. Cases will be invited to participate during clinical assistance in the recruitment centers and not eligible women will be invited to participate in the project by phone or public announcements. In the first contact, the patient will be fully informed about the purpose, methods, interventions and intended possible uses of the research, including the duration of clinical assessment. Telephone information of the investigator will be included in consent format. All samples will be stored at Hospital Clinic-IDIBAPS biobank. All these departments are part of Hospital Clinic in Barcelona, and participants could voluntarily retire whenever they decide without any repercussion. Research subjects will be informed about the results of each evaluation and surveys. As laboratory test takes more time for results, patients will be asked to send it by e-mail. In case of finding any abnormality, patients will be sent for medical evaluation at their own center of attention.

7.2.1 Patient information sheet and informed consent

Before inclusion in the clinical study, each patient must receive an explanation about the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, and any discomfort it may entail. Each subject will also receive a written explanation of the purposes, procedures, and potential hazards of the project (Appendix 17). Each patient must be informed that participation in the study is voluntary and that she may withdraw from the study at any

time. In case of acceptance, two written consent will be taken, written in non-technical language. One of them regarding to research project and other related to future uses of the bio-specimens collected. The informed consent forms are signed and dated by the participant and by the investigator.

7.2.3 Research Ethics Approval

Before initiation of the study, the protocol, all informed consents and additional material will be given to all researchers involved. The investigators named in the protocol have no financial or other competing interest that impacts their responsibilities towards the scientific value of the study.
The investigators agree to achieve and/or arrange for secure storage of study material and records for a minimum of 5 years after the close of the study.
All investigators involved in the study have to give to the PIs their curriculum vitae signed and the certificate of principles of GCP: these documents will be stored with study material.

Access to Data
During the study, access to the database and bio-bank samples will be limited to members of the study. All patient information will be both record in papers and later report in the database, in an anonymous form.

7.3 Publication policy

Findings of the study will be presented at conferences and published in several peer review journal.
The Chief Investigators, Prof Eduard Gratacós and Dr Fatima Crispi, will establish a writing group, which will include the investigators involved. For collaborations, the decision of publication policy will be taken by the Chief Investigator too.

8 Appendix

Appendix 1	Factores de riesgo basales que justifican control seriado (28±1, 32±1 y 37±1) del crecimiento fetal durante el tercer trimestre
Appendix 2	Cuestionario sobre la salud mental de la paciente (PHQ-9)
Appendix 3	Case Report Form
Appendix 4	Cuestionario sobre estrés percibido (Perceived Stress Scale, PSS)
Appendix 5	Cuestionario sobre ansiedad (State-Trait Anxiety Inventory, STAI)
Appendix 6	Cuestionario sobre estrés postraumático (Post-Traumatic Stress Disorder, PCL-5)
Appendix 7	Cuestionario sobre el optimismo (LOT)
Appendix 8	Cuestionario sobre el sueno (Pittsburg)
Appendix 9	Cuestionario sobre el estilo de vida (Mindful Attention Awareness Scale)
Appendix 10	Cuestionario sobre el autoevaluación prenatal
Appendix 11	Cuestionario sobre la depresión postpartum (Edinburgh)
Appendix 12	Cuestionario sobre la actividad física (Minnesota)
Appendix 13	Registro dietéticos de 7 días
Appendix 14	FFQ (Predimed Plus) + extra
Appendix 15	Cuestionario sobre la adherencia a la dieta Mediterranea (17 puntos)
Appendix 16	Cuestionario sobre la actividad física

IMPACT Barcelona

Improving Mothers for a better PrenAtal Care Trial

Intervencions per a la Millora PrenAtal del Creixement feTal a Barcelona

Chief Investigators: Eduard Gratacós
Fatima Crispi

Principal investigator: Francesca Crovetto

Date: 23rd October 2019, Version 2

Setting: BCNatal | Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Barcelona, Spain

Research line: Fetal cardiovascular programming

Table of Contents

1 Administrative information

This document provides information about an understanding of the background, rationale, objectives, and procedures for entering participants into the study, study population, interventions, methods, statistical analyses, ethical considerations, and administration of the study. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary.
The study will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), and the principles of Good Clinical Practice (GCP).

1.1 Structured study summary

Public title	IMPACT Bcn
Scientific title	IMPACT Barcelona: Improving Mothers for a better PrenAtal Care Trial
Contact for public queries	impactbcn@gmail.com
Contact for scientific queries	*Chief Investigators:* Prof Eduard Gratacós GRATACOS@clinic.cat Dr Fátima Crispi FCRISPI@clinic.cat *Principal investigator:* Dr Francesca Crovetto FCROVETTO@clinic.cat BCNatal - Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Barcelona, Spain
Setting of recruitment	Hospital Clinic and Hospital San Joan de Déu, Barcelona, Spain
Problem studied	Fetal growth restriction
Participants	Pregnant women at high risk to develop fetal growth restriction
Study type	Randomized Controlled Study 1:1:1 ratio, parallel, open blind
Interventions	1) Improvement of maternal nutritional status based on Mediterranean Diet 2) Reduction of maternal stress through Mindfulness Based Stress Reduction program
Sample size	1218 participants: 406 for each arm of randomization (No intervention, Nutritional Intervention, Mindfulness Intervention)
Inclusion and exclusion criteria	Inclusion: • Maternal age at recruitment ≥18 years • Viable singleton non-malformed fetus • Speak Spanish fluently • High risk pregnancies for fetal growth restriction • 19-23 weeks of gestation

	Exclusion: • Fetal anomalies including chromosomal abnormalities or structural malformations detected prenatally • Neonatal abnormalities diagnosed after birth • Maternal mental retardation or other mental or psychiatric disorders that impose doubts regarding the true patient´s willingness to participate in the study • No possibility to come to additional visits • Included in other RCT studies
Data of first enrolment	February 1st 2017
Study aims	1) To demonstrate that an improvement on maternal lifestyle, through intensive behavioral therapies based on a nutritional intervention with a Mediterranean diet or on stress reduction program with mindfulness techniques, have an impact in the reduction of the prevalence of fetal growth restriction in pregnancies at high risk to develop this condition. 2) To demonstrate that an improvement on maternal lifestyle and well-being, based on a nutritional intervention with a Mediterranean diet or on stress reduction program with mindfulness techniques, could reduce the prevalence of adverse perinatal outcome of pregnancies at high risk to develop fetal growth restriction. 3) To demonstrate that an interventional program to the mother have a positive impact on fetal programming, in terms of newborn neurodevelopment (at two years of age). 4) To demonstrate that an interventional program to the mother have a positive impact on fetal programming, in terms of cardiovascular profile later in life (at two years of age).

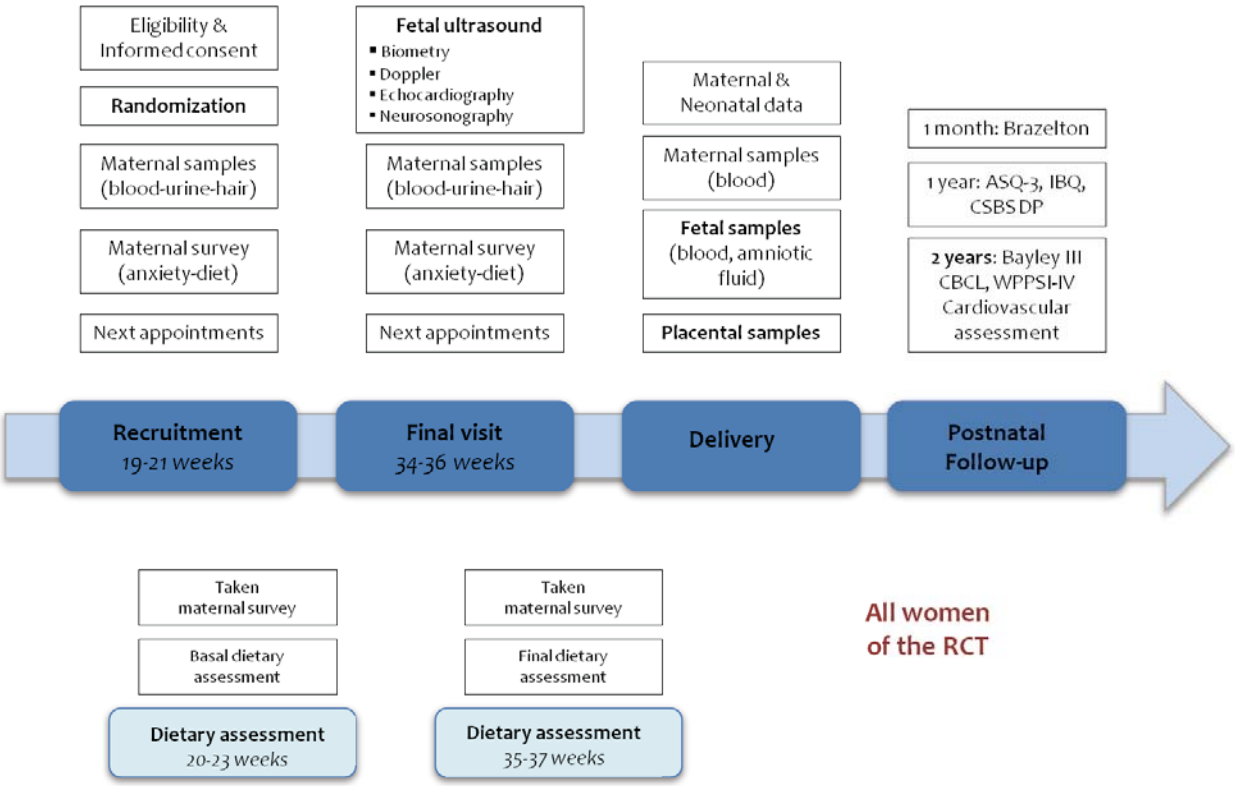
1.2 Roles and responsibilities

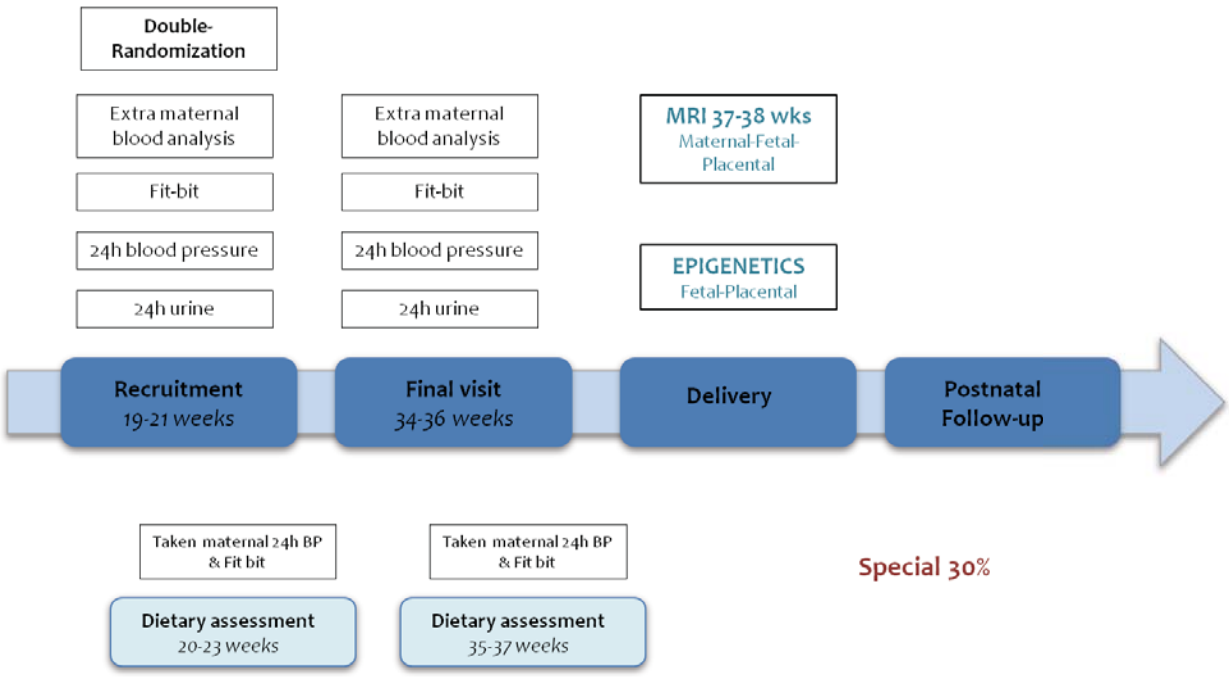
Name	Affiliation	Role
Prof Eduard Gratacos (EG)	BCNatal	Chief investigator, Director of Department of Obstetrics, Gynecology and Neonatology of Hospital Clinic Barcelona, Director of BCNatal Center
Dr Fatima Crispi (FC)	BCNatal	Co-Chief investigator, Scientific coordinator of BCNatal - Fetal Medicine Research Center
Dr Francesca Crovetto (FCr)	BCNatal	Principal investigator, specialist in Obstetrics and Fetal Medicine
Dr Cristina Paules (CP)	BCNatal	Assistant investigator, specialist in Obstetrics and Fetal Medicine
Estefania Callado (EC)	BCNatal	Project manager at BCNatal, project manager of the trial
Dr Maria Dolores Gomez (MDG)	BCNatal	Head of the Gynecology and Obstetrics Department at Hospital Sant Joan de Déu, BCNatal, co-supervisor of the recruitment at Hospital Sant Joan de Déu
Angela Arranz (AA)	BCNatal	Head of Nurses of the of Department of Obstetrics, Gynecology and Neonatology of Hospital Clinic Barcelona, responsible for nurses involved and postnatal follow-up
Dr Elisenda Eixarch (EE)	BCNatal	Coordinator of fetal neurology unit, specialist in Obstetrics and Fetal Medicine, responsible of the neuroimaging acquisition and analysis
Dr Rui Simoes (RS)	BCNatal	Postdoctoral researcher, Biochemist, responsible of the magnetic resonance spectroscopic analysis
Laura Segales (LS)	BCNatal	Researcher assistant, responsible of the recruitment
Marta Dacal (MD)	BCNatal	Researcher assistant, responsible of the recruitment and Mindfulness assistant
Miriam Osorio (MO)	BCNatal	Technician coordinator of the BCNatal Biobank
Cristina Miranda (CM)	BCNatal	Technician of the BCNatal Biobank
Roger Borras (RB)	IDIBAPS	Statistician, responsible of the analysis of the project
Gema Domenech (GM)	IDIBAPS	Statistician, responsible of the statistical analysis plan
Ferran Torres (FT)	IDIBAPS	Statistician, reviewer of the analysis of the project
Prof Ramon Estruch (RE)	IDIBAPS	Head of Research Group on Nutrition, Cardiovascular Disease and Aging, IDIBAPS/Hospital Clinic Barcelona,

		responsible of the Nutritional Intervention
Dr Rosas Casas (RC)	IDIBAPS	Biologist, co-responsible of the Nutritional Intervention
Dr Monica Domenech (MD)	IDIBAPS	Specialist in Internal Medicine, Hospital Clinic Barcelona Group on Nutrition, Cardiovascular Disease and Aging, IDIBAPS, involved in the Nutritional Intervention
Tania Freitas (TF)	IDIBAPS	Dietician involved in the Nutritional Intervention
Marina Sadurni (MS)	IDIBAPS	Dietician involved in the Nutritional Intervention
Carlos Galante (CG)	IDIBAPS	Dietician involved in the Nutritional Intervention
Rosa Mª Lamuela-Raventós (RML)	UB- School of Pharmacy	Associate Professor – Involved in biomarkers related to Nutritional Intervention
Anna Tresserra Rimabu (ATR)	UB- School of Pharmacy	Postdoctoral researcher– Involved in biomarkers related to Nutritional Intervention
José Fernando Rinaldi de Alvarenga (JFR)	UB- School of Pharmacy	PhD student– Involved in biomarkers related to Nutritional Intervention
Prof Eduard Vieta (EV)	H Clinic Bcn	Head of Psychiatric Service of Hospital Clinic Barcelona, supervisor of the Mindfulness Intervention
Anabel Martínez-Aràn (AM)	H Clinic Bcn	Psychologist of the Psychiatric Service of Hospital Clinic Barcelona, experts in psychotherapies, co-supervisor of the Mindfulness Intervention
Ivette Morilla (IM)	H Clinic Bcn	Psychologist of the Psychiatric Service of Hospital Clinic Barcelona, experts in psychotherapies, co-supervisor of the Mindfulness Intervention
Andrés Martín-Asuero (AM)	esMindfulness	Psychologist and founder of esMindfulness Institute in Barcelona, Instructor of MBSR certified by the University of Massachusetts, responsible of the Mindfulness Intervention
Maria Teresa Oller Guzmán (TO)	esMindfulness	Instructor of MBSR certified by esMindfulness Institute, co-resposible of the Mindfulness intervention
Amaia Helguera Antolinez (AH)	esMindfulness	Instructor of MBSR certified by esMindfulness Institute
Georgina Badosa (GB)	esMindfulness	Instructor of MBSR certified by esMindfulness Institute
Elvira Reche (ER)	esMindfulness	Instructor of MBSR certified by esMindfulness Institute
Dr Oscar Pozo (OP)	IMIM	Senior Researcher at Bioanalysis and Analytical Services Research Group of IMIM-Hospital del Mar Research Institute,

		responsible for the cortisol axis analysis of biological samples
Dr Rodriguez Jose	IMIM	Researcher at Bioanalysis and Analytical Services Research Group of IMIM-Hospital del Mar Research Institute, co-responsible for the cortisol axis analysis of biological samples
Dr Pujades Mitona	IMIM	Researcher at Bioanalysis and Analytical Services Research Group of IMIM-Hospital del Mar Research Institute, co-responsible for the cortisol axis analysis of biological samples
Simon Heath (SH)	CNAG	Team leader of the Statistical Genomics team at Centre Nacional d'Anàlisi Genòmica, co-responsible of the epigenomic analysis.
Ivo Gut (IG)	CNAG	Director at Centre Nacional d'Anàlisi Genòmica, co-responsible of the epigenomic analysis.
Mari Carmen Collado	IATA-CSIC	Senior researcher at Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC), responsible of the microbiome analysis

1.3 Study diagram




Double-Randomization
Extra maternal blood analysis
Fit-bit
24h blood pressure
24h urine
Extra maternal blood analysis
Fit-bit
24h blood pressure
24h urine
MRI 37-38 wks
Maternal-Fetal-Placental
EPIGENETICS
Fetal-Placental
Recruitment
19-21 weeks
Final visit
34-36 weeks
Delivery
Postnatal Follow-up
Taken maternal 24h BP & Fit bit
Dietary assessment
20-23 weeks
Taken maternal 24h BP & Fit bit
Dietary assessment
35-37 weeks
Special 30%

2 Abbreviations

AC	Abdominal Circumference
MTX	Acquisition Matrix
APO	Adverse Perinatal Outcome
ASQ	Ages and Stages Questionnaires
GABA	γ-Aminobutryric Acid
aIMT	Aortic Intima Media Thickness
AoI	Aortic Isthmus
ART	Assisted Reproductive Technologies
ADHD	Attention Deficit Hyperactivity Disorder
CDB	Biomedical Diagnostic Center
BPD	Biparietal Diameter
BW	Birth Weight
BP	Blood Pressure
BMI	Body Mass Index
cIMT	Carotid Intima-Media Thickness
CPR	Cerebroplacental Ratio
CHESS	Chemical Shift Selective module
CBC	Child Behavior Checklist
ABCA1	Cholesterol Transporter
CSBS DP	Communication and Symbolic Behavior Scales Developmental Profile Infant/Toddler Checklist
DMC	Data Monitoring Committee
DMN	Default Mode Network
DTI	Diffusion Tensor Imaging
DWI	Diffusion-weighted Images
DHA	Docosahexaenoic Acid
TE	Echo Time
EPDS	Edindburgh Postnatal Depression Scale
ET	Ejection Time
EFW	Estimated Fetal Weight
FL	Femur Length
FGR	Fetal Growth Restriction
FOV	Field Of View

FFQ	Food Frequency Questionnaires
FMBV	Fractional Moving Blood Volume
fMRI	Functional Magnetic Resonance Imaging
NR3C1	Gene of the Glucocorticoid Receptor
GCP	Good Clinical Practice
GLUT3	Glucose Transporter
GABA	Glutamate and γ-Aminobutryric Acid
HC	Head Circumference
IMIM	Hospital del Mar Medical Research Institute
11β-HSD2	11β-Hydroxysteroid Dehydrogenase Type 2
HPA	Hypothalamic Pituitary Adrenocortical
IBQ	Infant Behavior Questionnaire
INMA	Infancia y Medio Ambiente
IGF2	Insulin-Like Growth Factor 2
IUGR	Intrauterine Growth Restriction
IVIM	Intra-Voxel Incoherent Motion
ICT	Isovolumetric Contraction Time
IRT	Isovolumetric Relaxation Time
iSAP	Interim Statistical Analysis Plan
LEP	Leptin
LTL	Leukocyte Telomere Length
LOT	Life Orientation Test
LC-PUFA	Long-Chain Polyunsaturated Fatty Acid
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MD	Mediterranean Diet
MCA	Middle Cerebral Artery
MAAS	Mindful Attention Awareness Scale
MBSR	Mindfulness-Based Stress Reduction
MAPSE	Mitral Anular Plane Systolic Execution
RHEA	Mother-Child Cohort
MPI	Myocardial Performance Index

NAA	N-Acetyl-Aspartate
CNAG	National Centre of Genomic Analysis
NBAS	Neonatal Behavioral Assessment Scale
NSG	Neurosonography
NET	Noerpinephrine Transporter
NMR	Nuclear Magnetic Resonance
OR	Odds Ratio
PSS	Perceived Stress Scale
PBMC	Peripheral Blood Mononuclear Cells
PSQI	Pittsburgh Sleep Quality Index
PRESS	Point Resolved Spectroscopy
PCR	Polymerase Chain Reaction
PCL-5	Post-Traumatic Stress Disorder
PE	Preeclampsia
PI	Principal Investigator
PACE	Prospective Acquisition Correction
PI	Pulsatility Index
SAP	Statistical Analysis Plan
SC	Steering Committee
SLC6A4	Serotonin Transporter
SGA	Small for Gestational Age
SD	Standard Deviation
STAI	State-Trait Anxiety inventory
RF	Radio-Frequency
RCT	Randomized Controlled Trial
TR	Repetition Time
RCOG	Royal College of Obstetrics & Gynaecologists
TAPSE	Tricuspid Anular Plane Systolic Execution
UA	Umbilical Artery
UtA	Uterine Arteries
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition

3 Introduction

3.1 Two main protagonists

3.1.1 The fetus

In the past, the only person considered to be involved in pregnancy was the woman. Forty years ago, the fetus virtually did not exist in medicine. All obstetric medicine was focused on the mother and which pregnancy complications could occur to her. In late 70s, the introduction of ultrasound and the possibility to visualize the fetus in *uterus* brought about a true revolution in two aspects. For doctors, it allowed to diagnose in fetal life problems that until then had been known only in the newborn. For parents, it facilitated the recognition of the fetus as a person[1].

The combination of these two factors resulted in the development of a new concept, the fetus as a patient, and with it, the beginning of a subspecialty that we know today as fetal medicine. Fetal medicine is today a reality, and we can offer parents accurate assessment for many fetal problems. However, there are still many aspects to be clarified. Even if the fetus is now considered a patient, we have to avoid the tendency to think of the fetus as separate from the pregnant woman[2], obscuring the physical and psychological relationship between the pregnant woman and the fetus, the ways that maternal and fetal physiologies and welfare are linked, and perhaps most problematically, the woman herself with her background and her psychological status[1].

3.1.2 The mother

The physical care of pregnant women in the developed world has hugely improved over the past 100 years; however, the same has not been true of their emotional care[3]. Pregnancy is a complex and dynamic condition. Maternal psychological state changes produce a cascade of reactions, including changes in blood flow to the uterus as well as alterations to the intrauterine sensory environment experienced by the fetus. Given the intricate physiological relationship between the pregnant women and the fetus, it would be somewhat surprising if dynamic aspects of the maternal psychological environment did not serve to shape neurodevelopment of the fetus and ultimately that of the child[4]. However, because there are no direct neural connections between the pregnant woman and the fetus, the fetus requires transduction of a maternal physiological signal from a psychological state to experience it.

There is growing support for a central role of the prenatal period in the health and development of offspring throughout childhood and adult life. Considerable evidence from many prospective studies show that if the mother is depressed, anxious or stressed while she is pregnant, her child is more likely to experience a range of adverse neurodevelopmental outcomes such as an increased risk of behavioral, emotional and cognitive problems that do the children of other mothers[3,5]. Although genetics and postnatal care clearly affect these outcomes, evidence for an additional prenatal causal component is substantial.

3.1.3 A unique bond

The relationship between the fetus and her future mother is unique: the fetus as a patient depends completely and entirely from her; he is physiologically enmeshed with another patient, his

"environment" is the body of an autonomous agent. On the other side, the mother is completely involved by the presence of the fetus. The maternal bond, that is the relationship between the mother and her child, typically starts during pregnancy, and both physical and emotional factors influence the mother-baby bonding process.

Central to obstetrical practice are efforts to prevent, diagnose and treat conditions that can affect the mother and the future baby before birth. However, we still consider the mother and the fetus as two different and separate entities, and the majority of therapies are for preventing/monitoring women complications of pregnancy or, on the other side, for preventing/monitoring fetus diseases.
Nevertheless, we should start considering the unique relationship they have and start thinking that probably any effort to improve the maternal status could also improve the fetal condition. It seems very easy, but it is still one of the most neglected aspects of modern obstetrics.

3.2 Fetal Growth Restriction

Fetal growth restriction (FGR) is defined as the failure to achieve the endorsed growth potential and it affects 7-10% of all pregnancies[6,7]. Fetuses that are not fulfilling their growth potential have a 5- to 10-fold risk of dying in uterus[8,9]. Moreover, growth restricted fetuses have a higher risk of perinatal morbidity and mortality[10,11] and are also at higher risk of long-term disabilities and cognitive impairment[12,13,14,15].
Different terms have been used in this field of research: small-for-gestational-age (SGA) and intrauterine growth restriction (IUGR) for example, have often been used interchangeably, but not all small babies are growth restricted[16]. Although the concept of abnormal fetal growth is basic to the modern practice of perinatal medicine, the threshold below which the fetus or newborn is considered growth restricted varies between studies. Pediatricians define SGA as a statistical grouping of infants whose birth weight (BW) and/or length is at least two standard deviations below the mean for gestational age, which is approximately the 3rd percentile[16]. In obstetric practice, SGA is normally defined as an estimated fetal weight (EFW) below the 10th percentile[17], and IUGR when it shows signs of restriction (for example Doppler abnormalities or very severe restriction). Since these fetuses have a higher risk of mortality and morbidity, it is fundamental to use not only an obstetrical definition, but also fetal charts to identify this population[18]. For the purpose of this project, we decided to use the term of FGR, for which we considered those fetuses born with a BW below the 10th percentile, in which some could have shown signs of restriction since the fetal life and some may not.

