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**EFFECTOR: Effect of Bariatric Surgery Before Pregnancy on the Vascular Function in the Offspring – Protocol of a cross-sectional follow-up study**

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<td>Van De Maele, Karolien;</td>
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<td>Universitair Ziekenhuis Brussel, Department of pediatrics, division of pediatric endocrinology; Katholieke Universiteit Leuven Groep Biomedische Wetenschappen, Research unit Organ Systems, Department of Development and Regeneration Gies, Inge; Universitair Ziekenhuis Brussel, Department of pediatrics, division of pediatric endocrinology devlieger, roland; Universitaire Ziekenhuizen Leuven, Department of obstetrics and gynecology; Katholieke Universiteit Leuven Groep Biomedische Wetenschappen, Research unit Organ Systems, Department of Development and Regeneration</td>
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EFFECTOR: Effect of Bariatric Surgery Before Pregnancy on the Vascular Function in the Offspring – Protocol of a cross-sectional follow-up study

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K Van De Maele, I Gies, R Devlieger

ABSTRACT

BACKGROUND: The offspring of obese pregnant women is at risk for adverse metabolic, inflammatory and cardiovascular programming. Comprehensive long-term data is lacking on the contribution of changes in maternal weight after bariatric surgery before pregnancy compared to lifestyle interventions during pregnancy on the cardiovascular programming of the offspring.

METHODS: EFFECTOR is designed as a cross-sectional cohort follow-up study targeting the children of different groups of women who were previously studied during their pregnancy. Four subgroups of children are identified, based on maternal weight characteristics during pregnancy: normal weight women, obese women without any intervention and obese women who underwent a lifestyle intervention during or bariatric surgery before pregnancy. Data collection is performed as a single study visit at home. Data on socio-demographic situation, food habits and psychomotor development is obtained through questionnaires. During the home visit extensive anthropometric and vascular assessment is performed. Information on body composition is provided by assessment of body weight and height, bio-electrical impedance analysis, skin fold measurements and ultrasound. Endothelial function is measured by non-invasive peripheral arterial tonometry (EndoPAT 2000®). Biomarkers performed in blood and urine analysis conclude the data collection process.

TRIAL REGISTRATION: ClinicalTrials.gov NCT02992106. (First release 5/12/2016)
KEYWORDS: Maternal Obesity, Bariatric Surgery, Lifestyle intervention during pregnancy, Long-term follow-up offspring, Endothelial Function

What is known about this subject

Obesity and its associated cardiovascular and metabolic diseases impose a considerable challenge for the health care of the future generations.

Maternal obesity during pregnancy can result in an adverse cardiovascular and metabolic outcome in the offspring.

Endothelial cell dysfunction is the reversible, preliminary state of atherosclerosis that offers a window of opportunity for a health care intervention.

What this study wants to add

This study will be the first to provide data on endothelial cell function in the offspring of mothers who underwent bariatric surgery.

A better understanding of the long-term effects of certain interventions during pregnancy will provide guidance for future strategies in tackling the epidemic of childhood obesity.

Characterization of children with an increased risk for endothelial cell dysfunction could guide clinicians in identification of children who could benefit from early screening.
INTRODUCTION

According to the World Health Organization tackling childhood obesity is one of the major global public health challenges of this century. (1) Because of a consequently rising incidence of associated diseases e.g. cardiovascular diseases and diabetes, overweight and obesity are seen to be an origin of a continuously intergenerational challenge in the health care of future generations.

The research field on maternal obesity is of broad and current interest since there are known adverse outcomes for the offspring both in short- and long-term. There is an increased risk of large for gestational age or macrosomia, delivery by caesarean section, admission to a neonatal care unit and stillbirth. (2, 3) Several hypotheses have been formulated trying to identify the in utero origin of long-term adverse metabolic effects on the offspring, including a tendency to insulin resistance, adverse body composition and lipid profile and chronic state of low-grade inflammation. (4-9)

In order to influence these in utero programming processes, the pre-conception period and early pregnancy are considered as a window of opportunity for health care providers. Consensus on the long-term effects of a lifestyle intervention during pregnancy in the offspring is conflicting since most studies use a different methodology making it difficult to draw unambiguous conclusions. (10) Scarce data might point in the direction of less macrosomia and less obesity at childhood age. (3, 11, 12) Furthermore, bariatric surgery is widely used when the desired weight loss cannot be obtained through a lifestyle intervention. The current evidence indicates favorable neonatal and long-term outcomes with less macrosomia and large for gestational age, less childhood obesity, favorable lipid profile and less chronic inflammation. (13-15) Nevertheless further research is needed to confirm these observed trends.
The cardiovascular effects on the offspring are often mentioned in the same sentence as the adverse metabolic effects but this research area has been studied significantly less. Endothelial cell dysfunction is the preliminary state of atherosclerosis that offers a window of opportunity for a health care intervention since the process is still reversible. (16) Different hypotheses identify a probable fetal origin of the condition, programming the offspring of obese pregnant women for early development of cardiovascular disease through altered fetal hemodynamics and/or increased expression of vascular inflammation markers. (5, 9, 17)

The EFFECTOR-study is designed as a cross-sectional cohort follow-up study in the offspring of mothers followed in previous studies (11, 18-23). The main outcome is the endothelial function of the offspring measured by non-invasive peripheral arterial tonometry (EndoPAT 2000®). The overall objective is to show a difference in the endothelial function of the offspring of obese pregnant woman in favor of women who underwent bariatric surgery compared to mothers with obesity with or without a lifestyle intervention during pregnancy.