3.2.1 Fetal Programming

The question of the importance of prenatal environmental factors for development, behavior and health, has been scientifically studied from the 1940s onwards in humans. The seminal studies from David Barker have led to the concept of "development plasticity", describing a critical window during fetal development when the organs are sensitive to nutritional, hormonal and metabolic environment[19]. This is "*fetal programming*", that is when the environment in uterus during specific critical periods for different outcomes can affect the development of the fetus and the child in the long-term[20,21]. In the last years our research group gave a significant contribution in this field of research for growth restricted fetuses: these fetuses are at higher risk of adverse long-term consequences such as abnormal neural reorganization[22,23,24,25,26] and metabolic changes in the brain[27,28], as well as poor

neurological[12,23,29,30,] cardiovascular[31,32,] metabolic and endocrinological[33] outcomes. Thus, the antenatal identification of a growth restricted fetus is essential not only to reduce adverse perinatal outcome (APO)[34], but also for the potential window of opportunity to reduce the consequences of an adverse intrauterine environment.
With physical outcomes, the phenomenon of fetal programming is well established. Fetal programming, however, seems to be equally important for the development of psychopathology[35,36]; according to De Pietro *et al.*, early postnatal temperamental characteristics emerge during the prenatal period[37].

3.3 Origin of FGR

Several causes have been described to explain the origin of FGR including abnormal placenta development, tobacco use, maternal malnutrition, antiphospholipid syndrome, teratogen exposure, infections, and genetic/structural disorders. Our research group has contributed in the last years over the potential causes of FGR, particularly concerning placental insufficiency[38,39,40,41,42,43]. However, our knowledge of the spectrum of FGR continues to evolve and the hypothesis that FGR is a multi-phenotypic disease is the main objective of a project we have just conducted in our research center (*Phenomapping of Fetal Growth Restriction*[44]).

3.3.1 Nutrition in FGR

Nutrition in pregnancy plays an important role in the wellness of the mother and the fetus, and may further influence the health of the child later in life[45,46].
Dietary energy and nutrient requirements are generally increased to support increased maternal metabolism, blood volume and red cell mass expansion, and the delivery of nutrients to the fetus[47]. However, in a recent systematic review and meta-analysis of 90 dietary studies among pregnant women in developed countries (n=126,242), when compared to dietary recommendations in the specific countries, the intake of energy and fiber was generally lower, total fat and saturated fat higher, and carbohydrate intake was borderline or lower than recommendations[48]. Moreover, micronutrients intake during pregnancy, including folate, iron, zinc, calcium, vitamin D and essential fatty acid, was less than optimal[49].
Interventional studies with an understanding of optimal dietary patterns may provide promising results from both maternal and perinatal health. In this context it has been reported that a low adherence to the Mediterranean diet (MD) was related with lower BW[50]. The INMA (*Infancia y Medio Ambiente*) study in Spain and the RHEA (*Mother-Child Cohort*) study in Greece, have evaluated the adherence to the MD and fetal growth[51]. In the INMA cohort, a stronger adherence to the MD was associated with a low rate of FGR[52]. Although a huge amount of observational study, few Randomized Controlled Trial (RCT) studies were conducted to evaluate the effect of diet during pregnancy and their outcomes. One study from Norway randomized 290 pregnant women at 20 weeks' gestation to either continue their usual diet or to adopt a diet that promoted fish, low-fat meats and dairy products such as oil, grains, fruits, vegetables and legumes. The authors observed a modification in maternal lipid levels but not in cord blood or neonatal lipids, and a reduction of preterm delivery in those who were randomized for diet intervention[53].
Based on preliminary data from our cohort FGR fetuses (*Phenomapping of Fetal Growth Restriction*), the nutrition patterns of the mothers were often suboptimal, with lower consumption levels of

vegetable oil, fruit, milk and fish, and an increased consumption of salt (Figure 1A). We have also observed that growth-restricted fetus showed signs of malnutrition, with decrease in ferritin and zinc in the cord blood (Figure 1B).

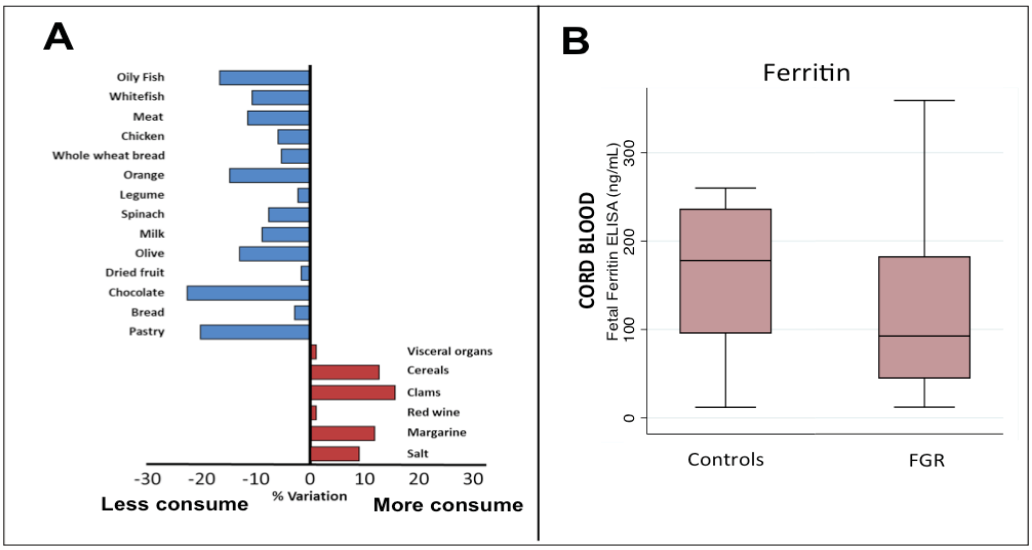


Figure 1A. *Dietary patterns in FGR relative to controls*

Figure 1B. *Levels of ferritin in the cord blood of FGR and controls*

In parallel, data from our experimental rabbit model of FGR indicated that dietary supplementation with long-chain polyunsaturated fatty acid (LC-PUFA), such as the docosahexaenoic acid (DHA), an omega-3 essential fatty acid, and lactoferrin, can improve BW, mortality and neurodevelopment of the offspring (Figure 2)[54].

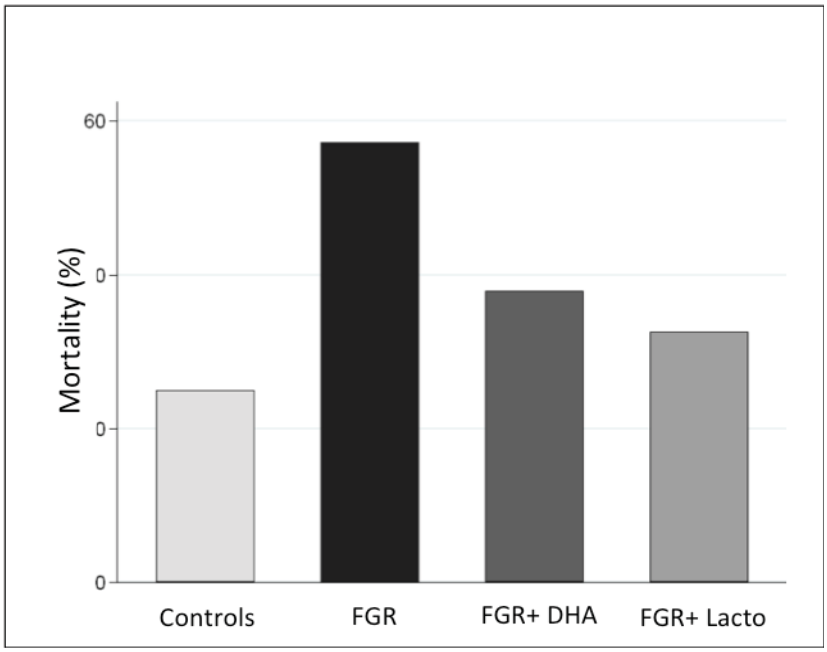


Figure 2. *Mortality in an animal model of FGR with different supplementation (DHA or Lactoferrin)*

3.3.2 Nutrition and fetal programming

Fetal neurodevelopment

Optimal fetal neurodevelopment is also dependent on specific nutrients from dietary sources, including DHA of which seafood is a major source. Fish is a rich source of nutrients such as LC-PUFA, protein, selenium, iodine, and vitamin D, which are considered to be beneficial for fetal growth and development[55] but, in contrast, fish is also a well-known route of exposure to pollutants such as dioxins, polychlorinated biphenyls, methylmercury, and other heavy metals, which may adversely affect fetal growth and gestational length[56,57]. Findings from prospective birth cohort studies on the relation between fish intake during pregnancy and fetal growth have been discrepant, with reports of either positive or null[58,59] or negative effects[57,60]. A recent meta-analysis among 19 European birth cohort studies with more than 150,000 women reported that fish's consumption, particularly for the blue fish, reduces the risk of preterm labor and increases BW[61]. Similarly, although in USA women are advised to limit the seafood intake during pregnancy up to 340 g per week to avoid fetal exposure to trace amounts of neurotoxins, a study based on a large cohort (11,875 pregnant women, ALSPAC cohort) reported that maternal seafood intake during pregnancy of less than 340 g per week was associated with increased risk of their children being in the lowest quartile for verbal intelligence quotient, suboptimum outcomes for social behavior, fine motor, communication and social development scores; on the contrary, a consumption of more than 340 of seafood per week was beneficial for the child's neurodevelopment[62].

Cardiovascular programming

FGR fetuses present cardiovascular remodeling already in uterus that persist into childhood[31,32,63]. Similarly, children who suffered FGR have signs of vascular dysfunction, including increased blood pressure (BP) and carotid intima-media thickness (cIMT)[31,64] which are well-known cardiovascular risk factors.

Nutrition has a strong effect on cardiovascular development during childhood[65]. Therefore, the effects of intrauterine environment on cardiovascular remodeling should be influenced by prenatal and postnatal factors. High dietary intake of LC-PUFA for example has been associated with lower cIMT values in children with FGR[66]. In a recent study from our group we have demonstrated that postnatal nutrition, based on breastfeeding and healthy-fat dietary, improves cardiovascular remodeling induced by FGR[67].

3.3.3 Stress in FGR

Another possible mechanism that may underlie FGR and consequently fetus programming is the increased exposure of the fetus to cortisol. Although glucocorticoids are essential for fetal development and tissue maturation, an overexposure can have negative effects and could occur for an increased maternal cortisol that crosses the placenta. During pregnancy, the corticotropin-releasing hormone is released also from the placenta into both the maternal and fetal compartments, and it has a positive feedback resulting in increased levels of cortisol in maternal blood (2-4 times higher) and the effects of glucocorticoids on the fetus may be amplified. However, the effects of maternal cortisol on the fetus are modulated by the presence of a placental enzyme, called *11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2)*, which oxidizes the cortisol into an inactive form, cortisone[68]. In a sort of way this enzyme protects the fetus from maternal cortisol. However, if there is less of this

barrier enzyme then the fetus will be exposed to more maternal cortisol. Some evidence in rat models shows that prenatal stress can down-regulate placental 11β-HSD2[69]. Recent studies in humans also found direct evidence that maternal prenatal anxiety and depression are associated with a down-regulation of 11β-HSD2 gene expression [70,71]. McTernan and colleagues reported a 25% reduction in 11β-HSD2 expression in placentas from FGR pregnancies [72].

Our preliminary results of the cohort of FGR fetuses (*Phenomapping of Fetal Growth Restriction*) are in line with these previous findings[73]: we found that FGR, particularly milder cases, seemed to be caused by higher levels of perceived stress and anxiety of the mothers, resulting in higher levels of cortisol in the amniotic fluid, that is the urine of the fetus, as reported in the figure 3A. Additionally, the levels of the gene expression of 11β-HSD2 in the placentas were lower in FGR cases compared to controls (figure 3B).

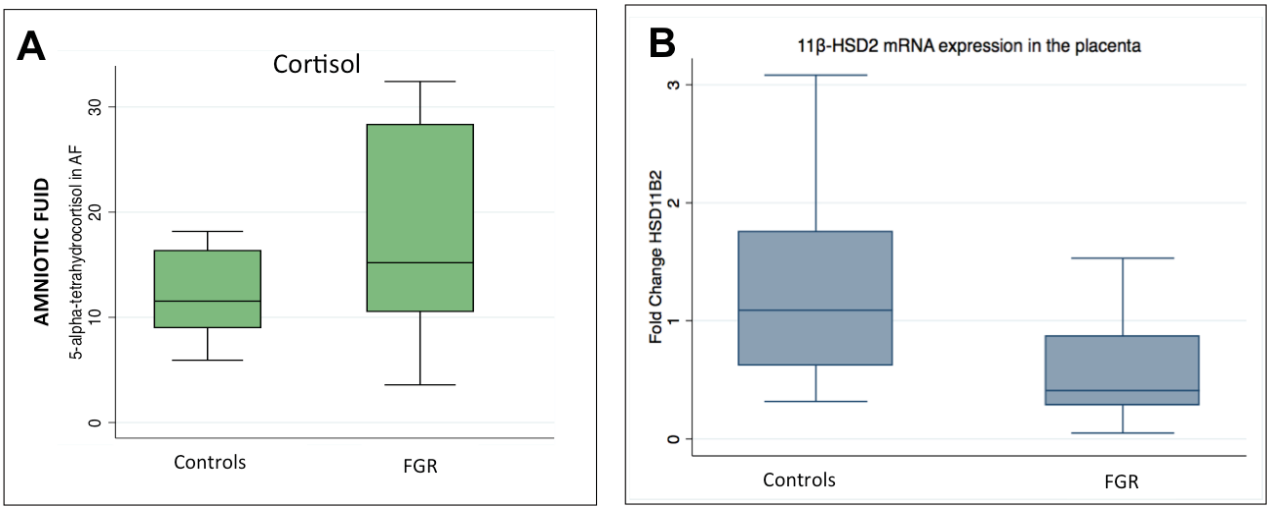


Figure 3A. *Expression of cortisol in the amniotic fluid of FGR and controls.*

Figure 3B. *Expression of* 11β-HSD2 gene in the placentas *of FGR and controls.*

3.3.4 Stress and fetal programming

A wide range of different outcomes has been shown to be associated with prenatal stress in studies examining children from birth until adulthood. Stress has been associated with lower BW, prematurity [74,75,76] and with a significantly smaller head circumference even if corrected by BW[77]. In the last few decades, several studies on prenatal stress have looked at neurodevelopmental and psychopathological outcomes. Newborns from mothers who reported stress during pregnancy showed poorer performance on the Neonatal Behavioral Assessment Scale (NBAS) than newborns of mothers who did not report stress symptoms[78]. Studies of toddlers and infants whose mothers had higher stress during pregnancy had a more difficult temperament[79], sleep problems[80], lower cognitive performance and increased fearfulness[81]. Evidence on children ages 3-16 years reported a link between maternal stress during pregnancy and an increased risk for child emotional problems such as anxiety, depression, symptoms of attention deficit hyperactivity disorder (ADHD) and conduct disorders[36,82,83,84,85].

The human fetal hypothalamic pituitary adrenocortical (HPA) axis is developed and functioning at 22 weeks of gestation, although its plasticity is maintained during the first two years of life[86,87]. Thus, maternal physical and psychological status during pregnancy has the potential to permanently alter the program of HPA axis functioning of their offspring. Human data in support of the role of glucocorticoids in mediating programming effects on neurodevelopment come from Finland, where the consumption of large amounts of licorice, that is an 11β-HSD2 inhibitor, is common. Maternal licorice consumption during pregnancy is associated with altered HPA axis activity in children, with those whose mothers consumed the most licorice during pregnancy having the highest circulating cortisol levels[88,89]. The mechanism through prenatal glucocorticoids overexposure leads to a wide variety of changes in tissue structure and function is probably linked with epigenetic.

3.4 Therapies for FGR

Several treatments have been proposed for FGR, as briefly summarized in table 1, but none of them has considered being successful.

Table 1. *Prenatal therapies for FGR (adapted from* Figueroa & Maulik 2006)

Bed rest
Maternal oxygen administration
Calcium channel blockers
Maternal nutritional interventions
Nutritional supplementation
Balanced energy/protein supplementation
High protein supplementation
Isocaloric protein supplementation
Salt restriction
Calcium supplementation
Folate supplementation
Iron supplementation
Magnesium supplementation
Zinc supplementation
Vitamin D supplementation
Fish oil supplementation
Plasma volume expansion
Nitric oxide donors

Bed rest

In the past, bed rest in hospital has been used in an attempt to improve many conditions in pregnancy, including FGR, with the idea to improve the utero-placental perfusion in attempt to improve fetal growth. This approach has largely been abandoned for no benefit and additionally for the onset of adverse events such as thromboembolism[91].

Maternal oxygen administration

Hypoxemia characterizes many instances of FGR; therefore, improving oxygen delivery to the fetus might improve FGR by improving transplacental nutrient transport. Nicolaides *et al.,* gave humidified oxygen continuously through a face mask to 5 women with FGR and showed that fetal hypoxemia could be corrected with maternal oxygen therapy[92]. A systematic review assessing the effect of maternal oxygen administration on perinatal outcomes in pregnancies affected by FGR found no significant effect on BW[93]. Continuous supplementation with oxygen is hard to achieve, and cessation can lead to abnormal fetal heart rate patterns.

Calcium channel blockers

Calcium channel blockers are a group of drugs that cause changes in smooth muscle and vascular contractility. They have been used in pregnancy as tocolytics. A systematic review of the use of calcium channel blockers in FGR identified one small study of 100 women where flunarizine seemed to increase BW, although this apparent effect has not been further evaluated in other larger studies[94].

Maternal nutritional interventions

Several different nutrients have been tried as treatment for FGR; overall the quality of the trials is variable and none of them gave a clear answer[95]. Table 2 gives more details about nutritional intervention[90]. Regarding all the other supplementations, more research is needed.

Table 2. *Nutritional intervention in FGR (adapted from* Figueroa & Maulik 2006)

Nutrition Intervention	Relative Risk
Nutritional supplementation with glucose	1.11 (0.64-1.92)
Nutritional supplementation with galactose	0.78 (0.39-1.54)
Nutritional advice	0.97 (0.97-2.11)
Balanced energy/protein supplementation	0.68 (0.56-0.84)
High protein supplementation	1.58 (1.03-2.41)
Isocaloric protein supplementation	1.35 (1.12-1.61)

Plasma volume expansion

During the course of a normal pregnancy there is almost a 50% increase in maternal intravascular volume. FGR has been associated with failure of the normal expansion of plasma volume in the mother. Plasma volume expansion, with hydroexyethylstarch or hemofusion, has been attempted in very few patients with FGR. However, there are no RCT in women with suspected FGR[96].

Nitric oxide donors

Nitric oxide donors, such as L-arginine, have been found to improve utero-placental blood flow because of their vasodilating effect. Some investigators have proposed the use of the nitric oxide test to identify pregnancies with FGR[97], but RCT are not available.

3.5 Prevention for FGR

As we have seen, none of the several treatments proposed for FGR is successful. Nowadays, the best thing we can do in prenatal life is to diagnose this condition and to manage it in order to reduce fetal and neonatal problems. However, our main goal as physicians is to treat conditions.
As a lot of complications during pregnancy are difficult to treat, in the last decades, fetal medicine research focused on the prevention of such conditions, instead of the treatment once they have been diagnosed. In particular, the main focus was on *primary prevention*, which is the prevention of disease in susceptible individuals or populations through health promotion and specific protection against certain risk factors. The key to prevention in any program is the recognition of those individuals who are at risk or are susceptible.
Regarding FGR, the Royal College of Obstetrics & Gynaecologists (RCOG) published in its guidelines for the investigation and management of FGR[98], a list of risk factors for FGR, as reported in table 3. Women are considered at risk if they have a major risk factor (odds ratio, OR >2.0) or at least three minor risk factors (OR ≤2.0).

Table 3. *Risk factors for FGR (adapted* Royal College of Obstetricians and Gynaecologist. 2013)

Risk category	**Risk factor**	**Odds Ratio (CI 95%)**
Age	Maternal age ≥35 years	1.4 (1.1-1.8)
	Maternal age >40 years	3.2 (1.9-5.4)
Parity	Nulliparity	1.89 (1.82-1.96)
Body Mass Index (BMI)	BMI <20	1.2 (1.1-1.3)
	BMI 25-29.9	1.2 (1.1-1.3)
	BMI ≥30	1.5 (1.3-1.7)
Maternal toxics	Smoker 1-10 cigarettes per day	1.54 (1.39-1.7)
	Smoker ≥11 cigarettes per day	2.21 (2.03-2.4)
	Cocaine	3.23 (2.43-4.3)
Assisted Reproductive Technologies (ART)	ART singleton pregnancy	1.6 (1.3-2.0)
Exercise	Daily vigorous exercise	3.3 (1.5-7.2)
Diet	Low fruit intake pre-pregnancy	1.9 (1.3-2.8)
Previous pregnancy history	Previous SGA baby	3.9 (2.14-7.12)
	Previous stillbirth	6.4 (0.78-52.56)
	Previous preeclampsia	1.31 (1.19-1.44)
	Pregnancy interval <6 months	1.26 (1.18-1.33)
	Pregnancy interval >60 months	1.29 (1.2-1.39)
Maternal medical history	Maternal SGA	2.64 (2.28-3.05)
	Chronic hypertension	2.5 (2.1-2.9)
	Diabetes with vascular disease	6 (1.5-12.3)

	Renal impairment	5.3 (2.8-10)
	Antiphospholipid syndrome	6.22 (2.43-16)
Paternal medical history	Paternal SGA	3.41 (1.17-10.27)
Current ongoing pregnancy	Heavy bleeding similar to menses	2.6 (1.2-5.6)
	Echogenic bowel	2.1 (1.5-2.9)
	Preeclampsia	2.26 (1.22-4.18)
	Mild gestational hypertension	1.3 (1.3-1.4)
	Severe gestational hypertension	2.5 (2.3-2.8)
	Low maternal weight gain	4.9 (1.9-12.6)
	PAPP-A <0.4 MoM	2.6

Therefore, the guidelines indicated that these women at risk to develop FGR should be referred for serial ultrasound measurements of fetal size and assessment of well-being from 26-28 weeks, but did not indicate any possible therapy they could do.

Based on our previously results of the cohort of FGR fetuses (*Phenomapping of FGR*) reported above, we identified two major characteristics that affect women whose fetuses do not grow appropriately during the prenatal period: malnutrition and stress. These two factors are related apparently with the mother, but their effects are seen also in the fetus, as shown our results on some micronutrients in the cord blood (under normal ranges) and on cortisol in the amniotic fluid (above normal range). Hence, we are proposing an ambitious RCT to evaluate the impact on fetal growth of two interventions that aim to improve maternal welfare (nutrition and stress reduction). Since we are dealing with pregnant women, we have focused on non-pharmacological interventions, based on changes in lifestyle.

3.5.1 Nutrition intervention: Mediterranean Diet

The traditional MD is the heritage of millennia of exchanges of people, cultures and foods of all countries around the Mediterranean basin. It is rich in plant food (cereals, fruit, vegetables, legumes, free nuts, seeds and olives), with olive oil as the principal source of added fat, with a high to moderate intakes of fish and seafood, moderate consumption of eggs, poultry and dairy products (cheese and yogurt), and low consumption of red meat. This healthy, tradition MD has been popularized since 1995 using the famous pyramid representation that graphically highlights the food groups to be consumed daily, weekly or less frequently (Figure 4). This pyramid is the result of an international consensus and it is based on the latest scientific evidences on nutrition and health published in several scientific articles in the recent decades[99]. Today it is recognized as centuries-old tradition that contributes to excellent health, provides a sense of pleasure and wellness and forms part of the world's collective cultural heritage. The diet, when consumed in sufficient amounts, provides the entire known essential micronutrients (vitamins and minerals), fiber and others. Olive oil is the principal source of fat and it contains a large proportion of monounsaturated fat, a relatively low proportion of saturated fat and it is also the source of the antioxidant vitamin E and other micronutrients.

Mediterranean diet with the supplementation of extra-virgin olive oil or nuts has been recently proposed among individuals at high cardiovascular risk, thanks to its efficacy to reduce by 30% the incidence of major cardiovascular events[100]. This was a great example of a behavioral intervention in a

population at high risk: the nutritional intervention was based on the idea to modify the food habits of people in a real-life context, without giving any additional drug supplementations[101].

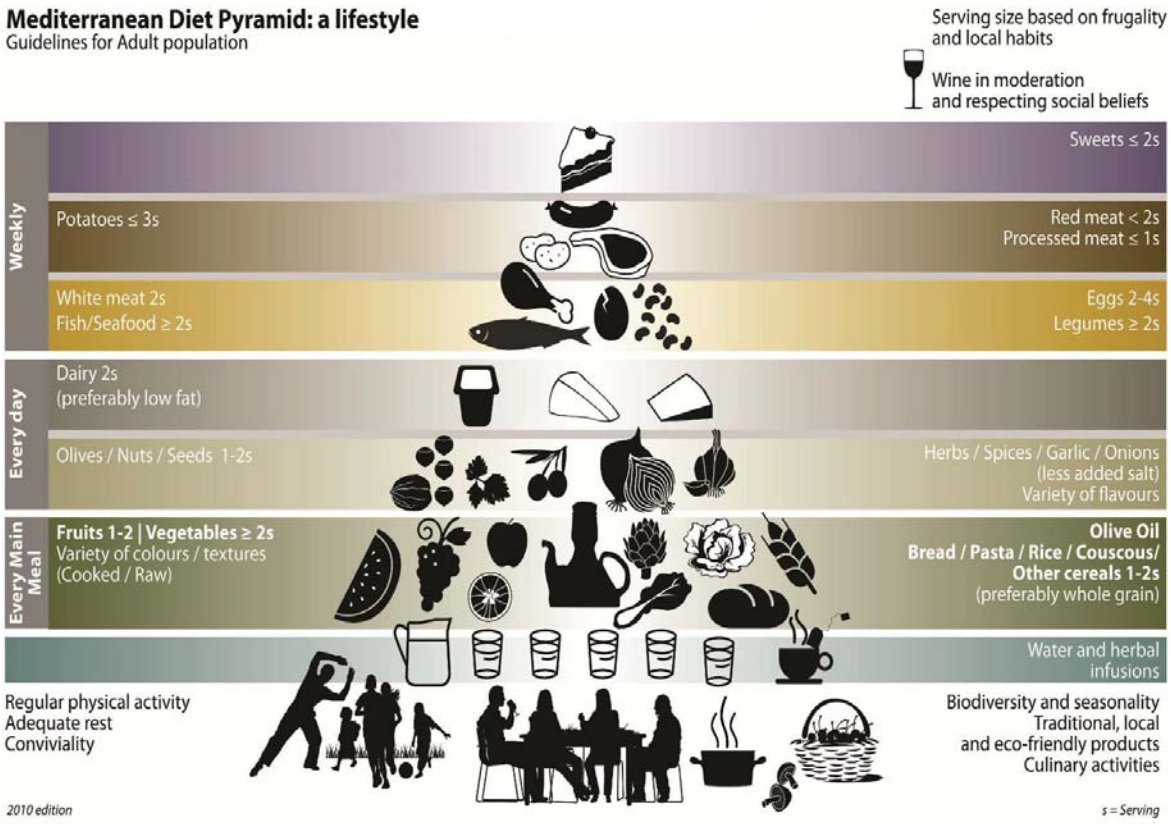


Figure 4. *Mediterranean diet pyramid*

3.5.2 Stress reduction intervention: Mindfulness Program

In recent years there has been increasing interest in the concept of mindfulness, which is a practice that has its roots in Buddhist meditation. Its definition coined by Jon Kabat-Zinn described mindfulness as *"paying attention in a particular way: on purpose, in the present moment, and non-judgmentally"*[102]. In other words, mindfulness corresponds to the higher-level awareness of present-moment sensory, affective, and cognitive experiences. Practice of mindfulness has entered the American mainstream in recent years, in part through the work of Jon Kabat-Zinn and his Mindfulness-Based Stress Reduction (MBSR) program, which he launched in 1979 at the University of Massachusetts Medical School[102,103]. Mindfulness-based interventions have been of increasing interest as a cost-effective, low-stigma, accessible treatment option for different psychological and medical symptoms, firstly with chronic pain, anxiety and depressive disorders[102,104,105] and then with other morbid conditions such as binge-eating in obesity[106], or reducing cardiovascular risk[107].

During pregnancy, mindfulness practice has been tested in RCT of 47 pregnant women who were recruited for elevated levels of stress during first or second trimester of pregnancy[108]; the authors reported an improvement of anxiety in those pregnant women who experienced the mindfulness program, but the effects were not sustained through follow-up at six weeks post-intervention. However, a limit of this study, aside from its small sample, was that in both groups women have been

conscious and motivated to reduce their high levels of prenatal stress and 30% of the control group took prenatal yoga classes during their pregnancies.
Another study also evaluated the effect of a mindfulness intervention during pregnancy on socio-emotional development and temperament in 10 months-old toddlers, reported that higher maternal mindfulness during pregnancy was associated with less infant self-regulation problems and less negative affectivity[101]. Finally, a pilot RCT in a tertiary maternity hospital in Australia, even if only with 32 patients in total, provided evidence on the feasibility of an antenatal mindfulness intervention to reduce psychological distress during pregnancy[109].
Mindfulness seems to have potential during pregnancy, considering this special period of woman's life. However, all previous studies considered as outcome only the reduction of maternal stress ignoring its potential benefits to the fetus.

3.6 Neuroimaging

Proper timing and guidance of neurogenesis, neuronal differentiation, apoptosis, synaptogenesis and myelination, are critical for the appropriate organization and functioning of the brain. These processes can be altered with environmental factors, such as viruses, tobacco, drugs, cortisol[110]. Neuroplasticity is the main characteristics of the brain, which is the organ built to change in response to environment and experience. The interaction between genes and environment/experience is fundamental for the development of cortical plasticity[111].

3.6.1 Neuroimaging in growth restricted fetuses

The brain is one of the most complex organs to rapidly develop and who presents a fastest rate of expression change during the fetal period[112]. Although early plasticity allows a proper adaptation to the environment that enables to survey, it might have negative effects later in life.
Neurodevelopmental impairment in FGR has been associated with specific neurostructural changes on magnetic resonance imaging (MRI) studies[113,114]. Several studies have demonstrated that growth restricted fetuses are associated with brain reorganization changes, showing alterations in brain microstructure and cortical development[23,24–26] as well as in brain metabolism[27,28,115]. Additionally, these changes were also seen in neonates[113] and one-year toddlers[116].
Going more in details, we have just demonstrated that FGR fetuses are characterized by a decrease in brain volume, even after adjusting for EFW percentile, a physiological brain asymmetry, a decreased gray matter volume, deeper measurements in some cortical sulci (insula fissure, left cingulate fissure)[24] and differences in insular cortical thickness[25] and in cerebellar and brain stem morphometries, that are associated with a suboptimal neonatal neurobehavior[28]. Both insula and cingulate are regions that play an important role in the limbic system[117], which is responsible for interoceptive awareness and higher cognitive functions; these areas are particularly vulnerable to sustained undernutrition and/or hypoxia.
Magnetic resonance spectroscopy (MRS) revealed differences in metabolite profiles of growth restricted fetuses. The most prominent example is decreased N-acetyl-aspartate (NAA, considered a surrogate marker of neuronal density and/or viability) to choline-compounds ratio, which in turn has been associated with corpus callosum development in these fetuses[28]. Interestingly, differences in

MRS have also been reported in FGR newborns at one-year of age[116]. We have also shown functional MRI alterations in toddlers that were restricted during fetal life, revealing an hyper-connected but sub-optimally organized functional brain networks[118], and also altered connectivity in motor and cortico-striatal-thalamic networks[119].

3.6.2 Neuroimaging and Mindfulness

While mindfulness-based interventions are increasingly applied in the therapeutic context[120], the investigation of neurobiology underlying the beneficial effects is still in its infancy[121,122,123,124]. The most promising studies were conducted on patients with anxiety disorders, generally associated with abnormalities in prefrontal activation, and altered relationship between activity of prefrontal regions and amygdala[125]. For example, lower activation of the ventrolateral prefrontal cortex and higher activation of the amygdala have been reported in patients with generalized anxiety disorders[126]. The ventrolateral prefrontal cortex is involved in inhibitory control[127] and its activation typically increases in people who can down-regulate their emotions[128–131]; in addition, it also modulates amygdala responses during emotion regulation processes[131]. In a study with functional MRI (fMRI) based on tasking evoking, Creswell *et al.* found that higher trait mindfulness were related with a greater activation of prefrontal areas, including ventrolateral and medial regions of the cortex, lower amygdala activation, and greater amygdala-prefrontal connectivity[132], suggesting a potential neural mechanism of mindfulness training. More recently, in a RCT of 26 patients affected by generalized anxiety disorders, in half of them who attended a MBSR program, the fMRI after 8 weeks of intervention revealed a decrease activation of the amygdala and grater activation in the ventrolateral prefrontal regions, suggesting that mindfulness practice leads to changes in fronto-limbic areas crucial for the regulation of emotion, and these changes corresponded also with reported symptoms improvements[122]. Therefore, there is increasing evidence that mindfulness training is associated with enhance activation and connectivity between several brain regions that are known to be crucial to successful emotion regulation, both for healthy and anxiety disorder populations, such as increased brain activation in sensory areas (insula, thalamus, anterior cingulate cortex and secondary somatosensory cortex) and decreased activation in an area that mediates executive control (the lateral prefrontal cortex) or in emotion-related areas (amygdala, hippocampus)[125].
A recent study on resting state in fMRI[133] investigated the association between mindfulness and functional connectivity of intrinsic brain activity among three central neurocognitive networks: the connections between Default Mode Network (DMN) and silence network, and between silence and central executive networks, were significantly associated with mindfulness scores. This might be one possible pathway for beneficial effects of mindfulness therapy.
Regarding MRS, a recent study reported different changes in some metabolites, such as an increasing myo-inositol in the posterior cingulate cortex and a decreasing glutamate and NAA in the left thalamus in a group of meditators compared to controls[134].

3.7 Epigenetics

Epigenetic is defined as the study of changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic processes, including DNA methylation, histone modification and non-coding RNAs, play a central role in regulating this

gene expression. Besides, these marks, though potentially reversible, are stable and could be long lasting.
There is growing evidence that the epigenome is particularly susceptible to a number of environmental factors, such as maternal diet and behavior during prenatal and early postnatal life, known as "environmental programming". They are key players in the development underling many of the processes of fetal programming, in addition to genetic factors and leading to long-term phenotypic alterations. Thus, improvement in maternal behavior and diet during pregnancy should be considered interventions that could impact the epigenome and consequently mitigate long-term phenotypic alterations[135].

3.7.1 Epigenetics and maternal behavior

Variations in maternal stress and behavior have been extensively correlated with stable alterations of DNA methylation and chromatine structure[136,137].
Maternal depression/anxiety in the third trimester was found to be associated with increased methylation of the gene which encoded the glucocorticoid receptor (NR3C1) in the cord blood of neonates and with increased salivary cortisol response to a visual new stimulus at 3 months of age[138]. The same methylation of the NR3C1 gene has been reported in children (10-19 years) whose mothers experienced intimate partner violence during pregnancy[139]. Besides, different animal studies reported that offspring of mothers that showed high levels of pup licking and grooming were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the glucocorticoid receptor promoter. These findings provide evidence that maternal care influences hippocampal glucocorticoid receptor expression and thus the HPA functions in the offspring[140,141,142,143].
Epigenetic variation in the placenta is emerging as a candidate mediator of environmental influence on placental functioning and a key regulator of pregnancy outcome. Some placental genes such as noerpinephrine transporter (NET), 11B-HSD2 and NR3C1 have been implicated in changes of HPA axis[144]. Regarding the placental 11β-HSD2 enzyme, we have reported above a recent study where prenatal maternal anxiety was correlated with a decreased 11β-HSD2 mRNA expression[71]; this decrease is thought to be mediated, at least partially, by epigenetic mechanisms such as DNA methylation[145]. In contrast, prenatal exposure to maternal depression was associated with decreased methylation of the placenta SLC6A4 gene, encoding the transmembrane serotonin transporter[146]. Besides, this decreased methylation for SLC6A4 in peripheral tissues, such as blood and saliva has been associated with early life adversity and depression.

3.7.2 Epigenetics and nutrition

Prenatal and early postnatal nutrition has been described as other critical environmental factor that could alter the epigenetic programming and could increase the susceptibility to chronic diseases[147]. Environmental exposures during early life may lead to an increased risk of obesity and metabolic syndrome later in life via alterations in DNA methylation. For example, in the Dutch Famine cohort, 60-year-old adults who were prenatally exposed to famine showed hypomethylation of whole blood of insulin-like growth factor 2 (IGF2) gene, and hypermethylation of two obesity-related non-imprinted genes (tumor necrosis factor, leptin) as compared with those whose mothers were not unexposed to the famine during pregnancy[148]. Recently, parental obesity and specifically paternal obesity was associated with IGF2 hypomethylation in umbilical cord blood leukocytes of newborns[149,150]. Furthermore, specific maternal characteristics, including gestational weight gain and gestational

diabetes, have also been associated with signature DNA methylation in cord blood and increased placental leptin gene methylation, respectively[151].
Regarding FGR, changes in the expression and the activity of the placental nutrient transporters have been associated to pregnancy pathologies such as this condition. It has been reported that maternal metabolic status determines adaptations of placental nutrient transporters thought the DNA metylation; the methylation of the cholesterol transporter ABCA1 appeared to be sensitive to in utero environment and may have long-term impact on lipid profile in cord blood [152].
In addition, a recent review has summarized the animal models demonstrating how dietary manipulation impacts perinatal programming[153].

3.7.3 Ageing

Telomeres are nucleoprotein structures consisting of 5-15 kilo base pairs of repetitive DNA sequences, located at the termini of the chromosomes. They are essential for chromosome stability and for cell survival[154,155]. Telomeres are progressively shortened with each cell division and also by environmental factors. Shortened telomeres promote cell cycle arrest, apoptosis, and genomic instability. The enzyme telomerase adds telomeric repeats to the ends of the chromosomes[156] and the human telomerase reverse transcriptase is the catalytic component of telomerase and it is considered to be the rate limiting factor in telomerase activity.
In recent years, ageing has emerged as a particularly attractive candidate among the molecular mechanisms underlying the link between stress and disease risk[157,158]. Telomere maintenance has relevance for long-term health. Shorten leukocyte telomere length (LTL) in humans has been associated with earlier mortality and morbidity[159]. It has been recently demonstrated a linking between maternal psychological stress exposure during prenatal life and shorter LTL in young adulthood[160]. More interestingly, this link has been reported to be evident as early as the time of birth: in a prospective cohort of 27 stressed-pregnant women, the analysis of cord blood peripheral blood mononuclear cells for LTL measurement reported a linear effect of pregnancy-stress on newborns LTL[161]. This is the first evidence in human being that maternal psychological stress may put forth a programming effect on the newborn telomere biology system, reflecting cellular aging.

3.8 Microbiota

There is increasing evidence from the interaction between microbiota (=the microbial community in a specific environment), diet and health. A structural and equilibrated gut microbiome (=the total genomic repertoire of microbiota) is needed for optimal health status, intervening in key host metabolic and immunological function[162]. Recent studies have shown that the physiologic, immune and metabolic changes that occur during pregnancy run in parallel with variations in microbial composition and diversity in the maternal microbiota[163,164].
Several studies indicate that adult's microbiota derived from the newborn's microbiota[165]. However, recent evidence shown that the development of the gut microbiota of the neonate is programmed during fetal life. The theory that during pregnancy the gut of fetus is sterile and that exposures to maternal microorganisms occur after birth, has been modified thanks to novel research, which report

that the firsts contacts that happed through the placenta and the amniotic fluid[166]; this means that the microbiota originates during pregnancy, and during pregnancy might be modulated.

3.9 Rational of the study

From a clinical prospective, given the high prevalence of FGR (7-10%) and its impact in future life, the critical goal is now to apply some strategies that could reduce the incidence of this disorder during prenatal life. Maternal psychological state as well as her dietary pattern seems to be fundamental for pregnancy environment and it probably shapes the neurodevelopment of the fetus and consequently of the child. We wonder if improving the maternal lifestyle and well-being with specific strategies we could obtain an improvement on fetal outcome.

4 Study aims

4.1 Hypothesis

Our **primary hypothesis** is that specific interventions improving maternal lifestyle and well-being have a positive impact on pregnancy outcomes as well as on fetal growth and development.

Our ***secondary hypothesis*** is that interventions to improve maternal lifestyle well-being have a positive impact on offspring's outcome later in life, in terms of neurodevelopment and cardiovascular profile, mediated by epigenetic changes in offspring.

4.2 Aims

1) To demonstrate that an improvement on maternal lifestyle and well-being, through intensive behavioral therapies based on nutritional intervention with a MD and stress reduction with MBSR program reduces the prevalence of FGR in pregnancies at high risk to develop this condition.

2) To demonstrate that an improvement on maternal lifestyle and well-being, through intensive behavioral based on nutritional intervention with a MD and stress reduction with MBSR program reduces the APO of pregnancies at high risk to develop FGR.

3) To demonstrate that an interventional program to the mother improves fetal brain development and infant neurodevelopment and cardiovascular profile.

4) To identify epigenetic changes that may explain the improvement of fetal outcome and programming.

4.3 Specific objectives

4.3.1 Principal objectives

For each of these principal objectives a sample size has been calculated (see below specific section 5.5.1).

1) The ***primary objective*** of this study is to evaluate whether an interventional program aiming at improving the maternal lifestyle and well-being in pregnancies at high risk to develop FGR (OR >2, 30% of risk for this category of women) could improve the fetal growth and subsequently reduces the prevalence of newborns with a BW below the 10th percentile (attend improvement: reduction of 30% of the prevalence of babies born with a BW <10th percentile: reduction from 30% to 20%).

   The ***secondary objectives*** are to:

2) Demonstrate a reduction of 50% (from 15% to 8%) of APO, defined according to the presence of any of the following neonatal measures:

   a. Preterm birth: delivery <37 weeks' gestation
   b. Preeclampsia (PE): defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy as BP ≥ 140mmHg or diastolic BP ≥ 90mmHg at least 4 h apart after 20 weeks of gestation and proteinuria of ≥300 mg in 24 h.
   c. Perinatal mortality: fetal or neonatal mortality (within 28 days of life).
   d. Severe FGR: BW <3rd percentile
   e. Metabolic acidosis: an umbilical artery pH below 7.10 and/or base excess >12 mEq/L in the newborn and/or an Apgar score at 5-minute below 7.0 assigned by the attending neonatologist or midwife.
   f. Major neonatal morbidity: presence of intraventricular hemorrhage grade III/IV, necrotizing enterocolitis, periventricular leucomalacia, sepsis, broncopulmonary dysplasia, hypoxic ischemic encephalopathy.

3) Demonstrate an improvement on offspring's neurodevelopment after maternal intervention, defined with Bayley test at two years of corrected postnatal age.

4) Demonstrate an improvement of offspring's cardiovascular profile after maternal intervention, defined with arterial BP assessed at two years of corrected postnatal age.

5) Demonstrate an improvement of cardiovascular risk factors such as glucose metabolism, BP, and lipid profile in the mothers after nutritional and stress-reduction interventions.

4.3.2 Other objectives

6) Demonstrate an improvement on offspring's fetal/neonatal neurodevelopment from mothers who attend the interventional program, revealed by different techniques of fetal MRI (cortical

development, brain volumes, spectroscopy assessment) and with NBAS at one month of corrected age.

7) Determine different epigenetic changes in fetal cord blood in pregnancies affected by FGR and in those who attend the interventional program as compared to non-complicated pregnancies with no intervention.

8) Demonstrate an improvement in placental perfusion by MRI and a decrease placental cell death in pregnancies who attend the interventional program as compared to pregnancies with no intervention.

9) Describe an improvement on maternal physical status during pregnancy thanks to a healthier nutrition based on MD, evaluated by different questionnaires and maternal metabolic profile and its impact on placental nutrient transport and fetal metabolic profile.

10) Describe an improvement on maternal psychological status during pregnancy thanks to an intervention of MBSR program, evaluated by different questionnaires, maternal brain metabolites and cerebral circuits assessed by maternal MRI.

11) Demonstrate that maternal gut microbiome could change thanks to a nutritional intervention based on MD or a stress reduction based on MBSR program.

12) Demonstrate that neonatal gut microbiome is different from mothers who attend different interventional programs (MD or MBSR or no intervention) at 1 month and at two years of age.

5 Methods

5.1 Study design

The project will be a RCT 1:1:1 ratio, parallel, open blind among women at higher risk to have a growth restricted fetus (30%) according to RCOG Guidelines[98] (see Appendix 1). These high-risk women will be randomized in order to evaluate an improvement in several outcomes thanks to different strategies apply to the mothers: a stress reduction program based on mindfulness techniques (MBSR) and/or a nutrition interventional program based on Mediterranean diet.

The study design of the RCT adheres to standard criteria for randomized trials, and it has been registered in the *Clinical Trials Gov* (https://clinicaltrials.gov/), # NCT03166332.

5.2 Study population

Women at high risk to have a growth restricted fetus according to the RCOG Guidelines[98] (Appendix 1).

5.2.1 Study setting

The study will take place in BCNatal, resulting from the integration of Hospital Clinic and Hospital Sant Joan de Déu in Barcelona (Spain), which is one of the most important university centers of maternal-fetal and neonatal medicine in Europe and a world-wide reference in this field. Both hospitals, with more than 7,000 deliveries per year, are university hospitals and tertiary referral institutions for high risk pregnancy. They are part of BCNatal Fetal Medicine Research center, a research center in fetal and perinatal medicine, recognized as one of the world's best research teams in the field that has as a main objective to demonstrate how prenatal life has an impact on childhood and adult life. The center counts with a multidisciplinary team of more than 70 members including doctors, engineers, biologists, nurses, psychologists as well as an own management structure.

5.3 Participants

5.3.1 Participant selection

Eligible participants will be pregnant women resulted at high risk to develop FGR during pregnancy (OR >2) according to the criteria of the RCOG[98]. Women will be selected at the moment of the routine second trimester ultrasounds scan (19-23 weeks' gestation) and will be randomized to three equally sized groups:

1) Nutrition program based on Mediterranean diet
2) Stress-reduction program based on Mindfulness techniques (MBSR)
3) Control group with no specific intervention (usual care)

Personnel who manage these patients in the clinical setting will be blinded for each group of randomization the patient is belonged to. Additionally, the subjects are asked not to reveal their group of randomization to any of the other subjects.

5.3.2 Participant inclusion criteria

- Maternal age at recruitment ≥18 years
- Speak Spanish fluently
- Viable singleton non-malformed fetus
- High-risk pregnancy to develop FGR
- 19-23 weeks of gestation

5.3.3 Participant exclusion criteria

- Fetal anomalies including chromosomal abnormalities or structural malformations detected by ultrasound prenatally
- Neonatal abnormalities diagnosed after birth
- Mental retardation or other mental or psychiatric disorders that impose doubts regarding the true patient´s willingness to participate in the study
- No possibility to come to additional visits
- Included in other RCT studies

5.4 Sample size estimation

5.4.1 Sample size rationale

Specific sample size estimation has been calculated for each of the principal objectives of the study explained above. We used for calculation the program "Power and Size Program version 3.1.2, 2014 (Department of Biostatistics, Vanderbilt University).

For fetal growth and neonatal growth centiles we use the customized charts of the Spanish population[18], adjusted by gestational age at delivery and gender.

1) Reduction of 30% of rate of neonates born with a BW <10th centile:
   Based on the criteria of the RCOG[98], 30% of pregnant women belonged to this high risk category will have a growth restricted fetus, defined with a BW<10th centile. The main outcome of this RCT is to reduce this prevalence by 30% (from 30% to 20%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 293 subjects for each arm of randomization. Considering a loss of 20%, the final sample size calculated is 367 participants for each group (total=1101 subjects).

2) Reduction of 50% of rate of APO:
   Based on evidence in the literature[167,168] the 15% of this population is considered at high risk will develop an APO during pregnancy. The second outcome of this RCT is to reduce this prevalence by 50% (from 15% to 8%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 325 subjects for each arm of randomization. Considering a loss of 20%, the final sample size calculated is 406 participants for each group (total=1218 subjects).

3) Improvement of Bayley score at two years of age:

Based on previous data from our group[169], the difference of the mean score at Bayley test at two years of age between controls and FGR was 6 (SD 14). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 87 subjects for each arm of randomization. Considering a loss of 30%, the final sample size calculated is 124 participants for each group (total=372 subjects).

4) Reduction of BP at two years of age:
Based on data from the guidelines for cardiovascular health and risk reduction in children and adolescents[170], the 90th centile for BP at two years of age is 102/57 mmHg. In a recent paper from our group[32], the prevalence of a mean BP >95th centile in FGR fetuses at 6 months of age was 41% compared to 5% of controls. In this trial we expect a prevalence of 20% of BP >95th centile at 2 years of age in the control group, and the objective is to reduce this prevalence by 50% (from 20% to 10%). For this purpose, with a power of 0.80 and null hypothesis of 0.05, we will need 199 subjects for each arm of randomization. Considering a loss of 30%, the final sample size calculated is 259 participants for each group (total=777subjects).

We estimate that a sample of 1218 women would be recruited to provide statistical power of 80% to detect a relative risk reduction of BW <10th percentile of 30% in each intervention group *versus* the control group with usual care.

5.5 Recruitment

At the moment of the routine second trimester ultrasound scan (19-23 weeks' gestation), all women are invited to check a list of risk factors based on the criteria establish by the RCOG (Appendix 1). These criteria set up a subgroup of women (approximately 30% of all pregnant women, 800 for year in each of our hospitals) that are at higher risk to develop a fetus with FGR. These guidelines establish that women are considered at risk if they meet a major risk factor (OR >2) or three or more minor risk factors (OR >1 for each) (see Appendix 1): the RCOG guidelines establishes that these women should be referred for serial ultrasound measurements of fetal size and assessment of well-being from 26-28 weeks' gestation[98]. In our hospitals, these women are scheduled for extra ultrasound assessments at 28- and 32-weeks' gestation; while women who do not fulfill these criteria are just submit to third trimester ultrasound at 37 weeks.

5.5.1 Enrolment visit

Participants at high risk to develop FGR are considered eligible for this clinical trial and the same day of the routine second trimester ultrasound scan they will be identified by one of the investigators involved in the recruitment (FCro, CP, LS, MD) for a formal screening visit.

The visit serves to identify inclusion/exclusion criteria in a more comprehensive manner (appendix 2) and to address all eligible patients about the purpose of the study and the voluntary nature of the participation. This 30-minute visit includes:

1) A face-to-face administration of questionnaires to inquire about psychosis, major depression and bipolar disorder (see document in the appendix 3). In case of positive answer to any psychosis or bipolar disorder, the patient will be considered not eligible from the study and referred to the Psychiatric Unit of our Hospital (responsible: Prof. Vieta). In case of a positive answer to the question number 9 of the PHQ-9, the patient will be considered not eligible from the study and the specific questionnaire for suicide (Palkel, appendix 3) will be given to her and she will be referred to the Emergency Psychiatric Unit.

2) If the candidate meets all the requirements (Appendix 2), an informed consent form will be given to her to be signed after a detailed explanation of all study's procedures. The informed consent comprised two parts, one for study participation (Appendix 4) and another for biochemical analyses and DNA collection for genetic analyses (Biobank informed consent, appendix 5, 6).

3) Demographic data, clinical history, anthropometric measures and biological samples will be taken (maternal hair, peripheral blood, urine, vaginal and fecal microbiota) (Case report form, appendix 7).

4) Randomization of the patient trough the website of the study, but in a part accessible only for researchers: depending on the arms in which she will be, a brief general explanation of the study and following appointments will be given to each woman (Appendix 8).

5) Several questionnaires will be given and explained to the patient for a self-report to be completed at home (see Appendix 9-15).

6) To a 30% of women double-randomized, additional measurements will be done: a fit-bit bracelet to use it for the next week in order to register their basal activity, 24-hours BP, and 24-hours urine collection (see paragraph 5.8.3 below for other assessments).

7) An individual visit (1 hour) with a dietitian for the compilation of two food questionnaires (Food frequency questionnaires, FFQ and adherence to the MD in 17-points) and a physical activity questionnaire (Appendix 16) will be scheduled for the all patients for the next 7-10 days: during this visit questionnaires, and eventually fit-bit bracelets, 24-hours BP and 24-hours urine, will be collected from each patient and from 30% of them double randomized, respectively.

8) The patient will be informed about the web site of the study (https://fetalmedbarcelona.org/impactbcn/), with a specific access depending on her arm of randomization, and about a mobile phone available for any problems related to the study.

9) If extra-appointments are necessary (depending of the group of randomization), they will be given to the patient.

5.5.2 Randomization

After obtaining the informed consent, the patient will be randomized in one of the three arms of this trial. The randomization sequence has been computer generated to assure balanced distribution within study arms; it is protected and managed by the computer technician of the foundation "Medicina Fetal Barcelona" which has no role in recruitment. The investigators enter data of the patient that is recruited in a web site, and the computer generates automatically the inclusion number and treatment assignment. An additional code is also generated, so the patient can have access in the official website of the study to a specific part according to her intervention (https://fetalmedbarcelona.org/impactbcn/).

5.6 Intervention treatments

The idea behind both interventions is focused on pregnant women's lifestyle and well-being: if women could do a significant change in their lives, this could lead to a benefit not only for themselves and their pregnancies, but might also reach their fetuses and consequently improve their growth and development. Both interventions are not based on pharmacological treatments, but on behavioral counseling and training, derived from robust scientific evidence and already validated in several important studies as explain below.

5.61 Dietary intervention

The dietary intervention is based on the results derived from the PREDIMED study[100], in which a MD supplemented with extra-virgin olive oil or mixed nuts improved main cardiovascular risk factors and significantly reduced incidence of major cardiovascular events.

One main focus of this intervention strategy is to change the dietary pattern in general instead focusing on changes in single food or macronutrients. Diet pattern is adapted to pregnant women and will be also adapted during the first individual assessment with the dietician to participant's weight, culture and food questionnaire. The intervention program is done by expert dieticians (TF, MS, CG) and supervised by the Research Group on Nutrition, Cardiovascular Disease and Aging from IDIBAPS - Hospital Clinic of Barcelona (RE, RC, MD).

The intervention is based on individual visit of 30 minutes assess every month and on monthly group classes of 1 hour, where theoretical information and discussion about MD will be done in order to identify problems and solution in diet implementation. Participants will receive extra-virgin olive oil (2 liters every month) and 15 g of walnuts per day at no cost (for a total of 450 gr every month). Every two weeks, woman will receive a telephone by the dietician in order to reinforce the intervention (Appendix 17). Each individual visit includes three steps: assessment, intervention and future directions. Specific materials (recipes, a quantitative 1-week shopping list of food items according to the season of the year, a weekly plan of meals with detailed menus...) will be given and will be also available on the web site in the Nutrition section (login with password given at the moment of randomization). During each monthly visit the 17item dietary screener to assess baseline adherence to the MD will be checked, and a 3-days register diet will be also evaluated (Appendix 18). In addition, BP and weigh gained will be also measured.

Adherence and effectiveness:
Adherence to the intervention will be assessed by an improvement adherence to the MD, based on an improvement of ≥3 points of their total final score of the 17-item dietary screener compared to their total initial score. The study population will be described according to adherence to the MD, depending on the score: ≥12 *high adherence*; 6-11 *moderate adherence*; <6 *low adherence*.
At the end of the treatment, biomarkers of compliance will be evaluated in a random sample of 30% of participants, including urinary hydrohytyrosol concentration (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid concentration (to confirm compliance of walnuts), as well us urinary total polyphenol excretion (as a measured of adhesion to overall Mediterranean dietary pattern) at the School of Pharmacy and Food Science at the University of Barcelona (RML).

Timeline for intervention: from 24 to 36 weeks' gestation (individual visits every 15 days + group intervention every month).

5.6.2 Mindfulness intervention

The mindfulness intervention is based on the MBSR program, which is an intervention developed in the late 1980s at the Center for Mindfulness at the University of Massachusetts Medical School by Dr. Jon Kabat-Zinn[102], that has been scientifically approved and recognized by university and health institutions. This program consists in 8 weeks of group classes of a 20-25 people, one full day and daily home practice. The intervention will be coordinated by the founder of the Institute of *esMindfulness* (AM), certified instructor by the Center of Mindfulness in Medicine, Health Care and Society, University of Massachusetts Medical School, Worcester, MA, USA, who is also trainer for mindfulness instructors, based on the principles and standards reported elsewhere[171] (http://umassmed.edu/cfm/training/principles--standards/). Weekly group classes of 2.5 hours and one full day will be conducted by female instructors from the *Istitute esMindfulness* (TO, AH, GB) with years of experience and certified. The program of MBSR we are going to use for this trial is based on the document of Standard of Practice of Center for Mindfulness, recognized by the Spanish Association of Mindfulness instructors (http://www.mbsr-instructores.org/).
The intervention is based on different exercises to cultivate awareness of internal present-moment experiences with an accepting, non-judgmental stance, management of stress techniques and other pleasurable activities focused on enhancing the adherence to the program. The program has been supervised by the head of Psychiatry Service of Hospital Clinic de Barcelona (EV).
After the randomization, before starting with the group of intervention, a presentation of the program will be done, in order to reinforce the importance of this treatment. Women will be also instructed for formal and informal meditation as home practices (e.g. present-focused awareness during eating, bathing, cleaning…) with the reminder of a specific book and a CD or USB with meditation guides. Before starting with a new section, the frequency of home-practice will be recorded in order to assess the adherence to the program (Appendix 19). Material will be also available on the web site in the Mindfulness section (login with password given at the moment of randomization).

Adherence and effectiveness:
Adherence to the intervention will be assessed by class attendance and time spent engaged in mindfulness meditation outside of class sessions. The treatment will be considered complete if at least 10 hours of meditation are done (both during class and at home), which we considered if the woman

has attended at least 6 section (out of 9 in total). Each group of intervention is composed of a maximum 25 women.
At the end of the treatment, biomarkers related to stress (cortisol axis) will be evaluated in maternal 24-hours urine and fetal amniotic fluid (fetal urine) in a random sample of 30% at the Bioanalysis and Analytical Services Research Group - IMIM (OP).
After the course, some extra session will be offered every two weeks until delivery, in order to keep in mind this intervention.

Timeline for intervention: from 24-26 to 32-34 weeks' gestation (group classes of 2.5 hours every week to a total of 8 sessions+1 full day)

5.7 Participant timeline

5.7.1 Promotion of adherence

Efforts to promote adherence begins at the earliest stages of the study. During the first visit at enrollment, participants are repeatedly provided with information about key features of the study. After randomization, participants will receive a detailed explanation of the study protocol by the specific trainer according to the arm included (dietician and mindfulness instructor respectively) in order to focus more on goals of the trial and to do the interventions as more efficient as possible.
In both intervention strategies is fundamental to do home practice: classes are useful to get some theoretical notions, but the majority of the treatment needs to be done in every-day life.
Nurses involved in the project will do phone calls at least twice during the treatment. In the website with a different access depending on the arm of randomization, several information and material will be also available.
For dietary intervention, the free distribution and supply of key foods (2 l of extra-virgin oil and 450 gr of walnuts per months) ensures a high adherence to the protocol. For mindfulness intervention, a book, meditation audio-types and a personal work note to register time spent in meditation and relaxation will be given free to ensure a high adherence. For women in the control group, usual care will be done.

5.7.2 Assessment of compliance

Home-practice is essential for both interventions, and people involved will reinforce this concept. To obtain also an objective evaluation, data from fit-bit bracelets will be analyzed in a random sample of 30% of participants.
For dietary intervention, the 17-item score will provide information about compliance and attainment of participants to MD intervention. Additionally, in random sample of 30% of participants, a blood sample and urine aliquots will be used to blindly ascertain the markers of compliance of walnut and extra-virgin olive oil, respectively, as well overall adherence to overall MD pattern. To relate these measurements to the time of intake, participants are asked the time since the last consumption of the specific food when blood and urine samples are taken.
The personal work note with mindfulness meditation and relaxation will be revised weekly during class in order to evaluate any problems and to reinforce the practice. Daily practice of mindfulness will be

considered and used for subsequent analysis (adherence: 10 hours' meditation, which means at least 6 class' attendance).
At the last visit during pregnancy a 3D ultrasound scan as a present will be given to each mother. Similarly, during postnatal follow-up, in every visit a small gift for the baby will be given (a different toy according to different ages).

5.7.3 Additional assessments to a random sample of 30% of women

In a randomly selection of 30% equally distributed among the three arms of the RCT, double randomized at the moment of recruitment, several extra assessments will be done:

1) Fit-bit assessment at the beginning (19-23 weeks) and at the end of the study (34-36 weeks)
2) 24-hours urine assessment at the beginning (19-23 weeks) and at the end of the study (34-36 weeks) for the analysis of cortisol (IMIM)
3) 24-hours BP assessment at the beginning (19-23 weeks) and at the end of the study (34-36 weeks)
4) Biomarkers of walnuts, extra-virgin olive oil and adherence to overall dietary patterns at the beginning and at the end of the trial (see below).
5) Maternal, fetal and placental MRI at 37-38 weeks: proposed to everybody depending on the MRI local schedule and select only those who accepted
6) Epigenetic studies in fetal and placental samples

Maternal 24 hours' blood pressure assessment:
A 24-hour BP monitoring will be performed using Spacelabs 90217 devices (Spacelabs1 Inc. Richmond, Washington, USA), scheduled every 20 minutes during the activity period and every 30 minutes during the resting period and after the appropriate recommendations to the patients. The periods of activity and resting are determined individually according to the bedtime and waking up of each patient. In addition, the duration of the monitoring (hours), the percentage of valid readings, and the mean values of systolic BP / diastolic BP will be recorded in the periods of activity, resting and in 24-hours. Records with a duration <24 hours, absence of an hourly reading and <70% of satisfactory readings will be excluded. According to the definition of ambulatory BP control, good ambulatory control is defined as mean values of 24 hours <130/80 mmHg, mean daytime ambulatory figures <135/85 mmHg and mean ambulatory nocturnal values <120/70 mmHg.

5.7.4 Desertion from the study

Data from patients enrolled in the trial who wants to desert it for any reason will be registered (see Appendix 20).

5.8 Measurements

All participants will receive an intervention divided in several phases:

1) Recruitment and randomization: 19-23 weeks
2) First nutritionist interview: 21-23 weeks
3) Final assessment, almost at the end of interventions: 34-36 weeks

4) Final nutritionist interview: 36-37 weeks
5) A random subgroup of women (30% of each arm) with specific assessments
6) Delivery
7) Postnatal follow-up

All patients will be part of each phase including the following interventions:

1. ***Recruitment and randomization: 19-23 weeks*** *(FC, CP, LS, DM)*
   a) Screening for depression and psychosis (PHQ-9) before enrollment
   b) Signature of informed consent form
   c) Register of socio-demographic data
   d) Maternal bio-sampling collection (blood, urine, hair, vaginal and fecal microbiome)
   e) Randomization
   f) Maternal lifestyle (diet/sleep/stress) assessment will be given
   g) 30% of women double randomized: fit-bit, 24-hour BP proposed
   h) Scheduled for: nutritionist (for all women), mindfulness first class (for mindfulness intervention)

2. ***First nutritional interview: 21-23 weeks***
   a) Collection of maternal lifestyle assessment (questionnaires: FFQ, 17-item score, physical activity)
   b) 30% of women double randomized: collection of fit-bit, 24-hour BP
   c) Interview for the assessment of FFQ, 17-items scale and physical activity
   d) Only for nutrition arms: specific suggestions and schedule for next appointments

3. ***Final assessment: 34-36 weeks***
   a) Feto-placental ultrasonographic assessment (EFW, Doppler, Echocardiography, Neurosonography).
   b) Maternal bio-sampling collection (blood, urine, hair, vaginal and fecal microbiota)
   c) Maternal lifestyle assessment (questionnaires, the same of the beginning) will be given
   d) 30% of women double randomized: fit-bit, 24-hour BP
   e) Scheduled for: dietitian (for all women), MRI (for 30% of women who accepted)

4. ***Final nutritional interview: 36-37 weeks***
   a) Collection of maternal lifestyle assessment (questionnaires)
   b) 30% of women double randomized: collection of fit-bit, 24-hour BP
   c) Interview for the assessment of FFQ, 17-items score and physical activity

5. ***MRI: 30% of women at 37-38 weeks***
   a) Maternal MRI (structural, spectroscopy, DTI, functional)
   b) Fetal MRI (structural, spectroscopy)
   c) Placental MRI (perfusion)

6. ***Delivery***
   a) Fetal bio-sampling (amniotic fluid, cord blood)

b) Placenta bio-sampling and tissue samples
c) Placenta pathology
d) Anthropometric evaluation of the neonate
e) Obstetrical outcomes

7. ***Postnatal follow-up:***

During every postnatal follow-up a bio-sampling of the mother and the newborn (hair) will be taken.

a) Neonatal morbidity and mortality
b) 1 month:
   - General data of the newborn (Appendix 21)
   - Brazelton tests (NBAS)
   - Microbiota samples
   - ***Mothers:*** Edinburgh evaluation (Appendix 22)

c) 12 moths (1 year):
   - General data of the child (Appendix 23)
   - Ages and Stages Questionnaires (ASQ): general questionnaire

d) 24 months (2 years): (checklist Appendix 24)
   - General data of the child (Appendix 25)
   - Bayley III test
   - Cardiovascular assessment: arterial BP, heart frequency
   - Microbiota samples
   - ***Mothers:*** general data (Appendix 25), questionnaires (Appendix 9-13, 16, 26), biological samples (urine, fecal microbiota), BP, heart frequency

e) 5 years:
   - General data of the child
   - CBCL
   - Wechsler Preschool and Primary Scale of Intelligence - Fourth Edition (WPPSI-IV)
   - Cardiovascular assessment: arterial BP, heart frequency, doppler carotid arterial measurements (cIMT)

5.8.1 Maternal lifestyle assessment

At the moment of recruitment, participants will receive a pack containing one of each of the questionnaires below (Appendix 9-15). The same questionnaires will be given during the final visit. All questionnaires are self-reported, a part from some diet quality assessments that will be assessed by the nutritionist itself (Appendix 16). The same questionnaires (Appendix 9-13, 16, 26) will be given during the postnatal assessment at 24 months of corrected postnatal age, see below.

Anxiety and stress: The *Perceived Stress Scale* (PSS) and the *State-trait Anxiety inventory* (STAI) are the best currently available instruments to evaluate the presence of anxiety and depression during pregnancy[172,173](Appendix 9 and 10).

Sleep quality: The *Pittsburgh Sleep Quality Index* (PSQI) is based on eighteen self-reported questions about the person own sleep quality (Appendix 11). The scale evaluates seven rated components, including, sleep subjective, quality, duration, disturbances, and latency, habitual sleep efficiency, use of sleeping medication, as well as daytime function. The score from each category is added to achieve a global score that range from 0 – 21. A cutoff score of 5 or above is indicative of a sleep disturbance. This scale has been recently validated in the obstetric population[174], in which an abnormal result was associated with an increased risk of preterm birth[175].

Quality of life and psychological assessment: The *Five Facet Minfulness Questionnaire (FFMQ)* is a questionnaire for measuring mindfulness, correlates positively with openness to experience, emotional intelligence, and negatively with psychological distress[176]; this scale is validated in Spanish[177] (Appendix 12). The *Well Being Index* from the World Health Organization, in its Spanish Version, evaluates through 5 questions the psychological wellness in general (Appendix 13).

Diet quality: Two questionnaires about physical activity are given to any woman (Appendix 14 and 16), one of them (Appendix 14) is the Minnesota, validated in a Spanish version[178]; in addition, the 7 days diary of their own diet will be also given to them (Appendix 15).

Diet quality (assessed by the dietitian): FFQ are the preferred dietary assessment method in most epidemiological studies mainly due to their low cost and easy administration, and they have been validated in many different populations. We plan to use a semi-quantitative FFQ of 146 food items to assess the usual daily intake of foods and nutrients (PREDIMED plus). In addition, the adherence to the MD within a 17-items scale (Appendix 16) will be registered for each participant.

Post-partum assessment: During the first postnatal follow-up (Brazelton test at 1-3 months of age), the *Edinburgh questionnaire* for the screening of post-partum depression will be given to all women (Appendix 22). If the final score will be >13, the patient will be sent to the specific psychiatric perinatal unit of the Hospital.

During the postnatal follow-up at 24 months, data on maternal and familiar life-style will be recorded (Appendix 25), as well as the same questionnaires given during pregnancy (Appendix 9-13, Appendix 16 for physical activity) will be assessed again. The nutrition assessment will be done with adherence to the MED diet within a 17-items scale modified as the one during pregnancy (Appendix 26).

5.8.2 Feto-placental ultrasonographic examination

Fetal biometry: includes evaluation of fetal growth using the Hadlock formula[179] based in a composite sonographic measurement of fetal head (biparietal diameter, BPD; head circumference, HC), abdominal circumference (AC) and femur length (FL). Fetal measurements will be performed following previously published techniques.

Fetoplacental hemodynamics: Umbilical artery (UA) pulsatility index (PI) will be performed from a free-floating cord loop. Normal UA will be considered as a PI below the 95th percentile[180]. Doppler PI for UA and mean PI from both uterine arteries (UtA) will be performed according to previously reported techniques[181–183]. The Aortic isthmus (AoI) PI will be measured either in a sagittal view of the fetal thorax with clear visualization of the aortic arch, placing the gate a few millimeters beyond the origin of the left subclavian artery; or in a cross sectional view of the fetal thorax, at level of the three vessel

and traquea view, placing the gate just the converge of the AoI and the arterial duct[184–186]. Prenatal Doppler ultrasound examinations will be performed in the absence of fetal movements. Pulse Doppler parameters will be performed automatically from three or more consecutive waveforms, with the angle of insonation as close to zero as possible.

Cerebral flow evaluation: The middle cerebral artery (MCA) PI will be obtained in a transversal view of the fetal head, at the level of its origin from the circle of Willis[187,188,189]. Three consecutive high-quality images with no artefacts will be recorded using previously reported parameters. The cerebroplacental ratio (CPR) will be calculated as a ratio of MCA PI to UA PI[190,191,192]. The MCA PI and CPR values below the 5th percentile will be considered indicative of cerebral blood flow redistribution[190,191,192].

Fetal cardiovascular assessment: Fetal cardiovascular remodeling will be assessed by measuring cardio-thoracic ratio, ventricular sphericity and wall thickness, tricuspid and mitral anular plane systolic execution (TAPSE and MAPSE) and myocardial performance index (MPI). Cardiac area will delineate in end-diastole from a 4-chamber view and divided by thoracic area in order to calculate cardio-thoracic ratio. Left and right sphericity indices will calculate by dividing the end-diastolic base to apex ventricular length by the transverse ventricular diameter measured in 2D in an apical or basal 4-chamber view[31]. Left, right and septal wall thicknesses will be measured from an apical or basal 4-chamber view at end-diastole. TAPSE and MAPSE will be calculated using M-mode real time from an apical or basal 4-chamber view, measuring the maximum displacement of the valvular rings between end systole and end-diastole[193]. The MPI will be measured in a cross sectional view of the fetal thorax, in an apical projection and at the level of the four-chamber view of the heart[194]. Briefly, the Doppler volume sample will be placed to include both the lateral wall of the ascending aorta and the mitral valve where the click corresponding to the opening and closing of the two valves can be clearly visualized. The isovolumetric contraction time (ICT), ejection time (ET), and isovolumetric relaxation time (IRT) will be calculated using the beginning of the mitral and aortic valves clicks as landmarks and the MPI will be calculated as follow: (ICT+IRT)/ET.

Fetal neurological assessment:
Systematic evaluation of cortical development has been described by Alonso *et al.*[195] and Pistorius *et al.*[196]*,* allowing quantification of brain sulcation in normal fetuses by ultrasound examination. Following this evidence, Egaña *et al.*[24] applied the same methodology to MRI technique in a population of FGR and controls.
A detailed **neurosonography (NSG)** will be performed at the moment of enrolment in the study population by one expert examinator (FC, CP) using a two-dimensional transabdominal and transvaginal approach. Fetal brain exam will be perform and standardize based on the ISUOG guidelines for fetal brain assessment[197] which include the following parameters:

- ✓ Axial planes:
  - *Transventricular plane*: lateral ventricle, parieto-occipital fissure, central sulcus, frontal and parietal area
  - *Transthalamic plane*: BPD, HC, Sylvian fissure, superior temporal fissure, frontal area, temporal area
  - *Transcerebellar plane*: Transverse cerebellar diameter, cerebellum, cisterna magna
- ✓ Sagittal planes:
  - *Midsagittal plane*: Corpus callosum, vermis
  - *Parasagittal plane*: Central sulcus, frontal area, parietal area

- ✓ Coronal planes:
  - *Transcaudate plane*: Anterior horns, cinculate sulcus, mesial area
  - *Transcerebellar plane*: Calcarine sulcus, occipital area

Neurosonographic images will be obtained prospectively and later will be analyze offline. Imaging post-processing and measurements will be performed using the semiautomatic software by expert examiners blinded to group randomization.

5.9 Magnetic Resonance Imaging

The final MRI protocol will be adjusted not to exceed 1.30h, including fetal brain, placenta and adult brain. This should include patient repositioning, between abdominal MRI (fetal brain and placenta) and head MRI (mother's brain). The decision of which one is the first will be depending of fetal movements and position.

5.9.1 Fetal neurodevelopmental examination by MRI

Estimation time: 30'

Fetal MRI will be performed at 36-38 weeks of gestation in all study population, in a clinical MR system operating at 3 Tesla, using a body array radio-frequency (RF) coil.

- ✓ Structural MRI:

Single-shot fast spin-echo T2-weighted sequences (repetition time, TR 1390 ms; echo time, TE, 160 ms; slice thickness 3 mm; field of view (FOV) 230 x 230 mm; pixel size 1.2x1.2 mm; no interslice-slice gap) will be acquired in the three orthogonal planes, oriented along the axis of the fetal brainstem, obtaining 2-loops of transverse, 2-loops of coronal and 2-loops of sagittal single shoot slices.
T2 fetal MRI data will be used to perform several measurements, including 3D reconstruction of fetal brain following a previously described methodology[198]:

- *2D measurements* including grading and measurements of the cortical development[24]
- *Reconstruction of the 3D volumes* by a semiautomatic process for the automatically measure of:
  - Brain tissue volume
  - Cortical gray matter
  - White matter volume
  - Cortical surface sulcation
  - Cortical thickness

- ✓ Spectroscopy MRI:

A localized single-voxel ^{1}H-MR spectroscopy of the fetal brain will be acquired. MRS data will be acquired from the frontal lobe based and with *Point Resolved Spectroscopy* (PRESS) localization, essentially as previously reported[27]: 20 x 20 x 40 mm voxel size, TR 2000 ms, TE 144 ms, 98

transients, and partial water suppression with a *Chemical Shift Selective module* (CHESS); a reference spectrum will also be acquired with 2 transients and no water suppression. To improve the performance of the technique at 3T, we will implement the dynamic acquisition mode with 13 measurements previously reported at 1.5 T[199]. Additional, we will test the feasibility of (i) using short echo-time (TE = 30 ms) for the frontal lobe detection of relevant metabolites with coupled spins, such as the glutamate-glutamine pool[200], and (ii) spectral editing for the detection of glutamate and γ-Aminobutryric Acid (GABA)[201,202].The MRS data obtained will be processed with the method of spectral sorting and alignment[199] by Matlab software and then by linear fitting in the frequency domain (LC Model) based on metabolite basis-sets available, essentially as reported before[27]; each metabolite will be quantified based on a reference water spectrum, as well as using metabolite ratios. Depending of the results obtained, we will also consider pattern-recognition analysis based on the entire spectral vectors, to identify potential additional features of interest for classification[203].

5.9.2 Placental examination by MRI

Estimation time: 15'

Placental imaging will be acquired during the same MRI session. Specifically, we will acquire reference T2-weighted and DWI over a larger field of view that includes the entire placenta. Diffusion-weighted imaging (DWI) will be acquired with 10 b-values (0, 10, 20, 40, 60, 150, 300, 500, 750, 1000 s/mm^2) and the data fitted with a bi-exponential *Intra-Voxel Incoherent Motion* (IVIM) model, to determine several biophysical parameters of the placenta, including the diffusion coefficient, perfusion fraction, and the pseudo-diffusion coefficient[204,205]. The voxel size of 3.4 x 3.4 x 3.4 will be placed closed to the cord insertion.

5.9.3 Maternal neurological examination by MRI

Estimation time: 30-40'

Maternal MRI will be performed in the same examination, in a clinical MR system operating at 3 Tesla, with Quantum gradients (30 mT/m) and a 8-channel head coil. This examination will include several sequences:

- ✓ Structural brain analysis:
  - o A structural T1-weighted magnetization prepared rapid gradient echo sequence (MPRAGE) will be used with: voxel size 0.8 x 0.8 x 0.8mm, TR = 2300 ms, TE = 2,08 ms, inversion time = 900 ms, 128 sagittal partitions, 0.83-mm slice thickness, square FOV of 240 mm, and acquisition duration of 5.58 min. The FreeSurfer 5.1.0 software package (Harved University, Boston, Massachusetts) will be used to create a 3-dimensional model of the cortical surface for measurement of cerebral and cerebellar gray and white matter volume, cortical thickness, and surface area.
  - o A structural T2-weighted magnetization will be done: voxel size 0.5 x 0.5 x 3 mm, TR = 5849 ms, TE = 95 ms, 3-mm slice thickness, square FOV of 240 mm, and acquisition duration of 3.03 min.

- ✓ Spectroscopy MRI:
  Localized brain 1H-MRS will additionally be performed on the mothers. Proton spectra (^{1}H-MRS) will be obtained from the cingulate anterior sulci region using single voxel PRESS: voxel size, 2 20 x 20 x 20 mm, TR 2000 ms, TE 30 ms; 98 transients, and partial water suppression with the CHESS module. A reference spectrum will be additionally acquired, with 2 transients and no water suppression. In this case, we will also acquire the data dynamically and additional acquire MEGA-PRESS to quantify glutamate and γ-Aminobutyric Acid (GABA)[201,202]. The data generated will be processed for individual metabolite quantification, by linear fitting with LC Model[116] and we will also consider pattern-recognition analysis based on the entire spectral vectors, to identify potential additional features of interest for classification[203].
- ✓ Resting state Functional brain analysis: Resting functional MRI scans will take 11 minutes, during which participants will be instructed to "keep your eyes closed and think of nothing in particular." Resting scans will consist of 190 interleaved oblique, 3 mm thick axial slices, covering the entire brain (repetition time = 6.0 s, echo time = 30 ms, flip angle = 90°, FOV 220 x 220, resulting in 1.7x1.7x3 mm isotropic voxels). *Prospective acquisition correction* (PACE) will be used to mitigate artifacts due to head motion.

- ✓ Diffusion Weighted Imaging (DWI): DWI generates contrast images from the changes on the diffusion of water molecules. DWI can reveal microscopic details of tissue architecture, such as the white matter tractography of the brain. In our protocol, this evaluation will take 14 minutes and it will consist of 37-38 directions, voxel 2.5x2.5x2.5 mm, with 2 b-values (800, 1500 s/mm^2) sampling the whole space[206]. TE=89ms, TR>7000ms. Non-weighted images (b=0) are also acquired and will be used to correct motion.

5.10 Biological samples

5.10.1 Sample collection and storage

Maternal blood and urine: Blood (30mL) will be drawn from an indwelling cannula in the brachial vein and kept in serum and EDTA treated tubes. Serum (16 mL), plasma (6 mL) and buffy coat (4 mL) will be separated by centrifugation at 3000 rpm for 10 minutes at 4°C and stored immediately at -80°C. Besides spot urine (16mL) and 24-h urine (16 mL) will be collected and store at -80°. These collections of urine and blood samples are carried at the time of enrolment and final assessment.
In all patients a blood exam (blood count, albumin, pre-albumine, lipid profile, iron assessment) and a basic urine exam will be done at the moment of enrollment and at the final visit.
In addition, to the 30% of women double-randomized, extra analysis will be done (hepatic and renal profile, folates, vitamin B12, vitamin D, minerals, apo-lipoprotein A1 and B, fibrinogen and homocistein).

Cord blood: 30mL of blood will be obtained from the umbilical vein after cord clamping at delivery and kept in serum and EDTA treated tubes. All samples will be processed within 1 hour. Serum, plasma

and buffy coat will be separated by centrifugation at 3000 rpm for 10 minutes at 4°C and stored immediately at -80°C.

Amniotic fluid: 5-10 mL of amniotic fluid will be retrieved during delivery when is possible and stored immediately at -80°C.

Maternal hair: A lock of hair approximately 3 mm thick will be cut from the vertex posterior region of the head as close to the scalp as possible and will be stored.

Microbiome samples: In all patients at the recruitment and during the final ultrasound assessment, non-invasive vaginal and fecal samples with swabs will be collected. Fecal samples from the mother will be also collected during the postnatal assessment at 24 months. Fecal sample of the newborn will be collected directly from the diaper during the postnatal assessments (at 1-3 months of age, Brazelton test, and at 24 months of age, Bayley III). Participants will be instructed on the self-collection procedure: either self-swab of the rectum or stool collection using disposable trays. For any self-swabbing procedure, participants must be instructed such that contamination from the skin around the rectal area, specimen handling, and sample collection equipment. All samples will be processed within the first 4h, frozen and stored at −80 °C for later analysis.

Placenta tissue samples: Upon delivery, placentas will be transported to the laboratory immediately after delivery, measured, weighed and sampled according to protocols previously described (includes a minimum of four sections of the placental disc, two sections of the umbilical cord, and two membrane rolls). Tissue sections will be formalin-fixed and paraffin-embedded, followed by H&E staining. Samples will thoroughly rinse in saline (NaCl 0.9%) to remove maternal blood. Besides, one section will be obtained to be stored at -80°C until DNA a protein extraction and other was collected in RNA later and stored at -80ºC for RNA extraction. Experienced placental pathologists blinded to the clinical diagnosis will evaluate all placentas to ascertain the presence of placental lesions.

Biological samples storage
Samples of plasma, serum, urine, and hair, will be collected from all patients at the moment of enrollment and final assessment. The total blood volume for banking of plasma, serum and DNA will not exceed 40 mL and will be collected according to the procedures outlined above. Cord blood, amniotic fluid and placental samples will be derived from one-time collection. Samples will be processed after collection and stored in -80°C freezers at Hospital Clinic-IDIBAPS and HSJDD biobank. All samples for specimen banking are stored in coded tubes without any other attached information that would allow identification of the individual from whom the sample is collected. All specimens are stored in secured, monitored and alarmed freezers at -80°C. Before inclusion in the clinical trial, each patient will sign two written consent. One of them regarding to research project and other related to future uses of the bio-specimens collected. Participants could voluntarily retire whenever they decide without any repercussion.

5.10.2 Biological analysis

5.10.2.1 Hormonal profile

Maternal blood, urine and hair will be collected twice: after the enrollment visit and at the final visit according to a common standard operating procedure and sent to the laboratory for measurement.
Additionally, the cortisol axis will be evaluated in several sampling (maternal hair and fetal amniotic fluid) collaborating with IMIM (Hospital del Mar Medical Research Institute).

5.10.2.2 Nutrimetabolomics in maternal and cord blood

Cord blood will be obtained immediately after delivery. Maternal venous blood will be also collected at the final visit for each mother-neonate pair. Samples will be collected in 10-mL EDTA tubes. Plasma will be separated immediately by centrifugation (5000X g, 10 min, 4°C) and stored a -80°C. Nuclear magnetic resonance (NMR)-based metabolite profiling is a quantitative nondestructive, noninvasive, non-equilibrium perturbing technique that provides detailed information on solution-state molecular structures, based on atom centered nuclear interactions and properties[207], it will be performed according to previously describe technique[208]. Metabolomics will be carried out at The Andalusian Centre for Nanomedicine and Biotechnology (BIONAND).
Additionally, we are planning to measure at the Biomedical Diagnostic Center (CDB) several vitamins both in maternal and fetal blood: vitamin A, vitamin B with the most important isoforms, vitamin D (25-hydroxyvitamin D) and vitamin E. We will also measure hemoglobin, mean corpuscolate volume, iron stores (serum ferritin), serum iron, transferrin and folates.
Adherence to nutritional intervention will be assessed by an improvement adherence to the MD, and biomarkers of compliance will be evaluated, including urinary hydrohytyrosol levels (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid levels (to confirm compliance of mixed nuts) by the Faculty of Pharmacy of the University of Barcelona.

5.10.2.3 Placenta pathology

Histopathological examinations of the placenta will be carried out on all for purposes of disease verification. An experienced and qualified pathologist will perform placental histological findings, which is essential for addressing all specific aims of the protocol. Initial evaluation of placenta histology is made under the light microscope in haematoxylin and eosin stained sections. Placental analyses will be performed at Hospital Clinic-IDIBAPS.

Placental patterns of placental maldevelopment and injury: Placental tissue sample collected according to our protocol. Samples will be used for studying the placental vascular assessment and the placental inflammatory profile by Redline classification[209], immunohistochemical analysis and Western blotting.

Placental ageing: Samples collected in the same way will be used for analyzing apoptosis, autophagy and senescence. Apoptosis will be measured through expression of different proteins involved in the p53 pathway as Caspase, p21, p53 or Bax by polymerase chain reaction (PCR). Senescence will be studied by determining the activity of telomerase, the telomere length and the cell senescence markers (p21, p16, SIRTs) by PCR. Autophagy will be determined thought inmunohistochemical analysis and Western blotting.

Maternal-fetal nutrient transport: amino acids, glucose and lipid transport will be studied through immunohistochemical analysis, PCR and Western blotting. Placental samples are going to be immunostained with antibodies to SLC38A1 to detect System A transporter, SLC2A1 for glucose transporter, ABCA1 for cholesterol transporter and FABP1 for fatty acids transporter.

5.10.2.4 Epigenetics

Methylation profiling: cord blood and placenta samples will be used for studying the methylation profiling using Methilome analysis. It will be provided quantitative methylation measurement of 850,000 methylation sites per sample at the single-CPG-site level. Cord blood DNA will be extracted from *peripheral blood mononuclear cells* (PBMC) that were obtained from buffy coat. Placental DNA will be extracted from the samples using the DNA commercial Kit. After that, DNA was bisulfite modified, amplified, fragmented and hybridized to perform the Methilome analysis.

Bisulfite pyrosequencing DNA methylation analysis: We will use the gold-standard pyrosequencing technology, an accurate and quantitative sequencing assay, to determine base-specific cytosine methylation levels at different loci within the CpG Island of gene promoters. Pyrosequencing assays combine sodium bisulfite DNA conversion chemistry, PCR, amplification, and sequencing by synthesis assay of the target sequence. Single analysis will be performed in placental or cord blood samples for different genes resulted significant at the first general analysis, and for several genes already known to be important, such as NET, 11B-HSD2, NR3C1, serotonin transporter (SLC6A4), cholesterol transporter (ABCA1), glucose transporter (GLUT3), IGF-2 and leptin (LEP).

5.10.2.5 Microbiome

Microbiome analysis of vaginal and fecal samples of the mothers and on fecal samples of the newborns will be performed in collaboration with the Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC) (Dr Mari Carmen Collado).

DNA extraction: Nucleic acids will be extracted from the fecal samples collected for this study using commercial kits. Isolated DNA concentrations will be measured and normalized using a Qubit® 2.0 Fluorometer (Life Technology, Carlsbad, CA, USA).

Sequencing: The 16S rDNA gene (V3-V4 region) will be amplified following Illumina protocols, and the libraries were sequenced using a 2x300pb paired-end run (MiSeq Reagent kit v3) on a MiSeq-Illumina platform according to the manufacturer's instructions. To rule out and control for potential reagent contamination, the reagents for DNA extraction and PCR amplification will be also sequenced as controls.

Bioinformatics: The taxonomic affiliation of the 16S rDNA sequences will be established using both standard procedures in the Ribosomal Database Project-II or QIIME pipeline (GreenGenes/Silva data

base). Alpha diversity indices (Chao1 and Shannon), beta diversity based on UNIFRAC (phylogenetic) and Bray Curtis distance (non-phylogenetic) among the samples, and PERMANOVA will be used to test for significance. The microbial profiles will be further compared using linear discriminant analysis, as well as effective size pairwise analysis in a Galaxy environment using an alpha cut-off of 0.05 and an effect size cut-off of 2.0.

5.11 Neonatal and infant postnatal follow-up

Anthropometric measurements: BW and head circumference will be extracted from maternity hospital records. At 1, 12 and 24 months of age, weight and height as well as head circumference will be measured at planned follow-up visits. Obesity will be defined as BMI above 90th percentile. Weight catch-up will be defined when changes in the Z score of the child weight compared to the Z score at birth are higher than 0.67[210].

Neurobehavioral assessment:

1. *The Neonatal Behavioral Assessment Scale (NBAS)* will be prospectively evaluated in all newborns at 40 weeks (+/- 1-3 months) corrected age by observers accredited by The Brazelton Institute (Harvard Medical School, Boston, USA). The observers will be blinded to the study group. The examination consists of six behavioral areas rated on a 1 to 9 scale where nine is the best performance for some areas and five for others[211]. In order to compare the evaluations of the infants' behavior, the items will be grouped into six clusters as follows: habituation (range 1-6), social anime organization (range 1-5), organization of the state, regulation of the state, autonomous nervous system, and motor area. The behavioral items will be converted in percentiles according to normal curves references for our population[212], and each area will be considered abnormal at a score below 5th percentile.

2. *Ages & Stages Questionnaires, 3rd Edition (ASQ-3),* will be evaluated at a corrected age of 12 months. It's the most widely used developmental screener across the globe, designed for the use by early educators and health care professionals. It relies on parents as experts and it evaluates the progress in children between the ages of one month to 5 ½ years. It takes just 10–15 minutes for parents to complete and 2–3 minutes for professionals to score.

3. The *Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III),* which is a revision of the Bayley Scales of Infant and Toddler Development[213] will be evaluated at a corrected age of 24 months. The Bayley-III is an individually administered instrument that assesses infant development across five domains, including cognitive, language and motor competencies. Parent reported questionnaires are incorporated into the Bayley-III to assess social-emotional and adaptive behaviors.

4. *The Child Behavior Checklist (CBCL)* will be evaluated at at 5 years. It is a widely the most used method of identifying problem behavior in children[214]. There are two versions of the checklist for caregivers, depending on the age of the youth. The preschool checklist (CBCL/1½-5) is intended for use with children aged 18 months to 5 years and it contains 100 problem behavior questions. There are two "broad band" scales that combine several of the syndrome scales: *Internalizing* problems sums the Anxious/depressed, Withdrawn-depressed, and Somatic complaints scores; *externalizing* problems combines Rule-breaking and Aggressive behavior. There also is a total problems score. The standard scores are scaled so that 50 is average for the youth's age and gender, with a standard deviation of 10 points. Higher scores indicate greater problems. For each syndrome, Internalizing and Externalizing problem scales, and the total score, scores can be interpreted as falling in the normal, borderline, or clinical behavior.

5. The Wechsler Preschool and Primary Scale of Intelligence – 4th Edition (WPPSI-IV) is an innovative measure of cognitive development for preschoolers and young children, testing measures intellectual abilities in young children.

Cardiovascular assessment:

1. *Blood Pressure:* systolic and diastolic BP will be obtained at two years of age by a trained physician from the brachial artery using a validated ambulatory automated Omron 5 Series device, while the infant is resting. Blood pressure percentiles were calculated according to published reference values[170]. Heart frequency will be also registered.

2. *Carotid intima media thickness (cIMT)*: At five years of age it will be measured using Vivid Q (General Electric Healthcare, Horten, Norway), with a 12L-RS linear-array 6.0-13.0 MHz transducer. Infants will be studied when resting quietly. cIMT measurement involves obtaining longitudinal clips of the far wall of the proximal abdominal aorta in the upper abdomen76, 77. cIMT measurements will be performed offline according to a standardized protocol based on a trace method with the assistance of a commercially available software (GE EchoPAC PC 108.1.x, General Electric Healthcare). To obtain cIMT, three end-diastolic frames will be selected across a length of 10 mm and analyzed for mean and maximum cIMT, and the average reading from these three frames will be calculated.

3. Infant e*chocardiography*: Cardiovascular child evaluation will be performed at five years of age using Vivid Q (General Electric Healthcare, Horten, Norway). Children will be studied when resting quietly. A complete two-dimensional M-mode and Doppler echocardiographic examination, with a 10S-RS phased-array 4.5-11.5 MHz transducer, will be performed to assess structural heart integrity and morphometry.

- *Cardiovascular morphometric parameters*: Left atrial area, left sphericity index and wall thicknesses.

- *Systolic function parameters*: stroke volumes, heart rate, cardiac output, shortening fraction, ejection fraction, mitral and tricuspid annular plane systolic excursion (MAPSE, TAPSE) and systolic annular peak velocities (S').

- *Diastolic function parameters*: IRT, peak early (A) and late (A) transvalvular filling velocities, E/A ratio, E deceleration time, A wave duration time, early-diastolic (E') and atrial contraction (A') annular peak velocities, E/E' ratio, E'/A' ratio, isovolumetric relaxation time by TDI (IRT').

5.12 Co-variables

Epidemiological data: Parental demographics, both parents' education and professional status, maternal age, ethnicity, pre-gestational BMI smoking status, and socioeconomic level.

Maternal and obstetrical history: past medical history (chronic hypertension, diabetes, renal disease, autoimmune disease, coagulation disorders, etc.), previous preeclampsia, FGR, or fetal death, therapies before or during pregnancy.

Actual pregnancy: Assisted reproductive technologies, any treatment, any complication, blood analysis (Hemoglobin…), any practice of yoga o meditation, any diet or nutrition restriction done during pregnancy.

Maternal sleep quality: Pittsburgh sleep questionnaire.

Maternal stress exposure: The State-trait Anxiety inventory, the Perceived Stress Scale

Dietary patterns: Survey of dietary and toxic exposure (tobacco, drugs in urines).

Maternal Physical characteristics: blood pressure at the time of diagnosis, weight gain, BMI.

Maternal organ function: proteins in urine 24 hours (at enrollment and at 34 weeks), protein-to-creatinine ratio, hepatic enzymes, hemoglobin, and hematocrit at the time of enrollment and delivery.

Antenatal ultrasound findings: Estimated fetal weight and Doppler ultrasound measurements (UA PI, MCA PI, AoI, means PI of UtAs, and CPR).

Perinatal data: pregnancy complications (gestational hypertension, preeclampsia, eclampsia or HELLP syndrome), exposure to corticoids, perinatal death, gestational age at delivery, route of delivery, induction to labor, and emergency cesarean section.

Neonatal data: Gender, BW, weight percentile, Apgar score at 1 and 5 minutes, cord arterial and venous birth pH, base excess, pO_2, head circumference and percentile.

Postnatal data: BW and weight percentile, head circumference and percentile at 1, 6, 12, 24 months of age, Brazelton test at 1-3 months, Bayley test and cardiovascular profile assessment at 24 months.

6 Statistical Analysis

The primary analysis will be based on the intention-to-treat population, which is defined as all randomized participants regardless of compliance with the protocol. A secondary analysis will be performed according to effective treatment. One interim analysis of the primary outcome data will be conducted and the P value will be adjusted by Bonferroni; this analysis will be performed once the half of the sample size is obtained and results will be shown only to the Data Monitoring Committee (DMC) members and blinded to all the investigators. Early termination of the study should be considered only if the intervention effect is really great after the first interim analysis (>50% of reduction of BW<10^{th} percentile in one of the interventions groups). Specific details of the interim analysis are specified in a separate document (iSAP).
A detail statistical plan analysis (SAP) has been done before any further analysis and by a different statistician (GC) of the one that will be involved in the interim and in the final analysis (RB). A separate document will be provided.

6.1 Data Monitoring Committee (DMC)

The DMC is composed of 3 to 5 members, designed by the Chief Investigators. It is a group of independent experts that provide assessment on aspects affecting the progress, safety data and preliminary results.
For this RCT, the DMC will evaluate the results of the interim analysis, in which the DMC members are asked to endorse or not the recommendations of the statistician concerning continuation or discontinuation of the trial. The final decision will be communicated to the Chief and Principal Investigators.

7 Management

7.1 Data management and quality control of data

The participant has to agree on the handling of her personal information and their newborn within the informed consent form. Principal investigators will monitor data safety and confidentiality of patient information at all times. Subjects participating in the study will not be identified by name in any public available written or oral reports. Paper records and case report forms will be maintained in locked cabinets, rooms, or in computer files protected through the use of computer passwords. Only authorized personnel will have access to this data. Human specimens are stored under codes in locked freezers and laboratories. Only PIs and listed co-investigators can make the linkage between coded specimens and human data.

Quality assurance and quality control measurements: To reduce the "learning curve effect", several pilot scans will be performed, to standardize the technique and image views and strict scanning protocols will be established. A single high-level, high-resolution machine will be used to collect all the ultrasound and Doppler measurements. Ultrasound data collection will be monitored by a random selection of ultrasound scans by the supervisor of the study (FC).

Monitoring: To assess the reliability of data included in the questionnaires and database, an internal monitor will review medical records and material of the study. Steps will be taken to insure security and confidentiality, including distribution by certified mail and enactment of a return policy of all reports.

7.2 Ethical aspects

Standards from 1947 Nuremberg Code and 1964 Helsinki Declaration will be considered. This study will be conducted according to globally accepted standards of GCP. Data will be used only for scientific proposes, including publications or scientific-academic meeting. Confidentiality and anonymity will be ensured. Name of participant will not appear in any cases. In the first contact, the patient will be fully informed about the purpose, methods, interventions and intended possible uses of the research, including the duration of clinical assessment. Telephone information of the investigator will be included in consent format. All samples will be stored at Hospital Clinic-IDIBAPS biobank. All these departments are part of Hospital Clinic in Barcelona, and participants could voluntarily retire whenever they decide without any repercussion. Research subjects will be informed about the results of each evaluation and surveys. In case of finding any abnormality, patients will be sent for medical evaluation at their own center of attention.

7.2.1 Patient information sheet and informed consent

Before inclusion in the clinical study, each patient must receive an explanation about the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, and any discomfort it may entail. Each subject will also receive a written explanation of the purposes, procedures, and potential hazards of the project (See Consent Inform, Appendix4-6). Each patient must be informed that participation in the study is voluntary and that she may withdraw from

the study at any time. In case of acceptance, two written consent will be taken, written in non-technical language. One of them regarding to research project and other related to future uses of the bio-specimens collected. The informed consent forms are signed and dated by the participant and by the investigator.

7.2.3 Research Ethics Approval

Before initiation of the study, the protocol, all informed consents and additional material will be given to all researchers involved. The investigators named in the protocol have no financial or other competing interest that impacts their responsibilities towards the scientific value of the study.
The investigators agree to achieve and/or arrange for secure storage of study material and records for a minimum of 5 years after the close of the study.
All investigators involved in the study have to give to the PIs their curriculum vitae signed and the certificate of principles of GCP: these documents will be stored with study material.

Access to Data
During the study, access to the database and bio-bank samples will be limited to members of the study. All patient information will be both record in papers and later report in the database, in an anonymous form.

7.3 Publication policy

Findings of the study will be presented at conferences and published in several peer review journals.
A steering committee (SC), which members will be designed by the Chief Investigators, will establish a list of potential papers and the names of the investigators involved. For collaborations, the SC will take the decision of publication policy too.

8 Bibliography

1. Lyerly AD, Little MO, Faden RR. A critique of the “fetus as patient”. *Am J Bioeth*. 2008;8(7):42-4-6. doi:10.1080/15265160802331678
2. Lyerly AD, Mahowald MB. Maternal-fetal surgery for treatment of myelomeningocele. *Clin Perinatol*. 2003;30(1):155-165. doi:10.1016/S0095-5108(02)00080-5
3. Glover V. Maternal depression, anxiety and stress during pregnancy and child outcome; What needs to be done. *Best Pract Res Clin Obstet Gynaecol*. 2014;28(1):25-35. doi:10.1016/j.bpobgyn.2013.08.017
4. Dipietro JA. Maternal stress in pregnancy: Considerations for fetal development. *J Adolesc Heal*. 2012;51(2 SUPPL.). doi:10.1016/j.jadohealth.2012.04.008
5. Van Den Bergh BRH. Developmental programming of early brain and behaviour development and mental health: a conceptual framework. *Dev Med Child Neurol*. 2011;53(SUPPL.4):19-23. doi:10.1111/j.1469-8749.2011.04057.x
6. Figueras F, Gardosi J. Intrauterine growth restriction: New concepts in antenatal surveillance, diagnosis, and management. *Am J Obstet Gynecol*. 2011;204(4):288-300. doi:10.1016/j.ajog.2010.08.055
7. Figueras F, Savchev S, Triunfo S, Crovetto F, Gratacos E. An integrated model with classification criteria to predict small-for-gestational-age fetuses at risk of adverse perinatal outcome. *Ultrasound Obstet Gynecol*. 2015;45(3):279-285. doi:10.1002/uog.14714
8. Richardus JH, Graafmans WC, Verloove-Vanhorick SP, et al. Differences in perinatal mortality and suboptimal care between 10 European regions: Results of an international audit. *BJOG An Int J Obstet Gynaecol*. 2003;110(2):97-105. doi:10.1046/j.1471-0528.2003.02053.x
9. Kady SM, Gardosi J. Perinatal mortality and fetal growth restriction. *Best Pr Res Clin Obs Gynaecol*. 2004;18(3):397-410.
10. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med*. 1999;340(16):1234-1238. doi:10.1056/NEJM199904223401603
11. Breeze ACG, Lees CC. Prediction and perinatal outcomes of fetal growth restriction. *Semin Fetal Neonatal Med*. 2007;12(5):383-397. doi:10.1016/j.siny.2007.07.002
12. Larroque B, Bertrais S, Czernichow P, Léger J. School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study. *Pediatrics*. 2001;108(1):111-115. doi:10.1542/peds.108.1.111
13. O’Keeffe MJ, O’Callaghan M, Williams GM, Najman JM, Bor W. Learning, cognitive, and attentional problems in adolescents born small for gestational age. *Pediatrics*. 2003;112(2):301-307. doi:10.1542/peds.112.2.301
14. Leitner Y, Fattal-Valevski A, Geva R, et al. Neurodevelopmental outcome of children with intrauterine growth retardation: a longitudinal, 10-year prospective study. *J Child Neurol*. 2007;22(5):580-587. doi:10.1177/0883073807302605
15. Arcangeli T, Thilaganathan B, Hooper R, Khan KS, Bhide A. Neurodevelopmental delay in small babies at term: A systematic review. *Ultrasound Obstet Gynecol*. 2012;40(3):267-275. doi:10.1002/uog.11112
16. Lee PA, Chernausek SD, Hokken-Koelega ACS, Czernichow P, International Small for Gestational Age Advisory Board. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics*. 2003;111(6 Pt 1):1253-1261. doi:10.1542/peds.111.6.1253
17. Cox S. ACOG Practice Bulletin No. 12: Interuterine Growth Restriction. *ACOG Pract Bull*. 2000;(12). doi:10.1097/AOG.0b013e3181e8b073
18. Figueras F, Meler E, Iraola A, et al. Customized birthweight standards for a Spanish population. *Eur J Obstet Gynecol Reprod Biol*. 2008;136(1):20-24. doi:10.1016/j.ejogrb.2006.12.015
19. Barker DJ., Godfrey K., Gluckman P., Harding J., Owens J., Robinson J. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993;341(8850):938-941. doi:10.1016/0140-6736(93)91224-A
20. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England

and Wales. *Lancet*. 1986;1(8489):1077-1081. doi:S0140-6736(86)91340-1 [pii]
21. Barker D. The fetal and infant origins of adult disease. *BMJ Br Med J*. 1990;301(156):1111. doi:10.1136/bmj.301.6761.1111
22. Sanz-Cortés M, Figueras F, Bonet-Carne E, et al. Fetal brain MRI texture analysis identifies different microstructural patterns in adequate and small for gestational age fetuses at term. *Fetal Diagn Ther*. 2013;33(2):122-129. doi:10.1159/000346566
23. Sanz-Cortes M, Egaña-Ugrinovic G, Zupan R, Figueras F, Gratacos E. Brainstem and cerebellar differences and their association with neurobehavior in term small-for-gestational-age fetuses assessed by fetal MRI. *Am J Obstet Gynecol*. 2014;210(5). doi:10.1016/j.ajog.2013.12.008
24. Egaña-Ugrinovic G, Sanz-Cortes M, Figueras F, Bargalló N, Gratacós E. Differences in cortical development assessed by fetal MRI in late-onset intrauterine growth restriction. *Am J Obstet Gynecol*. 2013;209(2). doi:10.1016/j.ajog.2013.04.008
25. Egaña-Ugrinovic G, Sanz-Cortes M, Figueras F, Couve-Perez C, Gratacõs E. Fetal MRI insular cortical morphometry and its association with neurobehavior in late-onset small-for-gestational-age fetuses. *Ultrasound Obstet Gynecol*. 2014;44(3):322-329. doi:10.1002/uog.13360
26. Egaña-Ugrinovic G, Sanz-Cortés M, Couve-Pérez C, Figueras F, Gratacós E. Corpus callosum differences assessed by fetal MRI in late-onset intrauterine growth restriction and its association with neurobehavior. *Prenat Diagn*. 2014;34(9):843-849. doi:10.1002/pd.4381
27. Sanz-Cortes M, Simoes R V, Bargallo N, Masoller N, Figueras F, Gratacos E. Proton Magnetic Resonance Spectroscopy Assessment of Fetal Brain Metabolism in Late-Onset "Small for Gestational Age" versus "Intrauterine Growth Restriction" Fetuses. *Fetal Diagn Ther*. 2014:108-116. doi:10.1159/000365102
28. Sanz-Cortes M, Egaña-Ugrinovic G, Simoes R V, Vazquez L, Bargallo N, Gratacos E. Association of brain metabolism with sulcation and corpus callosum development assessed by MRI in late-onset small fetuses. *Am J Obstet Gynecol*. 2015;212(6):804.e1-8. doi:10.1016/j.ajog.2015.01.041
29. Eixarch E, Meler E, Iraola A, et al. Neurodevelopmental outcome in 2-year-old infants who were small-for-gestational age term fetuses with cerebral blood flow redistribution. *Ultrasound Obstet Gynecol*. 2008;32(7):894-899. doi:10.1002/uog.6249
30. Figueras F, Oros D, Cruz-Martinez R, et al. Neurobehavior in term, small-for-gestational age infants with normal placental function. *Pediatrics*. 2009;124(5):e934-e941. doi:10.1542/peds.2008-3346
31. Crispi F, Bijnens B, Figueras F, et al. Fetal growth restriction results in remodeled and less efficient hearts in children. *Circulation*. 2010;121(22):2427-2436. doi:10.1161/CIRCULATIONAHA.110.937995
32. Cruz-Lemini M, Crispi F, Valenzuela-Alcaraz B, et al. Fetal cardiovascular remodelling persists at 6 months of life in infants with intrauterine growth restriction. *Ultrasound Obstet Gynecol*. 2016;48(3):349-356. doi:10.1002/uog.15767
33. Verkauskiene R, Figueras F, Deghmoun S, Chevenne D, Gardosi J, Levy-Marchal M. Birth weight and long-term metabolic outcomes: Does the definition of smallness matter? *Horm Res*. 2008;70(5):309-315. doi:10.1159/000157878
34. Lindqvist PG, Molin J. Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound Obstet Gynecol*. 2005;25(3):258-264. doi:10.1002/uog.1806
35. Diego MA, Jones NA, Field T, et al. Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosom Med*. 2006;68(5):747-753. doi:10.1097/01.psy.0000238212.21598.7b
36. Rice F, Harold GT, Boivin J, Hay DF, van den Bree M, Thapar A. Disentangling prenatal and inherited influences in humans with an experimental design. *Proc Natl Acad Sci U S A*. 2009;106(7):2464-2467. doi:10.1073/pnas.0808798106
37. DiPietro JA, Costigan KA, Gurewitsch ED. Fetal response to induced maternal stress. *Early Hum Dev*. 2003;74(2):125-138. doi:10.1016/j.earlhumdev.2003.07.001
38. Cruz-Martinez R, Figueras F, Oros D, et al. Cerebral blood perfusion and neurobehavioral performance in full-term small-for-gestational-age fetuses. *Am J Obstet Gynecol*. 2009;201(5). doi:10.1016/j.ajog.2009.05.028
39. Cruz-Martinez R, Figueras F, Hernandez-Andrade E, Puerto B, Gratacós E. Longitudinal brain perfusion changes in near-term small-for-gestational-age fetuses as measured by spectral Doppler indices or by fractional moving blood volume. *Am J Obstet Gynecol*. 2010;203(1). doi:10.1016/j.ajog.2010.02.049

40. Oros D, Figueras F, Cruz-Martinez R, et al. Middle versus anterior cerebral artery Doppler for the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational-age fetuses with normal umbilical artery Doppler. *Ultrasound Obstet Gynecol*. 2010;35(4):456-461. doi:10.1002/uog.7588
41. Cruz-Martínez R, Figueras F, Hernandez-Andrade E, Oros D, Gratacos E. Fetal brain Doppler to predict cesarean delivery for nonreassuring fetal status in term small-for-gestational-age fetuses. *Obstet Gynecol*. 2011;117(3):618-626. doi:10.1097/AOG.0b013e31820b0884
42. Savchev S, Figueras F, Cruz-Martinez R, Illa M, Botet F, Gratacos E. Estimated weight centile as a predictor of perinatal outcome in small-for-gestational-age pregnancies with normal fetal and maternal Doppler indices. *Ultrasound Obstet Gynecol*. 2012;39(3):299-303. doi:10.1002/uog.10150
43. Savchev S, Figueras F, Sanz-Cortes M, et al. Evaluation of an optimal gestational age cut-off for the definition of early- and late-onset fetal growth restriction. *Fetal Diagn Ther*. 2014;36(2):99-105. doi:10.1159/000355525
44. Miranda J, Crispi F, Paules C, Noell G, Crovetto F, Cañellas N, Youssef L, Simoes R, Eixarch E, Faner R GE. Identification of Phenotypes of Fetal Growth Restriction using a Network-Based Clustering Approach. *Prep*. 2019;(In preparation).
45. Godfrey KM, Barker DJP. Fetal nutrition and adult disease. In: *American Journal of Clinical Nutrition*. Vol 71. ; 2000.
46. Mason JB, Shrimpton R, Saldanha LS, et al. The first 500 days of life: Policies to support maternal nutrition. *Glob Health Action*. 2015;8(1). doi:10.3402/gha.v7.23623
47. Kaiser L, Allen LH. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. *J Am Diet Assoc*. 2008;108(3):553-561. doi:10.1016/j.jada.2008.01.030
48. Blumfield ML, Hure AJ, MacDonald-Wicks L, Smith R, Collins CE. Systematic review and meta-analysis of energy and macronutrient intakes during pregnancy in developed countries. *Nutr Rev*. 2012;70(6):322-336. doi:10.1111/j.1753-4887.2012.00481.x
49. Blumfield ML, Hure AJ, MacDonald-Wicks L, Smith R, Collins CE. A systematic review and meta-analysis of micronutrient intakes during pregnancy in developed countries. *Nutr Rev*. 2013;71(2):118-132. doi:10.1111/nure.12003
50. Timmermans S, Steegers-Theunissen RP, Vujkovic M, et al. The Mediterranean diet and fetal size parameters: The Generation R Study. *World Rev Nutr Diet*. 2014;109:102-103. doi:10.1159/000356110
51. Chatzi L, Mendez M, Garcia R, et al. Mediterranean diet adherence during pregnancy and fetal growth: INMA (Spain) and RHEA (Greece) mother-child cohort studies. *Br J Nutr*. 2012;107(1):135-145. doi:10.1017/S0007114511002625
52. Rodriguez-Bernal CL, Rebagliato M, Iniguez C, et al. Diet quality in early pregnancy and its effects on fetal growth outcomes: the Infancia y Medio Ambiente (Childhood and Environment) Mother and Child Cohort Study in Spain. *Am J Clin Nutr*. 2010;91(6):1659-1666. doi:ajcn.2009.28866 [pii]\n10.3945/ajcn.2009.28866
53. Khoury J, Henriksen T, Christophersen B TS. Effect of a cholesterol-lowering diet on maternal, cord, and neonatal lipids, and pregnancy outcome: a randomized clinical trial.tle. *Am J Obs Gynecol*. 2005;193(4):1292-1301.
54. Illa M, Brito V, Eixarch E, Pla L, Muñoz-Moreno E, Serrano G. Figueras F, Gines S GE. No Title. In: *Abstracts of the 26th World Congress on Ultrasound in Obstetrics and Gynecology, Rome, Italy*. ; 2016.
55. Philibert A, Vanier C, Abdelouahab N, Chan HM, Mergler D. Fish intake and serum fatty acid profiles from freshwater fish. *Am J Clin Nutr*. 2006;84:1299-1307.
56. Mahaffey KR, Clickner RP, Jeffries RA. Methylmercury and omega-3 fatty acids: Co-occurrence of dietary sources with emphasis on fish and shellfish. *Environ Res*. 2008;107(1):20-29. doi:10.1016/j.envres.2007.09.011
57. Halldorsson TI, Thorsdottir I, Meltzer HM, Nielsen F, Olsen SF. Linking exposure to polychlorinated biphenyls with fatty fish consumption and reduced fetal growth among Danish pregnant women: A cause for concern? *Am J Epidemiol*. 2008;168(8):958-965. doi:10.1093/aje/kwn204
58. Oken E, Wright RO, Kleinman KP, et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. *Environ Health Perspect*. 2005;113(10):1376-1380. doi:10.1289/ehp.8041
59. Gaillard R, Bakker R, Steegers EA, Hofman A, Jaddoe VW. Maternal Lifestyle and Pregnancy

Complications: The Generation R Study. In: *Maternal Lifestyle and Pregnancy Complications The Generation R Study*. ; 2011:66-82.
60. Halldorsson TI, Meltzer HM, Thorsdottir I, Knudsen V, Olsen SF. Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women. *Am J Epidemiol*. 2007;166(6):687-696. doi:10.1093/aje/kwm133
61. Leventakou V, Roumeliotaki T, Martinez D, et al. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. *Am J Clin Nutr*. 2014;99(3):506-516. doi:10.3945/ajcn.113.067421
62. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet*. 2007;369(9561):578-585. doi:10.1016/S0140-6736(07)60277-3
63. Cruz-Lemini M, Crispi F, Valenzuela-Alcaraz B, et al. A fetal cardiovascular score to predict infant hypertension and arterial remodeling in intrauterine growth restriction. *Am J Obstet Gynecol*. 2014;210(6). doi:10.1016/j.ajog.2013.12.031
64. Demicheva E, Crispi F. Long-term follow-up of intrauterine growth restriction: Cardiovascular disorders. *Fetal Diagn Ther*. 2014;36(2):143-153. doi:10.1159/000353633
65. Gibbs BG, Forste R. Socioeconomic status, infant feeding practices and early childhood obesity. *Pediatr Obes*. 2014;9(2):135-146. doi:10.1111/j.2047-6310.2013.00155.x
66. Skilton MR, Mikkilä V, Würtz P, et al. Fetal growth, omega-3 (n-3) fatty acids, and progression of subclinical atherosclerosis: preventing fetal origins of disease? The Cardiovascular Risk in Young Finns Study. *Am J Clin Nutr*. 2013;97(1):58-65. doi:10.3945/ajcn.112.044198
67. Rodriguez-Lopez M, Osorio L, Acosta R, et al. Influence of breastfeeding and postnatal nutrition on cardiovascular remodeling induced by fetal growth restriction. *Pediatr Res*. 2016;79(1-1):100-106. doi:10.1038/pr.2015.182.
68. Murphy VE, Fittock RJ, Zarzycki PK, Delahunty MM, Smith R, Clifton VL. Metabolism of Synthetic Steroids by the Human Placenta. *Placenta*. 2007;28(1):39-46. doi:10.1016/j.placenta.2005.12.010
69. Mairesse J, Lesage J, Breton C, et al. Maternal stress alters endocrine function of the feto-placental unit in rats. *Am J Physiol Endocrinol Metab*. 2007;292:E1526-E1533. doi:10.1152/ajpendo.00574.2006
70. Glover V, Bergman K, Sarkar P, O'Connor TG. Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. *Psychoneuroendocrinology*. 2009;34(3):430-435. doi:10.1016/j.psyneuen.2008.10.005
71. O'Donnell KJ, Bugge Jensen A, Freeman L, Khalife N, O'Connor TG, Glover V. Maternal prenatal anxiety and downregulation of placental 11β-HSD2. *Psychoneuroendocrinology*. 2012;37(6):818-826. doi:10.1016/j.psyneuen.2011.09.014
72. McTernan CL, Draper N, Nicholson H, et al. Reduced placental 11beta-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *J Clin Endocrinol Metab*. 2001;86(10):4979-4983. doi:10.1210/jcem.86.10.7893
73. Miranda J, Maciás-Redondo S, Paules C, Gomez-Gomez A; Crovetto F, Basso A, Youssef L G-RMEEPOSJCFGE. The association between the medical diagnosis of fetal growth restriction and maternal antenatal stress: a prospective cohort study. *Under Submiss*. 2019;(Under submission).
74. Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: A prospective investigation. *Am J Obstet Gynecol*. 1993;169(4):858-865. doi:10.1016/0002-9378(93)90016-C
75. Rondó PHC, Ferreira RF, Nogueira F, Ribeiro MCN, Lobert H, Artes R. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr*. 2003;57(2):266-272. doi:10.1038/sj.ejcn.1601526
76. Sable MR, Wilkinson DS. Impact of Perceived Stress, Major Life Events and Pregnancy Attitudes on Low Birth Weight. *Fam Plann Perspect*. 2000;32(6):288-294. doi:10.2307/2648197
77. Lou HC, Hansen D, Nordentoft M, et al. Prenatal stressors of human life affect fetal brain development. *Dev Med Child Neurol*. 1994;36(9):826-832. doi:10.1111/j.1469-8749.1994.tb08192.x
78. Diego M a., Field T, Hernandez-Reif M, Cullen C, Schanberg S, Kuhn C. Prepartum, Postpartum, and Chronic Depression Effects on Newborns. *Psychiatry*. 2004;67(1):63-80. doi:10.1521/psyc.67.1.63.31251

79. Austin MP, Hadzi-Pavlovic D, Leader L, Saint K, Parker G. Maternal trait anxiety, depression and life event stress in pregnancy: Relationships with infant temperament. *Early Hum Dev*. 2005;81(2):183-190. doi:10.1016/j.earlhumdev.2004.07.001
80. O'Connor TG, Caprariello P, Blackmore ER, Gregory AM, Glover V, Fleming P. Prenatal mood disturbance predicts sleep problems in infancy and toddlerhood. *Early Hum Dev*. 2007;83(7):451-458. doi:10.1016/j.earlhumdev.2006.08.006
81. Bergman K, Sarkar P, O'Connor TG, Modi N, Glover V. Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy. *J Am Acad Child Adolesc Psychiatry*. 2007;46(11):1454-1463. doi:10.1097/chi.0b013e31814a62f6
82. O'Connor TG, Heron J, Golding J, Beveridge M, Glover V. Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. *Br J Psychiatry*. 2002;180(JUNE):502-508. doi:10.1192/bjp.180.6.502
83. O'Connor T, Heron J, Golding J, Glover V. Maternal antenatal anxiety and behavioural / emotional problems in children: a test of a progamming hypothesis. *J Child Psychol Psychiatry*. 2003;44(7):1025-1036. doi:10.1111/1469-7610.00187
84. Van den Bergh BRH, Marcoen a. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8-and 9-year-olds. *Child Dev*. 2004;75(4):1085-1097. doi:10.1111/j.1467-8624.2004.00727.x
85. Van den Bergh BRH, Van Calster B, Pinna Puissant S, Van Huffel S. Self-reported symptoms of depressed mood, trait anxiety and aggressive behavior in post-pubertal adolescents: Associations with diurnal cortisol profiles. *Horm Behav*. 2008;54(2):253-257. doi:10.1016/j.yhbeh.2008.03.015
86. Mesiano S, Jaffe RB. Role of growth factors in the developmental regulation of the human fetal adrenal cortex. *Steroids*. 1997;62(1):62-72. http://www.ncbi.nlm.nih.gov/pubmed/9029717.
87. Tarullo AR, Gunnar MR. Child maltreatment and the developing HPA axis. *Horm Behav*. 2006;50(4):632-639. doi:10.1016/j.yhbeh.2006.06.010
88. Räikkönen K, Pesonen AK, Heinonen K, et al. Maternal licorice consumption and detrimental cognitive and psychiatric outcomes in children. *Am J Epidemiol*. 2009;170(9):1137-1146. doi:10.1093/aje/kwp272
89. Räikkönen K, Seckl JR, Heinonen K, et al. Maternal prenatal licorice consumption alters hypothalamic-pituitary-adrenocortical axis function in children. *Psychoneuroendocrinology*. 2010;35(10):1587-1593. doi:10.1016/j.psyneuen.2010.04.010
90. Figueroa R, Maulik D. Prenatal therapy for fetal growth restriction. *Clin Obstet Gynecol*. 2006;49(2):308-319. doi:10.1097/00003081-200606000-00012
91. Gulmezoglu AM, Hofmeyr GJ. Bed rest in hospital for suspected impaired fetal growth. *Cochrane database Syst Rev*. 2000;(2):CD000034. doi:10.1002/14651858.CD000034
92. Nicolaides K., Bradley R., Soothill P., Campbell S, Bilardo C., Gibb D. Maternal Oxygen Therapy for Intrauterine Growth Retardation. *Lancet*. 1987;329(8539):942-945. doi:10.1016/S0140-6736(87)90292-3
93. Say L, Gülmezoglu AM, Hofmeyr GJ. Maternal oxygen administration for suspected impaired fetal growth. *Cochrane Database Syst Rev*. 2009;(4). doi:10.1002/14651858.CD000137
94. Gulmezoglu AM, Hofmeyr GJ. Calcium channel blockers for potential impaired fetal growth. *Cochrane Database Syst Rev*. 2000;(2):CD000049. doi:10.1002/14651858.CD000049
95. Say L, Gülmezoglu a M, Hofmeyr GJ. Maternal nutrient supplementation for suspected impaired fetal growth. *Cochrane Database Syst Rev*. 2003;(1):CD000148. doi:10.1002/14651858.CD000148
96. Gulmezoglu AM, Hofmeyr GJ. Plasma volume expansion for suspected impaired fetal growth. *Cochrane Database Syst Rev*. 2000;(2):CD000167. doi:10.1002/14651858.cd000167
97. Sieroszewski P, Suzin J, Karowicz-Bilińska A. Ultrasound evaluation of intrauterine growth restriction therapy by a nitric oxide donor (L-arginine). *J Matern Fetal Neonatal Med*. 2004;15(6):363-366. doi:10.1080/14767050410001725280
98. Royal College of Obstetricians and Gynaecologist. The Investigation and Management of the Small for Gestational Age Fetus. *Green-top Guidel No 31*. 2013;(31):1-34.
99. Bach-Faig A, Berry EM, Lairon D, et al. Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr*. 2011;14(12A):2274-2284. doi:10.1017/S1368980011002515
100. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med*. 2013;368(14):1279-1290. doi:10.1056/NEJMoa1200303

101. van den Heuvel MI, Johannes MA, Henrichs J, Van den Bergh BRH. Maternal mindfulness during pregnancy and infant socio-emotional development and temperament: The mediating role of maternal anxiety. *Early Hum Dev*. 2015;91(2):103-108. doi:10.1016/j.earlhumdev.2014.12.003
102. Kabat-Zinn J, Massion AO, Kristeller J, et al. Effectiveness of a meditation-based stress reduction program in the treatment of anxiety disorders. In: *American Journal of Psychiatry*. Vol 149. ; 1992:936-943. doi:10.1037/a0022272
103. Miller JJ, Fletcher K, Kabat-Zinn J. Three-year follow-up and clinical implications of a mindfulness meditation-based stress reduction intervention in the treatment of anxiety disorders. *Gen Hosp Psychiatry*. 1995;17(3):192-200. doi:10.1016/0163-8343(95)00025-M
104. Hofmann SG, Sawyer AT, Witt AA, Oh D. The effect of mindfulness-based therapy on anxiety and depression: A meta-analytic review. *J Consult Clin Psychol*. 2010;78(2):169-183. doi:10.1037/a0018555
105. Hoge EA, Bui E, Marques L, et al. Randomized controlled trial of mindfulness meditation for generalized anxiety disorder: effects on anxiety and stress reactivity. *J Clin Psychiatry*. 2013;74(8):786-792. doi:10.4088/JCP.12m08083
106. Ruffault A, Carette C, Lurbe i Puerto K, et al. Randomized controlled trial of a 12-month computerized mindfulness-based intervention for obese patients with binge eating disorder: The MindOb study protocol. *Contemp Clin Trials*. 2016;49:126-133. doi:10.1016/j.cct.2016.06.012
107. Loucks EB, Britton WB, Howe CJ, Eaton CB, Buka SL. Positive Associations of Dispositional Mindfulness with Cardiovascular Health: the New England Family Study. *Int J Behav Med*. 2014;22(4):540-550. doi:10.1007/s12529-014-9448-9
108. Guardino CM, Dunkel Schetter C, Bower JE, Lu MC, Smalley SL. Randomised controlled pilot trial of mindfulness training for stress reduction during pregnancy. *Psychol Health*. 2014;29(3):334-349. doi:10.1080/08870446.2013.852670
109. Woolhouse H, Mercuri K, Judd F, Brown SJ. Antenatal mindfulness intervention to reduce depression, anxiety and stress: a pilot randomised controlled trial of the MindBabyBody program in an Australian tertiary maternity hospital. *BMC Pregnancy Childbirth*. 2014;14:1-16. doi:10.1186/s12884-014-0369-z
110. Levitt P. Prenatal effects of drugs of abuse on brain development. *Drug Alcohol Depend*. 1998;51:109-125. doi:S0376-8716(98)00070-2 [pii]
111. Grossman AW, Churchill JD, McKinney BC, Kodish IM, Otte SL, Greenough WT. Experience effects on brain development: Possible contributions to psychopathology. *J Child Psychol Psychiatry Allied Discip*. 2003;44(1):33-63. doi:10.1111/1469-7610.t01-1-00102
112. Colantuoni C, Lipska BK, Ye T, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature*. 2011;478(7370):519-523. doi:10.1038/nature10524
113. Batalle D, Eixarch E, Figueras F, et al. Altered small-world topology of structural brain networks in infants with intrauterine growth restriction and its association with later neurodevelopmental outcome. *Neuroimage*. 2012;60(2):1352-1366. doi:10.1016/j.neuroimage.2012.01.059
114. Eikenes L, Martinussen MP, Lund LK, et al. Being born small for gestational age reduces white matter integrity in adulthood: a prospective cohort study. *Pediatr Res*. 2012;72(6):649-654. doi:10.1038/pr.2012.129
115. Sanz-Cortes M, Ratta GA, Figueras F, et al. Automatic Quantitative MRI Texture Analysis in Small-for-Gestational-Age Fetuses Discriminates Abnormal Neonatal Neurobehavior. *PLoS One*. 2013;8(7). doi:10.1371/journal.pone.0069595
116. Simões R V., Cruz-Lemini M, Bargalló N, Gratacós E, Sanz-Cortés M. Brain metabolite differences in one-year-old infants born small at term and association with neurodevelopmental outcome. *Am J Obstet Gynecol*. 2015;213(2):210.e1-210.e11. doi:10.1016/j.ajog.2015.04.011
117. Mutschler I, Wieckhorst B, Kowalevski S, et al. Functional organization of the human anterior insular cortex. *Neurosci Lett*. 2009;457(2):66-70. doi:10.1016/j.neulet.2009.03.101
118. Batalle D, Muñoz-Moreno E, Tornador C, et al. Altered resting-state whole-brain functional networks of neonates with intrauterine growth restriction. *Cortex*. 2016;77:119-131. doi:10.1016/j.cortex.2016.01.012
119. Eixarch E, Muñoz-Moreno E, Bargallo N, Batalle D, Gratacos E. Motor and cortico-striatal-thalamic connectivity alterations in intrauterine growth restriction. *Am J Obstet Gynecol*. 2015;214(6):725.e1-725.e9. doi:10.1016/j.ajog.2015.12.028

120. Grossman P, Niemann L, Schmidt S, Walach H. Mindfulness-based stress reduction and health benefits: A meta-analysis. *J Psychosom Res*. 2004;57(1):35-43. doi:10.1016/S0022-3999(03)00573-7
121. Smith JC. Alterations in brain any immune function produced by mindfulness meditation: Three caveats. *Psychosom Med*. 2004;66(1):148-149. doi:10.1097/01.psy.0000116716.19848.65
122. Hölzel BK, Hoge EA, Greve DN, et al. Neural mechanisms of symptom improvements in generalized anxiety disorder following mindfulness training. *NeuroImage Clin*. 2013;2(1):448-458. doi:10.1016/j.nicl.2013.03.011
123. Gard T, Hölzel BK, Sack AT, et al. Pain attenuation through mindfulness is associated with decreased cognitive control and increased sensory processing in the brain. *Cereb Cortex*. 2012;22(11):2692-2702. doi:10.1093/cercor/bhr352
124. Goldin P, Ziv M, Jazaieri H, Hahn K, Gross JJ. MBSR vs aerobic exercise in social anxiety: fMRI of emotion regulation of negative self-beliefs. *Soc Cogn Affect Neurosci*. 2013;8(1):65-72. doi:10.1093/scan/nss054
125. Kim MJ, Loucks RA, Palmer AL, et al. The structural and functional connectivity of the amygdala: From normal emotion to pathological anxiety. *Behav Brain Res*. 2011;223(2):403-410. doi:10.1016/j.bbr.2011.04.025
126. Monk CS, Telzer EH, Mogg K, et al. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with GAD. *Arch Gen Psychiatry*. 2008;65(5):568-576.
127. Lee JH, Tsunada J, Cohen YE. A model of the differential representation of signal novelty in the local field potentials and spiking activity of the ventrolateral prefrontal cortex. *Neural Comput*. 2013;25(1):157-185. doi:10.1162/NECO_a_00388
128. Wager TD, Davidson ML, Hughes BL, Lindquist MA, Ochsner KN. Prefrontal-Subcortical Pathways Mediating Successful Emotion Regulation. *Neuron*. 2008;59(6):1037-1050. doi:10.1016/j.neuron.2008.09.006
129. Lydiard RB. The role of GABA in anxiety disorders. *J Clin Psychiatry*. 2003;64 Suppl 3:21-27.
130. Amiel JM, Mathew SJ. Glutamate and anxiety disorders. *Curr Psychiatry Rep*. 2007;9(4):278-283. doi:10.1007/s11920-007-0033-7
131. Ochsner KN, Ray RD, Cooper JC, et al. For better or for worse: neural systems supporting the cognitive down-and up-regulation of negative emotion. *Neuroimage*. 2004;23(2):483-499. doi:10.1016/j.neuroimage.2004.06.030
132. Creswell JD, Way BM, Eisenberger NI, Lieberman MD. Neural correlates of dispositional mindfulness during affect labeling. *Psychosom Med*. 2007;69(6):560-565. doi:10.1097/PSY.0b013e3180f6171f
133. Doll A, Hölzel BK, Boucard CC, Wohlschläger AM, Sorg C. Mindfulness is associated with intrinsic functional connectivity between default mode and salience networks. *Front Hum Neurosci*. 2015;9:461. doi:10.3389/fnhum.2015.00461
134. Fayed N, Lopez del Hoyo Y, Andres E, et al. Brain Changes in Long-Term Zen Meditators Using Proton Magnetic Resonance Spectroscopy and Diffusion Tensor Imaging: A Controlled Study. *PLoS One*. 2013;8(3). doi:10.1371/journal.pone.0058476
135. Lester BM, Conradt E, Marsit C. Introduction to the Special Section on Epigenetics. *Child Dev*. 2016;87(1):29-37. doi:10.1111/cdev.12489
136. Grote NK, Bridge JA, Gavin AR, Melville JL, Iyengar S, Katon WJ. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Arch Gen Psychiatry*. 2010;67(10):1012-1024. doi:10.1001/archgenpsychiatry.2010.111
137. Diego MA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, Gonzalez-Quintero VH. Prenatal depression restricts fetal growth. *Early Hum Dev*. 2009;85(1):65-70. doi:10.1016/j.earlhumdev.2008.07.002
138. Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008;3(2):97-106. doi:10.4161/epi.3.2.6034
139. Radtke KM, Ruf M, Gunter HM, et al. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Transl Psychiatry*. 2011;1(7):e21. doi:10.1038/tp.2011.21

140. Weaver ICG, Cervoni N, Champagne F a, et al. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7(8):847-854. doi:10.1038/nn1276
141. Meaney MJ, Szyf M. Maternal care as a model for experience-dependent chromatin plasticity? *Trends Neurosci*. 2005;28(9):456-463. doi:10.1016/j.tins.2005.07.006
142. Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: Life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci*. 2005;7(2):103-123. doi:http://dx.doi.org/10.1523/JNEUROSCI.3652-05.2005
143. Turecki G, Meaney MJ. Effects of the Social Environment and Stress on Glucocorticoid Receptor Gene Methylation: A Systematic Review. *Biol Psychiatry*. 2016;79(2):87-96. doi:10.1016/j.biopsych.2014.11.022
144. Conradt E, Lester BM, Appleton AA, Armstrong DA, Marsit CJ. The roles of DNA methylation of NR3C1 and 11??-HSD2 and exposure to maternal mood disorder in utero on newborn neurobehavior. *Epigenetics*. 2013;8(12):1321-1329. doi:10.4161/epi.26634
145. Jensen Pena C, Monk C, Champagne FA. Epigenetic effects of prenatal stress on 11beta-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PLoS One*. 2012;7(6):e39791. doi:10.1371/journal.pone.0039791
146. Devlin AM, Brain U, Austin J, Oberlander TF. Prenatal exposure to maternal depressed mood and the MTHFR C677T variant affect SLC6A4 methylation in infants at birth. *PLoS One*. 2010;5(8). doi:10.1371/journal.pone.0012201
147. Lucas A. Programming by Early Nutrition: An Experimental Approach. *J Nutr*. 1998:407-410.
148. Tobi EW, Lumey LH, Talens RP, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*. 2009;18(21):4046-4053. doi:10.1093/hmg/ddp353
149. Soubry A, Schildkraut JM, Murtha A, et al. Paternal obesity is associated with IGF2hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. *BMC Med*. 2013;11(1):29. doi:10.1186/1741-7015-11-29
150. Vidal AC, Murphy SK, Murtha AP, et al. Associations between antibiotic exposure during pregnancy, birth weight and aberrant methylation at imprinted genes among offspring. *Int J Obes*. 2013;37(7):907-913. doi:10.1038/ijo.2013.47
151. Morales E, Groom A, Lawlor DA, Relton CL. DNA methylation signatures in cord blood associated with maternal gestational weight gain: results from the ALSPAC cohort. *BMC Res Notes*. 2014;7(1):278. doi:10.1186/1756-0500-7-278
152. Houde AA, Guay SP, Desgagné V, et al. Adaptations of placental and cord blood ABCA1 DNA methylation profile to maternal metabolic status. *Epigenetics*. 2013;8(12):1289-1302. doi:10.4161/epi.26554
153. Lecoutre S, Breton C. The cellularity of offspring's adipose tissue is programmed by maternal nutritional manipulations. *Adipocyte*. 2014;3(4):256-262. doi:10.4161/adip.29806
154. Blasco M. Telomerase beyond telomeres. *Nat Rev Cancer*. 2002;2(8):627-633. doi:10.1038/nrc862
155. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci U S A*. 1988;85(18):6622-6626. doi:10.1073/pnas.85.18.6622
156. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345(6274):458-460. doi:10.1038/345458a0
157. Sahin E, Colla S, Liesa M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*. 2011;470(7334):359-365. doi:10.1038/nature09787
158. Jaskelioff M, Muller FL, Paik J-HH, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011;469(7328):102-106. doi:10.1038/nature09603
159. Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosisSamani, N. J., Boultby, R., Butler, R., Thompson, J. R., & Goodall, A. H. (2001). Telomere shortening in atherosclerosis. Lancet, 358(9280), 472–473. http://doi.org/10.1016/S0140-6736(01)05633-1. *Lancet*. 2001;358(9280):472-473. doi:10.1016/S0140-6736(01)05633-1
160. Entringer S, Epel ES, Kumsta R, et al. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *ProcNatlAcadSciUSA*. 2011;108(33):E513-E518. doi:10.1073/pnas.1107759108
161. Entringer S, Epel ES, Lin J, et al. Maternal psychosocial stress during pregnancy is associated with

newborn leukocyte telomere length. *Am J Obstet Gynecol*. 2013;208(2). doi:10.1016/j.ajog.2012.11.033
162. Dzidic M, Boix-Amorós A, Selma-Royo M, Mira A, Collado M. Gut Microbiota and Mucosal Immunity in the Neonate. *Med Sci*. 2018. doi:10.3390/medsci6030056
163. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell*. 2012;150(3):470-480. doi:10.1016/j.cell.2012.07.008
164. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr*. 2008.
165. Siezen RJ, Kleerebezem M. The human gut microbiome: Are we our enterotypes? *Microb Biotechnol*. 2011. doi:10.1111/j.1751-7915.2011.00290.x
166. Thum C, Cookson AL, Otter DE, et al. Can Nutritional Modulation of Maternal Intestinal Microbiota Influence the Development of the Infant Gastrointestinal Tract? *J Nutr*. 2012. doi:10.3945/jn.112.166231
167. Nicolaides KH, Bakalis S, Akolekar R, Gallo DM, Poon LC. Umbilical and fetal middle cerebral artery Doppler at 30-34 weeks' gestation in the prediction of adverse perinatal outcome. *Ultrasound Obstet Gynecol*. 2015;45(4):409-420. doi:10.1002/uog.14822
168. Miranda J, Triunfo S, Rodriguez-Lopez M, Sairanen M, Kouru H, Parra-Saavedra M, Crovetto F, Figueras F, Crispi F GE. Performance of a third trimester combined screening model for the prediction of adverse perinatal outcome. *Ultrasound Obstet Gynecol*. 2016. doi:10.1002/uog.17317
169. Savchev S, Sanz-Cortes M, Cruz-Martinez R, et al. Neurodevelopmental outcome of full-term small-for-gestational-age infants with normal placental function. *Ultrasound Obstet Gynecol*. 2013;42(2):201-206. doi:10.1002/uog.12391
170. National Heart, Lung and BI. *Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report*. Vol 128.; 2011. doi:10.1542/peds.2009-2107C
171. Crane RS, Kuyken W, Hastings RP, Rothwell N, Williams JMG. Training Teachers to Deliver Mindfulness-Based Interventions: Learning from the UK Experience. *Mindfulness (N Y)*. 2010;1(2):74-86. doi:10.1007/s12671-010-0010-9
172. Julian LJ. Measures of Anxiety. *Arthritis Care*. 2011;63(0 11):1-11. doi:10.1002/acr.20561.Measures
173. Nast I, Bolten M, Meinlschmidt G, Hellhammer DH. How to measure prenatal stress? A systematic review of psychometric instruments to assess psychosocial stress during pregnancy. *Paediatr Perinat Epidemiol*. 2013;27(4):313-322. doi:10.1111/ppe.12051
174. Mindell JA, Cook RA, Nikolovski J. Sleep patterns and sleep disturbances across pregnancy. *Sleep Med*. 2015;16(4):483-488. doi:10.1016/j.sleep.2014.12.006
175. Okun ML, Schetter CD, Glynn LM. Poor sleep quality is associated with preterm birth. *Sleep*. 2011;34(11):1493-1498. doi:10.5665/sleep.1384
176. Baer R a, Smith GT, Hopkins J, Krietemeyer J, Toney L. Using self-report assessment methods to explore facets of mindfulness. *Assessment*. 2006;13(1):27-45. doi:10.1177/1073191105283504
177. Cebolla A, García-Palacios A, Soler J, Guillen V, Baños R, Botella C. Psychometric properties of the Spanish validation of the Five Facets of Mindfulness Questionnaire (FFMQ). *Eur J Psychiatry*. 2012;26(2):118-126. doi:10.4321/S0213-61632012000200005
178. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. *Am J Epidemiol*. 1994;139(12):1197-1209. doi:10.1016/j.hrthm.2012.06.008
179. Hadlock FP. Estimation of fetal weight with the use of head, body, and femur measurements—A prospective study. *AJOG*. 1985;151(3):333-337.
180. Arduini D, Rizzo G. Normal values of pulsatility index from fetal vessels: A cross-sectional study on 1556 healthy fetuses. *J Perinat Med*. 1990;18(3):165-172. doi:10.1515/jpme.1990.18.3.165
181. Cruz-Martinez R, Savchev S, Cruz-Lemini M, Mendez A, Gratacos E, Figueras F. Clinical utility of third-trimester uterine artery Doppler in the prediction of brain hemodynamic deterioration and adverse perinatal outcome in small-for-gestational-age fetuses. *Ultrasound Obstet Gynecol*. 2015;45(3):273-278. doi:10.1002/uog.14706
182. Vergani P, Roncaglia N, Andreotti C, et al. Prognostic value of uterine artery Doppler velocimetry in growth-restricted fetuses delivered near term. In: *American Journal of Obstetrics and Gynecology*. Vol 187. ; 2002:932-936. doi:10.1067/mob.2002.127137

183. Ghosh GS, Gudmundsson S. Uterine and umbilical artery Doppler are comparable in predicting perinatal outcome of growth-restricted fetuses. *BJOG An Int J Obstet Gynaecol*. 2009;116(3):424-430. doi:10.1111/j.1471-0528.2008.02057.x
184. Del Río M, Martínez JM, Figueras F, et al. Reference ranges for Doppler parameters of the fetal aortic isthmus during the second half of pregnancy. *Ultrasound Obstet Gynecol*. 2006;28(1):71-76. doi:10.1002/uog.2827
185. Rizzo G, Capponi A, Vendola M, Pietrolucci ME, Arduini D. Use of the 3-vessel view to record Doppler velocity waveforms from the aortic isthmus in normally grown and growth-restricted fetuses: comparison with the long aortic arch view. *J Ultrasound Med*. 2008;27(11):1617-1622. http://www.ncbi.nlm.nih.gov/pubmed/18946101.
186. Fouron JC, Gosselin J, Amiel-Tison C, et al. Correlation between prenatal velocity waveforms in the aortic isthmus and neurodevelopmental outcome between the ages of 2 and 4 years. *Am J Obstet Gynecol*. 2001;184(4):630-636. doi:10.1067/mob.2001.110696
187. Figueras F, Fernandez S, Eixarch E, et al. Middle cerebral artery pulsatility index: Reliability at different sampling sites. *Ultrasound Obstet Gynecol*. 2006;28(6):809-813. doi:10.1002/uog.2816
188. Forouzan I, Tian ZY. Fetal middle cerebral artery blood flow velocities in pregnancies complicated by intrauterine growth restriction and extreme abnormality in umbilical artery Doppler velocity. *Am J Perinatol*. 1996;13(3):139-142. doi:10.1055/s-2007-994311
189. Crimmins S, Desai A, Block-Abraham D, Berg C, Gembruch U, Baschat AA. A comparison of Doppler and biophysical findings between liveborn and stillborn growth-restricted fetuses. *Am J Obstet Gynecol*. 2014;211(6):669.e1-699.e10. doi:10.1016/j.ajog.2014.06.022
190. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. *Obstet Gynecol*. 1992;79(3):416-420. doi:10.1097/00006250-199203000-00018
191. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. *Obstet Gynecol*. 1987;69(5):705-709. http://www.ncbi.nlm.nih.gov/pubmed/2952911.
192. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. *Ultrasound Obstet Gynecol*. 2003;21(2):124-127. doi:10.1002/uog.20
193. Gardiner HM, Pasquini L, Wolfenden J, et al. Myocardial tissue Doppler and long axis function in the fetal heart. *Int J Cardiol*. 2006;113(1):39-47. doi:10.1016/j.ijcard.2005.10.029
194. Hernandez-Andrade E, Benavides-Serralde JA, Cruz-Martinez R, Welsh A, Mancilla-Ramirez J. Evaluation of conventional doppler fetal cardiac function parameters: E/A ratios, outflow tracts, and myocardial performance index. *Fetal Diagn Ther*. 2012;32(1-2):22-29. doi:10.1159/000330792
195. Alonso I, Borenstein M, Grant G, Narbona I, Azumendi G. Depth of brain fissures in normal fetuses by prenatal ultrasound between 19 and 30 weeks of gestation. *Ultrasound Obstet Gynecol*. 2010;36(6):693-699. doi:10.1002/uog.7660
196. Pistorius LR, Stoutenbeek P, Groenendaal F, et al. Grade and symmetry of normal fetal cortical development: A longitudinal two- and three-dimensional ultrasound study. *Ultrasound Obstet Gynecol*. 2010;36(6):700-708. doi:10.1002/uog.7705
197. Malinger G, Ben-Sira L, Lev D, Ben-Aroya Z, Kidron D, Lerman-Sagie T. Fetal brain imaging: a comparison between magnetic resonance imaging and dedicated neurosonography. *Ultrasound Obstet Gynecol*. 2004;23(4):333-340. doi:10.1002/uog.1016
198. Jiang S, Xue H, Counsell S, et al. In-utero three dimension high resolution fetal brain diffusion tensor imaging. In: *Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*. Vol 4791 LNCS. ; 2007:18-26.
199. Simões R V., Sanz-Cortes M, Muñoz-Moreno E, et al. Feasibility and Technical Features of Fetal Brain Magnetic Resonance Spectroscopy (Mrs) in 1.5t Scanners. *Am J Obstet Gynecol*. 2015;213(5):741-742. doi:10.1016/j.ajog.2015.06.033
200. Sanz-Cortes M, Simoes R V., Bargallo N, Masoller N, Figueras F, Gratacos E. Proton magnetic resonance spectroscopy assessment of fetal brain metabolism in late-onset “small for gestational age” versus “intrauterine growth restriction” fetuses. *Fetal Diagn Ther*. 2015;37(2):108-116. doi:10.1159/000365102
201. Mullins PG, McGonigle DJ, O ’gorman RL, et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA Cardiff Symposium on MRS of GABA. *Neuroimage*.

2014;86:43-52. doi:10.1016/j.neuroimage.2012.12.004
202. O'Gorman RL, Michels L, Edden RA, Murdoch JB, Martin E. In vivo detection of GABA and glutamate with MEGA-PRESS: Reproducibility and gender effects. *J Magn Reson Imaging*. 2011;33(5):1262-1267. doi:10.1002/jmri.22520
203. Simões R V., Muñoz-Moreno E, Carbajo RJ, et al. In vivo detection of perinatal brain metabolite changes in a rabbit model of intrauterine growth restriction (IUGR). *PLoS One*. 2015;10(7). doi:10.1371/journal.pone.0131310
204. Siauve N, Chalouhi GE, Deloison B, et al. Functional imaging of the human placenta with magnetic resonance. *Am J Obstet Gynecol*. 2015;213(4):S103-S114. doi:10.1016/j.ajog.2015.06.045
205. Barbieri S, Donati OF, Froehlich JM, Thoeny HC. Comparison of Intravoxel Incoherent Motion Parameters across MR Imagers and Field Strengths: Evaluation in Upper Abdominal Organs. *Radiology*. 2015;0(0):151244. doi:10.1148/radiol.2015151244
206. Caruyer E, Lenglet C, Sapiro G, Deriche R. Design of multishell sampling schemes with uniform coverage in diffusion MRI. *Magn Reson Med*. 2013;69(6):1534-1540. doi:10.1002/mrm.24736
207. Beckonert O, Keun HC, Ebbels TMD, et al. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc*. 2007;2(11):2692-2703. doi:10.1038/nprot.2007.376
208. Sanz-Cortes M, Carbajo RJ, Crispi F, Figueras F, Pineda-Lucena A GE. Metabolomic profile of umbilical cord blood plasma from early and late intrauterine growth restricted (IUGR) neonates with and without signs of brain vasodilation. *PLoS One*. 2013;8(12):e80121.
209. Redline RW. Classification of placental lesions. *Am J Obstet Gynecol*. 2015;213(4):S21-S28. doi:10.1016/j.ajog.2015.05.056
210. Skilton MR, Raitakari OT, Celermajer DS. High intake of dietary long-chain ??-3 fatty acids is associated with lower blood pressure in children born with low birth weight: NHANES 2003-2008. *Hypertension*. 2013;61(5):972-976. doi:10.1161/HYPERTENSIONAHA.111.01030
211. Nugent, J. K. & Brazelton TB. *Evaluation and Assessment.*; 2000.
212. Costa R, Figueiredo B, Tendais I, Conde A, Pacheco A, Teixeira C. Brazelton Neonatal Behavioral Assessment Scale A psychometric study in a Portuguese sample. *INFANT Behav Dev*. 2010;33(4):510-517. doi:10.1016/j.infbeh.2010.07.003
213. Macha T, Petermann F. Bayley Scales of Infant and Toddler Development, Third Edition - Deutsche Fassung. *Zeitschrift fur Psychiatr Psychol und Psychother*. 2015;63(2):139-143. doi:10.1177/0734282906297199
214. Achenbach TM, Rescorla LA. The Achenbach System of Empirically Based Assessment (ASEBA) for Ages 1.5 to 18 Years. *use Psychol Test Treat Plan outcomes Assess Vol 2 Instruments Child Adolesc (3rd ed)*. 2004:179-213. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=psyc4&NEWS=N&AN=2004-14949-007.

9 Appendix

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Improving Mothers for a better PrenAral Care Trial BarCeloNa (IMPACT BCN)

Summary of changes between protocol version 1 and version 2

1. Incorporation of names of several people involved in the trial (1.2)
2. Incorporation of microbiota analyses (rationale 3.8, collection of biological samples 5.10.1 and analysis 5.10.2.5)
3. Incorporation of an additional maternal evaluation test and measure at the time of postnatal follow-up at 24 months (lifestyle questionnaires, blood pressure: 5.8.7)
4. Incorporation of diffusion weighted imaging to MRI analysis (5.9)
5. Additional information of data monitoring committee (6.1)
6. Clarification of publication policy (7.3)
7. Incorporation of additional details to the mindfulness notebook. Such information was already included in the program from the beginning in separated booklets (Appendix 19)
8. Correction of several grammar errors across the document

Statistical Analysis Plan

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Project	***TITLE**: IMPACT Barcelona "Improving Mothers for a better PrenAtal Care Trial"* ***CODE**:* NCT03166332 ***Final Protocol**:* Version 1, 1st November 2016
Sponsor	*Fetal Medicine Research Center, BCNatal Hospital Clínic and Hospital Sant Joan de Déu, Universitat de Barcelona*
Principal Investigator	*Eduard Gratacós*
Institution	*Medical Statistics Core Facility IDIBAPS - Hospital Clínic de Barcelona*
Date and version	*16/03/2019, final version*

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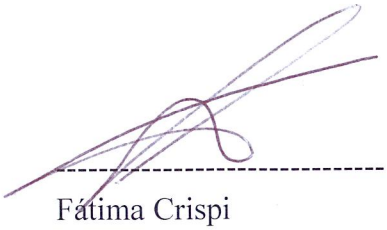

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3 SCOPE OF ANALYSIS PLAN

This Statistical Analysis Plan (SAP) covers the final analysis of all data related to the primary, secondary outcomes and specific variables (see section 7.4 for more details) collected in this trial, whereas the Interim Analysis Plan will be treated in another document. This analysis will be analyzed by the assigned project statistician. The SAP will follow the general regulatory recommendations given in the ICHE91 guidance, as well as other specific guidance on methodological and statistical issues2.

An agreed version of the SAP with the sponsor representative and project statistician will be available before database lock.

4 SOFTWARE METHODS

The SAS System3 (Release 9.4 or an upgraded version) or R version 3.5.2 (or upgrade version) will be used to analyze the data sets.

5 STUDY OBJECTIVE

- Primary endpoint
  1) Evaluate whether an interventional program, based on Mediterranean Diet (MD) or Mindfulness Based Stress Reduction (MBSR), in pregnancies at high risk to develop fetal growth restriction (30%), could reduce by 30% the prevalence of newborns with a birth weight (BW) below the 10th centile (attend improvement: reduction from 30% to 20%).
- Secondary endpoints:
  2) Compare the effectiveness of the interventions in terms of reduction of 50% (from 15% to 8%) of adverse perinatal outcome (APO), defined according to the presence of any of the following perinatal measures:
     a) Preterm birth: delivery <37 weeks' gestation
     b) Preeclampsia (PE): defined as systolic blood pressure (SBP) ≥ 140mmHg or diastolic blood pressure (DBP) ≥ 90mmHg at least 4 h apart after 20 weeks of gestation and proteinuria of ≥300 mg in 24 h
     c) Perinatal mortality: fetal or neonatal mortality (within 28 days of life)
     d) Severe fetal growth restriction: birth weight <3rd centile
     e) Metabolic acidosis: an umbilical artery pH below 7.10 and/or base excess >12 mEq/L in the newborn and/or an Apgar score at 5-minute below 7.0 assigned by the attending neonatologist or midwife
     f) Major neonatal morbidity: presence of intraventricular hemorrhage grade III/IV, necrotizing enterocolitis, periventricular leukomalacia, sepsis, bronchopulmonary dysplasia, hypoxic ischemic encephalopathy; days in neonatal intensive care unit >10.
  3) Evaluate the effectiveness of the interventions in Bayley score at two years of corrected postnatal age of the newborns.

4) Evaluate the effectiveness of the interventions in terms of SBP, DBP, mean arterial pressure and Heart Rate at two years of corrected postnatal age of the newborns.

6 TRIAL CHARACTERISTICS

6.1 STUDY DESIGN

The current study is randomized following a 1:1:1 ratio, parallel, open blind from a single center.

High risk pregnancies for Fetal Growth Restriction (FGR) are selected according to the criteria of the Royal College of Obstetricians and Gynecologists (RCOG) at mid gestation (19-23 weeks). Participants are randomized (ratio 1:1:1) to three different arms: 1) nutrition intervention based on MD with a supplementation of olive oil and walnuts, 2) Stress reduction program bases on Mindfulness techniques, MBSR, 3) usual care without any intervention.

The main hypothesis is that these two interventions during pregnancy could improve the growth of the fetus and consequently reduce the prevalence of FGR by 30% (prevalence in this population: 30%; expected prevalence: 20%). Secondary hypotheses include a reduction of adverse perinatal outcomes (mainly preeclampsia and preterm delivery), and an improvement of neurodevelopment and cardiovascular remodeling/function at two years of age.

The study design adheres to standard criteria for randomized trials, and it has been registered in the *Clinical Trials Gov*(https://clinicaltrials.gov/), # NCT03166332.

6.2 JUSTIFICATION OF SAMPLE SIZE

The study sample size (n=1218, 406 per arm) was designed on a conservative basis so as to guarantee an 80% statistical power for the most restrictive secondary endpoint (reduction of 50% of rate of adverse perinatal outcome). The sample size required for that secondary endpoint was actually more restrictive than the one required for the primary end-point (reduction of 30% of rate of neonates born with a BW <10th centile, n=1101, 367 per arm). Please refer to section 5.4 of the study protocol for more details.

Specific sample size estimation was calculated for each of the principal objectives of the study. We used for calculation the program "Power and Size Program version 3.1.2, 2014 (Department of Biostatistics, Vanderbilt University).

RANDOMIZATION PROCEDURE

Eligible participants are pregnant women resulted at high risk to develop FGR during pregnancy (odds ratio, OR>2) according to the criteria of the RCOG4. Women are selected at the moment of the routine second trimester ultrasounds scan (19-23 weeks' gestation) and randomly allocated in a 1:1:1 ratio to one of the three treatment groups:

1) Nutrition program based on MD
2) MBSR
3) No intervention (usual care)

The randomization sequence has been computer generated; it is protected and managed by the computer technician of the foundation "*Medicina Fetal Barcelona*" which has no role in recruitment. The investigators enter data of the patient that is recruited in a web site, and the computer generates automatically the inclusion number and treatment assignment. An additional code is also generated, so the patient can have access in the official website of the study to a specific part according to her intervention (https://fetalmedbarcelona.org/impactbcn/).

In a 30% random sample (of three treatment groups) will be evaluated the following characteristics: biomarkers of compliance with the nutrition program including urinary hydrohytyrosol concentration (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid concentration (to confirm compliance of walnuts), urinary total polyphenol excretion (as a measured of adhesion to overall Mediterranean dietary pattern) and biomarkers related to stress (cortisol axil) evaluated by means of maternal 24-hours urine.

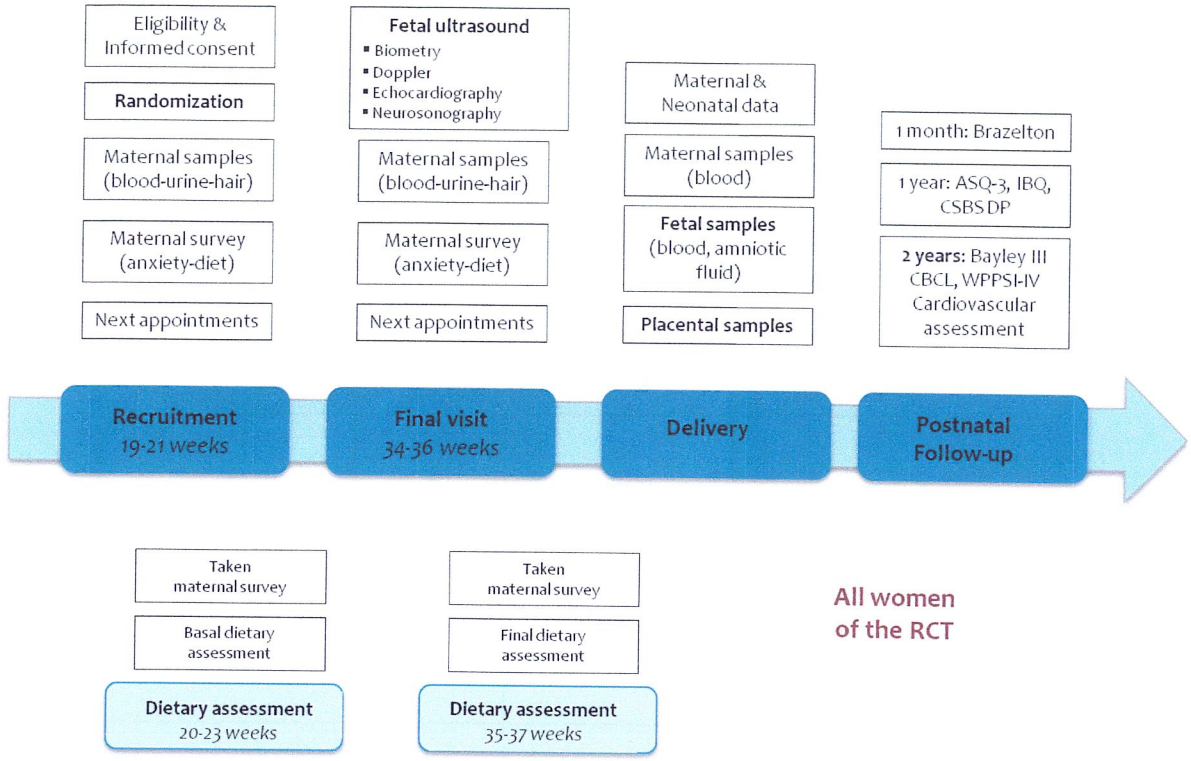
6.3 STATISTICAL INTERIM ANALYSIS AND MULTIPLICITY ADJUNTMENTS

The analysis will follow the principles specified in the ICHE91 and the CPMP/EWP/908/99 Points to Consider on Multiplicity issues in Clinical Trials guidelines5.
The study fixed final sample size is 1218 and an interim analysis is planned at the 50% of the study recruitment using the O'Brien-Fleming approach. The alpha adjusted nominal level will be 0.0015 one-sided (0.0031 two-sided) at the interim look, and 0.0245 one-sided (0.0490 two-sided) at the final analysis.
The inferential analysis for the main variable (proportion of newborns with a BW below the 10th centile) will be performed by means OR using a logistic regression model, with a 95% IC. The method of Hochberg will be use for the adjusted p-values for the comparison of each experimental arm versus control i.e. the result p-values of two comparisons will be ordered descending, the following decisions about the positive results of the study will be considered:

- If the highest p-value will be lower than the threshold (0.0490), the study is positive for the two experimental arms *vs.* control.
- If the highest p-value will be higher than the threshold (0.0490), the study is not positive for this and the next variable p-value should be lowest than the 0.0245 (0.0490/2) to be able to conclude the positive comparison of this experimental arm *vs.* control.

For the secondary analyses no alpha adjusted will be made, all secondary statistical tests will be applied with a 0.05 two-sided significance level due to the exploratory purpose.

6.4 FLOW CHART OF TRIAL PROCEDURES



CONTROL GROUP

Participants in this group follow the usual obstetrical care of the hospital. An initial and a final personal interview with the nutritionist are programmed, in order to undertake questionnaires on MD, but no more information is given. Participants are also invited to undertake blood samples analysis at the beginning and at the end of the study, and to do a specific ultrasound around 32-35 weeks' gestation.
The next table is the visit calendar programmed for these group participants.

Activity	Day	Hour	Assistance
Recruitment			
Initial nutritionist interview			
Final analytics			
3D final ultrasound			
Final nutritionist interview			
RMN			
1 month follow-up			
1 year follow-up			
2 year follow-up			

NUTRITION GROUP

The nutrition intervention is based on the results derived from the PREDIMED study6, and it is conducted by two expert dieticians and supervised by the Research Group on Nutrition, Cardiovascular Disease and Aging from IDIBAPS - Hospital Clinic of Barcelona (Prof. R. Estruch).

The intervention is based on individual visit of 30 minutes assess every month and on monthly group classes of 1 hour. Participants will receive extra-virgin olive oil (2 litters every month) and 15 g of walnuts per day at no cost (given every month for a total of 450 gr). Every two weeks woman will receive a telephone by the dietician in order to reinforce the intervention. Specific materials (recipes, a quantitative 1-week shopping list of food items according to the season of the year, a weekly plan of meals with detailed menus...) will be given and will be also available on the official web site of the trial in the Nutrition section (login with password given at the moment of randomization). During

each monthly visit, the 17-item dietary screener to assess baseline adherence to the MD will be checked, and a 3-days register diet will be also evaluated. In addition, blood pressure and weigh gained will be also measured.

Adherence and effectiveness:

Adherence to the intervention will be assessed by an improvement adherence to the MD, based on an improvement of ≥3 points of their total final score of the 17-item dietary screener compared to their total initial score. The study population will be described according to adherence to the MD, depending on the score: ≥12 high adherence; 6-11 moderate adherence; <6 low adherence.

At the end of the treatment, biomarkers of compliance will be evaluated in a random sample of 30% of participants, including urinary hydrohytyrosol concentration (to confirm compliance of extra-virgin oil) and plasma alpha-linoleic acid concentration (to confirm compliance of walnuts), as well as urinary total polyphenol excretion (as a measured of adhesion to overall Mediterranean dietary pattern).

The next table is the visit calendar programmed for these group participants.

Activity		Day	Hour	Assistance
Recruitment				
Nutrition	1st visit			
	1st group session			
	2nd visit			
	2nd group			
	3rd visit			
	3th group session			
	4th visit			
	4th group session			
Final analytics				
3D final ultrasound				
RMN				
1 month follow-up				
1 year follow-up				
2 year follow-up				

MINDFULNESS GROUP

The mindfulness intervention is based on the MBSR program, which is an intervention developed in the late 1980s at the Center for Mindfulness at the University of Massachusetts Medical School by Dr. Jon Kabat-Zinn, that has been scientifically approved and recognized by university and health institutions. This program consists in 8 weeks of 2.5 hours of group classes of a 20-25 people, one full day and daily home practice. The intervention will be coordinated by co-founders of the *Institute of Mindfulness*, with instructors certified by the Center of Mindfulness in Medicine, Health Care and Society, University of Massachusetts Medical School, Worcester, MA, USA. The program has been supervised by the head of Psychiatry Service of Hospital Clinic de Barcelona (Prof. E. Vieta).
The course takes place in the hospital one day a week of 2.5 hours for 8 weeks and with one full day. Materials (specific book and a CD or USB with meditation guides) are given at the presentation of the course at no cost.
After the course, some extra sessions are offered every two weeks until delivery, in order to keep in mind this intervention.

Adherence and effectiveness:
Adherence to the intervention will be assessed by class attendance and time spent engaged in mindfulness meditation outside of class sessions. The treatment will be considered complete if at least 10 hours of meditation are done (both during class and at home), which we considered if the woman has attended at least 6 section (out of 9 in total). Each group of intervention is composed of a maximum 25 women. At the end of the treatment, biomarkers related to stress (cortisol axis) will be evaluated in maternal 24-hours urine in a random sample of 30%.
The next table is the visit calendar programmed for these group participants.

Activity		Day	Hour	Assistance
Recruitment				
Initial nutritionist / psychologist interview				
	Orientation session			
	1st session			
	2nd session			
	3rd session			
	4th session			
	5th session			
	6th session			
	7th session			
	8th session			
	Mindfulness Day			
Final analytics				

3D final ultrasound			
Final nutritionist interview			
RMN			
1 month follow-up			
1 year follow-up			
2 year follow-up			

7 STATISTICAL ANALYSIS

7.1 ANALYSIS POPULATION

7.1.1 Description of populations

There will be the following populations for this study:

- The Intention to Treat population (ITT) is defined as all randomized patients.
- Modified Intention to Treat population (mITT) is defined as all randomized patients with neonates who did not ended with a congenital malformation, diagnosed during pregnancy or in the postnatal period.
- Population per protocol (PP) is defined as all subject included in mITT that participated to at least one visit without protocol deviation that might impact the study's main assessments. The protocol deviation considered in this study is the low compliance with treatments. The next compliance criteria will be a reason for exclusion:
    - MBSR group: the patient attends less than 6 of 9 sessions (<67 %).
    - NUTRITION group: the patient has a MD score at final assessment <3 to the initial one.

The patient who withdrew informed consent will be excluded from all populations.

The main study analysis will be performed using the mITT, pre-randomization and baseline analyses and primary and secondary efficacy analyses.

The primary outcome analysis will also be performed using:

- ITT population as a supportive.
- PP population to test the robustness of the results.

No inferential analysis will be performed for the baseline comparability.

7.1.2 Procedure to definition of the final study population

The precise reason for excluding participants from each population will be fully defined and documented independently of the randomization codes during the Data Blind Review and in a database partial lock for the variables related to the primary endpoint and first secondary endpoint analyses. The objective is to carry out the population selection and definition of the final study population as well as a preliminary assessment of the quality of the trial data.

7.2 HANDLING OF MISSING VALUES

The handling of missing data will follow the principles specified in the ICHE9[1] and the CPMP/EWP/1776/99 Rev1.Guideline on Missing Data in confirmatory trials[7].

Formal imputations will be only performed for the main variable (proportion of FGR newborns (BW <10th centile)) where missing data will be imputed by the follow two strategies:

- The main missing imputation strategy: the worst case will be imputed for all causes of missing data, i.e BW < 10th centile. *(Cod: 166, 1130)*
- The secondary missing imputation strategy: multiple imputation with the observed rates in the control group in all cases.

The main analysis will be considered the worst-case imputation and the multiple imputation will be a sensitivity analysis.

For the remaining efficacy variables, no formal imputation will be performed, and the available data only (ADO) approach will be used.

7.3 FLOW DIAGRAM

A flow diagram will be performed according to ICHE3 and the consort statement in order to summarize the number of patients at study losses by time at each stage. Patients screened, eligible, consented, randomized, receiving their allocated treatment, withdrawing, lost to follow up, and included in the different populations sets defined in the section 7.1.1.

7.4 VARIABLES

7.4.1 Demographic characteristics, pre-randomization and baseline

The following pre-randomization characteristics will be analyzed:

- Informed consent
- Eligibility criteria according to RCOG criteria (major and minor criteria):
  - Number of major criteria and number of minor criteria (the eligibility criteria are at least one major criteria or ≥3 minor criteria).
  - Individually major criteria Yes/No: maternal age >40 years, obesity (body mass index, BMI ≥35), smoking ≥11 cigarettes/day, daily vigorous exercise, drugs abuse, previous pregnancy history (previous small-for-gestational-age, SGA, such as newborn <p10, previous stillbirth or previous PE, PE <34 weeks), medical history (chronic hypertension, diabetes mellitus, renal disease or autoimmune disease), first trimester data (uterine arteries pulsatility index, PI, >p95, high risk of PE (>1/75) or PAPP-A <0.4 MoMs), echogenic bowel, heavy bleeding similar to menses, maternal SGA newborn (<p10), paternal SGA newborn (<p10).
  - Individually minor criteria Yes/No: maternal age >35 years, assistive reproductive technologies, nulliparity, BMI <20 (or 25-35), smoking 1-10 cigarettes/day, previous pregnancy history (Previous PE ≥34 weeks, Pregnancy interval <6 months or Pregnancy interval >5 years)

- Inclusion and Exclusion criteria.
- Randomization: assigned treatment.
- Maternal characteristics:
    - Demographic data: last menstrual period (LMP) corrected by ultrasound, age at recruitment, ethnicity, socio-economic status, social condition, BMI before pregnancy, categorized BMI (underweight: BMI <18; normal weight: BMI ≥18 and BMI <25; overweight: BM I≥25 and BMI <30; obesity: ≥30).
    - Medical history: chronic hypertension, diabetes mellitus, renal disease, autoimmune disease, psychiatric disorders and thyroid disorders.
    - Obstetrical history: nulliparous, previous preterm birth, previous PE, previous SGA and previous stillbirth.
    - Assisted reproductive technologies
    - Life-style during pregnancy: smoking status, categorized smoking status (<10 day; 10-20 day; >20), alcohol, drugs, sport and Yoga/Pilates.

7.4.2 Efficacy variables

7.4.2.1 Primary efficacy variable

The primary outcome (see section 5) will be analyzed by means following variable

- Proportion of FGR newborns (BW <10th centile). The centile will be calculated adjusted mainly by gestational age at delivery and gender according to Figueras F. *et al.* "Customized birth weight standards for the Spanish population"[11]. Neonatal charts by Figueras F. *et al.* will be used.

7.4.2.2 Secondary efficacy variables

7.4.2.2.1 Secondary efficacy variables related to the main and secondary endpoints

The following variables will be analyzed as a secondary due to relationship with the primary outcome and secondary outcomes (described in section 5):

- Related to the primary outcome:
    - Birth weight (g)
    - Gestational age delivery
    - Gender
    - Birth weight centile
    - Prenatal diagnosis of FGR newborns (prenatally, not prenatally, prenatally but not confirmed postnatally)

- The first secondary outcome will be analyzed by means of proportion of subjects with at least one of the adverse perinatal of the following six conditions:
    - Preterm birth (<37 weeks)
    - Preeclampsia
    - Perinatal mortality defined as Stillbirth or Neonatal death (<28 days)
    - Severe fetal growth restriction newborns (BW <3rd centile)
    - Metabolic acidosis
    - Major neonatal morbidity

- Related to the previous secondary outcome:
    - Categorized preterm birth (severe: <32weeks; moderate: < 34weeks; mild: ≥34 weeks)
    - Early or late PE (Early: delivery <34 weeks; late: delivery ≥ 34 weeks)
    - Mild/moderate PE (Mild: no criteria of severity; Severe: presence of one criteria of severity, which requires perfusion with magnesium sulfate drug)
    - PE with/without FGR
- The second secondary outcome of neurodevelopment Bayley score at two years of corrected postnatal age will be evaluated by means of their four items total score (raw and scaled):
    - Cognitive score
    - Language score
    - Motor score
    - Social-emotional score
- The third secondary outcome of newborns cardiovascular system: SBP, DBP, Mean arterial pressure and Heart Rate.

7.4.2.2.2 Secondary efficacy variables related to perinatal and neonatal evaluation

The following variables are the list of secondary variables related to perinatal and neonatal evaluations:

- Gestational age at recruitment
- Treatment during the pregnancy: aspirin, corticoids, iron, magnesium sulfate, general vitamins, specific supplementation of docosahexaenoic acid (DHA) and others.
- Gestational Diabetes Mellitus (GDM)

- Premature Rupture of the Membranes (PROM)
- Induction of labor or elective caesarean section (CS)
- Mode of delivery
- Gestational age at delivery
- Apgar and categorized Apgar (<7 ;≥7)
- pH umbilical artery and categorized pH umbilical artery (<7.15; ≥7.15)
- Base excess umbilical artery and categorized base excess umbilical artery (<12;≥12)
- Neonatal Intensive Care Unit (NICU) admission and days in NICU

7.4.2.2.3 Secondary efficacy variables related to nutritional assessment

The following variables will be analyzed in order to evaluate de nutritional assessment:

- Evaluation of food consumption by MD questionnaire:
  - MD by item
  - Total Med diet score
  - Categorized total Med diet score (low adherence: 0-5; medium adherence: 6-11; high adherence: >11)
  - Improvement of at least 3 points from baseline
- Food Frequency Questionnaires (FFQ): Carbohydrates, Proteins, Lipids, Ethanol, Energy, Saturated fatty acids, Monosaturated fatty acids, Polyunsaturated fatty acids, Cholesterol, Sugars, Fiber, Polysaccharides, Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Zinc, Vitamin A, Retinoids, Carotenoids, Vitamin D, Vitamin E, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B9, B12 vitamin and Vitamin C (this questionnaire will be analyzed by Dr. Estruch's group)
- Physical activity questionnaire
- Minnesota physical activity questionnaire (this questionnaire will be analyzed by Dr. Estruch's group)
- Metabolites of oil consumption evaluation only in a subgroup of patients (see section 6.4): Urine hidroxitirosol and relative metabolites, OH-TY (ng/mL), 4'-O-Glu-OH-TY (ng/mL), 3'-O-Glu-OH-TY (ng/mL) and total OH-TY (ng/mL)
- Walnuts consumption evaluation only in a subgroup of patients (see section 6.4): Blood metabolites, omega 6 and omega 3

7.4.2.2.4 Secondary efficacy variables related to MBSR assessment

- Perceived Stress Scale (PSS) total score

- State-Trait Anxiety Inventory (STAI) Scale:
  - Subscale SA (state anxiety)
  - Subscale TA (trait anxiety)
- Five Facet Mindfulness Questionnaire (FFMQ) total score and the following subscale:
  - Observing
  - Describing
  - Acting Awareness
  - Nonjudging
  - Nonreactivity
- WHO Well Being Index total score
- Pittsburgh sleep quality index (PSQI) total score
- Cortisol axis only in a subgroup of patients (see section 6.4): F(total), F(Gluc), F(libre), 20aDHF, 20bDHF(total), 20bDHF(Gluc), 20bDHF(libre), 5aTHF, 5bTHF, 6OHF, E(total), E(Gluc), E(libre), 20aDHE, 20bDHE, 5bTHE and 6OHE.

7.4.2.2.5 Other secondary variables

- Final evaluation (end of study)

7.5 STATISTICAL METHODS

7.5.1 Descriptive Analysis

Results will be presented by study product with descriptive statistics appropriate to the nature of the variables:

- Continuous variables: Mean, 95%CI of Mean (95% mean confidence interval), SD (standard deviation), minimum, P25 (percentile 25), Median, P75 (percentile 75), maximum and N. Per group and globally.
- Categorical variables: total column %, each category N. Per group and globally.
- Ordinal variables with few categories (less than 10) will be described using two tables: one including continuous variables descriptive parameters (as long as the interpretation is reasonable) and the other including categorical variables descriptive parameters. For ordinal variables with >10 categories, the same approximation used for continuous variables will be applied.

Where applicable, these summaries will be provided by visit including the absolute differences from baseline.

7.5.2 Inferential Analysis

The main efficacy variable, proportion of newborns with a BW below the 10^{th} centile will be analyzed and compared between groups by means OR using a logistic regression model, with a 95% IC.

Hochberg will be use for the adjusted p-values for the comparison of each experimental arm *vs.* control (see section 6.3) only for the main efficacy variables, for the rest of inferential analysis the statistical tests will be applied with 0.05 two-sided significance without alpha correction due to the exploratory purpose.

For the secondary variables measures ones (related to perinatal/neonatal outcomes and Bayley) the following strategy will be followed:

- For the binary variables the treatment comparison will be made using same logistic regression model using for the main analysis.
- For the rest of categorical variables Fisher exact (nominal variables) or Mann-Whitney U test (ordinal variables) will be use for the treatment comparisons.
- For the continuous variables' the treatment comparison will be made by means of ANOVA model using de absolutes.

For the secondary variables measures twice (cardiovascular variables, nutritional assessment and MBSR assessment) the following strategy will be followed:

- Non-baseline comparability will be performed.
- For the binary variables the treatment comparison will be made at final time point using same logistic regression model using for the main analysis.

- For the rest of categorical variables Fisher exact (nominal variables) or Mann-Whitney U test (ordinal variables) will be use for the treatment comparisons at final time point.
- For the continuous variables will be analyzed by linear model or analysis of covariance (including the baseline values as a covariate) and the treatment group as fixed effect. The dependent variables will be the absolute values (or absolute change from baseline when applicable). Treatment effects will be estimated by means of Least Square Means (LSM) and their standard error (SE) and 95% CI. Differences between treatments will be estimated by differences between LS means and their SE and 95%CI.

7.5.3 Demographic characteristics, pre-randomization and baseline

Descriptive statistics and listings per treatment group for each variable of section 7.4.1 will be performed. This analysis will be performed using the mITT.

No inferential analysis will be performed for the baseline comparability.

7.5.4 Efficacy variables

7.5.4.1 Primary efficacy analysis

The primary efficacy analysis will be conducted to evaluate the proportion of FGR newborns (BW< 10^{th} centile).

The treatment comparison will be performed by means of OR using a logistic regression model, with a 95% IC using the impute data with the worst case on mITT set.

The Hochberg method or the adjusted p-values for the comparison of each experimental arm *vs.* control (see section 6.3 and 7.5.2 for more details).

The same analysis will be performed using the following approaches:

- As a sensitivity analysis:
  - The imputed data with the multiple imputation strategy on mITT set.
  - The ADO data on mITT set.
- As a supportive analysis:
  - Worst case imputed data on ITT set.
  - Multiple imputation data on ITT set.
  - ADO data on ITT set.
- To test the robustness of the mITT results:
  - Worst case imputed data on PP set.
  - Multiple imputation data on PP set.
  - ADO data on PP set.

7.5.4.2 Secondary efficacy analysis

The second efficacy variables listed in section 7.4.2.2 will be described and listed, also the treatment comparison will be performed according to the nature of the variable and taking into account if it is

collected ones or twice, see section 7.5.2 for more details. For these statistical tests will be applied with 0.05 two-sided significance without alpha correction due to the exploratory purpose.

All secondary analysis will be performed using the ADO approach on mITT set.

8 SUBGROUP ANALYSIS

The following subgroups are declared of special interest and they will be investigated for the proportion of FGR newborns (BW <10th centile):

- Age: ≤40/>40
- Ethnicity: White/No White
- Socio-economic status: Low (no study or primary study and/or unemployed) /High (secondary study and employed)
- Pre-pregnancy BMI: <30/≥30
- Chronic hypertension: Yes/No
- Diabetes: Yes, gestational/Yes, pregestational/No
- Assisted reproductive technologies: Yes/No
- Parity: Nulliparous/Multiparous
- High risk of preeclampsia at first trimester screening: Yes/No
- Previous obstetrical history (preeclampsia, FGR, stillbirth or preterm birth): Yes/No
- Smoking during pregnancy: Yes/No
- Yoga/relaxation during pregnancy: Yes/No
- Baseline score for adherence by means of Total Med Diet Score (MD): low: 0-11/high: 12-17
- Baseline score for Stress by means of State-Trait Anxiety Inventory (STAI) Scale: Low/High (cut-off: 75th centile of the baseline values of the entire population)
- Baseline score for Stress by means of Perceived Stress Scale (PSS) total score: Low/High (cut-off: 75th centile of the baseline values of the entire population)

The same logistic regression model using for the main analysis will be applied to test the treatment and subgroup interaction (including subgroup and treatment per subgroup in the model). The subgroup interaction will be statistically significant considering a significant level of 10%, nevertheless the primary analysis will be performed separately by each category of subgroups as exploratory. These analyses will be shows by means of forest plot.

No other subgroup analyses are planned. In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, hey will follow the principles and regulatory recommendations.

This SAP not includes predictive models.

9 CHANGES TO THE PLANNED ANALYSIS

The initial definition of the secondary endpoints 3 and 4 have been reworded according to the general objective of the study "comparison between active groups and control group". The final definition considering for the statistical analysis will be the follow:

3) Evaluate the effectiveness of the interventions in Bayley score at two years of corrected postnatal age of the newborns.
4) Evaluate the effectiveness of the interventions in terms of SBP, DBP and heart rate at two years of corrected postnatal age of the newborns.

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Table 17. Proportion of FGR newborns (BW<10th centile). Descriptive analysis. Worst imputed data on ITT set.
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Table 75. WHO Well Being Index total score. Descriptive table. mITT set.
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Table 77. Cortisol axis by parameter (subgroup of patients). Descriptive table. mITT set.
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12 REFERENCES

1. CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500002928, last access: 12-Jan-2018.

2. EMEA Scientific Guidelines for Human Medicinal Products, Clinical Efficacy and Safety Guidelines, General Guidelines. URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000085.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac0580027549&jsenabled=true, last access: 12-Jan-2018.

3. SAS version 9.4 software, SAS Institute Inc., Cary, NC, URL: http://www.sas.com/, last access, 21Feb2014.

4. Royal College of Obstetricians and Gynaecologist. The Investigation and Management of the Small for Gestational Age Fetus. Green-top Guidel No 31. 2013;(31):1-34.

5. CPMP/EWP/908/99. Points to Consider on Multiplicity issues in Clinical Trials. URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001220.jsp&mid=WC0b01ac05807d91a4, last access: 09-Dec-2016.

6. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-1290. doi:10.1056/NEJMoa1200303.

7. CPMP/EWP/1776/99 Rev1. Guideline on Missing Data in confirmatory trials. URL:http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500096793, last access: 21-Apr-2013.

8. Verbeke G, Molenberghs G. Linear Mixed Models for longitudinal Data. New York: Springer-Verlag, 2000.

9. Brown H, Prescott R. Applied Mixed Models in Medicine. New York: J. Wiley & Sons, 1999.

10. Molenberghs G, Kenward MG. Missing data in clinical Studies. Chichester, West Susex: John Wiley & Sons, Ltd., 2007.

11. Figueras F. et al. Customized birthweight standards for a Spanish population. European Journal of Obstetrics & Gynecology and Reproductive Biology 136 (2008) 20–24.