METHODS & ANALYSIS

- Study outcomes

The primary aim of the study is to show a better endothelial function (measured by peripheral arterial tonometry) in the offspring of mothers who underwent bariatric surgery before pregnancy compared to the endothelial function in the offspring of mothers with obesity during pregnancy.

Our secondary outcome consists of an assessment of body composition, metabolic and inflammatory profile in the offspring after bariatric surgery compared to the other subgroups.

- Study design

The study is designed as a cross-sectional cohort study and the subjects are children of women that were recruited previously in different studies and have a maximum age of 11 years at the start of our study. The children are assigned to a specific subgroup based on the characteristics
of their mother, but all subgroups undergo the same prospective data collection procedure. There is no randomization or blinding procedure.

- **Study population and recruitment**

The EFFECTOR-study population will consist of children whose mothers were recruited in previous Flemish studies, supervised by the Ethical Committee of the University Hospital of Leuven. All of these mothers were well informed about possible follow-up studies at the time of the original study. For reasons of clarification the children are arbitrarily assigned to a subgroup according to the characteristics of the original maternal cohort, identified as can be seen in table 1.

The mothers will be contacted by letter or e-mail, including an information brochure. Afterwards, they will be contacted by phone by an investigator to inform them thoroughly and answer all their questions. The inclusion process will start with the eldest children, going gradually down to the youngest ones.

- **Power calculation and statistics**

The main primary outcome for the endothelial cell function is the reactive hyperemia index (RHI) measured by the EndoPAT device: a continuous variable, possibly not normally distributed. The power calculation is based on the existing literature and the subgroups where we expect the largest difference. (9, 24) Assuming a mean of 1.6 in the offspring of obese mothers without intervention compared to 1.9 in the normal weight group, with a standard deviation of 0.05, assuming a participation rate of one in three (35%) i.e. 45 children of obese mothers without intervention and 58 children in the control group, we have a power of 0.82 to demonstrate a statistical significant difference between the two groups (two-sided significance level < 0.05, Mann-Whitney test). If the actual difference in RHI would be larger, for example 0.5 (1.4 versus 1.9), than the power of the test increases to 0.99.

A participation rate of 35% is feasible, based on reported response rates in literature and a high response rate (almost 50%) in a follow-up study that is actually ongoing on the children.
one of the original cohorts. With a participation rate of 35%, we subsequently have 80 children of obese mothers with lifestyle intervention and 87 children of mothers after bariatric surgery, so in total 270 children. Comparing the other subgroups will be exploratory, no formal hypotheses are available in the literature.

The statistical analysis will be performed by using an intention-to-treat principle. The statistical software package IBM SPSS version 24 will be used for the analyses. Descriptive statistics will be used for the description of the study population at baseline. RHI will be assessed both as continuous variable and dichotomized (cutoff 1.67 for defining low and high RHI). As continuous outcome, a linear regression model will be used, after assessing the need for transforming the RHI into a symmetric distribution (Box-Cox transformations). As binary outcome, logistic regression models will be used. Explanatory variables are at least the four groups (obese no intervention, obese lifestyle intervention, bariatric and control), children’s age, children’s BMI, children’s metabolic profile, children’s eating behavior, socio-demographic characteristics.

- Data collection process

1. Secondary data analysis

After obtaining a written informed consent, this section of data collection will ensue by collecting available data from the previous studies. The collection of these data took place in a prospective manner at the time of the previous studies. The collection of these data will give us the opportunity to set a point of origin for all the children that will participate in our study.

The secondary data consists of the following data: maternal pre-pregnancy weight, pregnancy weight gain, pregnancy- and birth outcomes such as pregnancy duration and birth weight, relevant medical (and obstetric) history (previous bariatric surgery, identification of any pregnancy lifestyle intervention, preeclampsia); biochemical data (white blood cell count, glucose metabolism screening and CRP at time of delivery).

2. Prospective data collection
Data will be obtained by performing a single home visit for approximately 90 minutes to gather additional data independently from the age of the child at that time (depends on the year when the mother was included in the previous study). An overview of the study intervention can be seen in figure 1. This intervention is identical for all the subgroups of the cohort and will contain following parts:

**Peripheral arterial tonometry**

Pulse wave amplitude will be measured by using a finger plethysmograph (peripheral arterial tonometry) by using the EndoPAT device from Itamar Medical Ltd. Briefly explained, both index fingers of the patient are placed in the pneumatic probes. First the device performs 5 minutes of baseline measurement and thereafter a blood pressure cuff occludes the arterial flow of the arm for 5 minutes. After a rapid deflation of the cuff a reactive hyperemia takes place, which is a measure for the arterial endothelial function. There is a concomitant software package. Children on growth hormone treatment will be excluded for the test with the EndoPAT, since this drug influences the test result (25). If there is an infection ongoing, the examination will be postponed for 2 weeks. Since taking a blood sample can influence the test results as well, the blood sample will be done after the test with the EndoPAT device. (25)

**Anthropometric data**

The anthropometric data will consist of weight and height, four site skinfold measurement, neck-, waist- and hip circumference, body composition by BIA, ultrasound and blood pressure. Standing height will be measured using a portable stadiometer with a movable headboard and vertical backboard (seca 217, SECA, Hamburg, Germany). The body weight and total body fat percentage will be assessed by using a BIA (Tanita MC-780U). BMI will be calculated from measured weight and height and expressed as SDS according to the Flemish growth charts. Pubertal development will be assessed according to Tanner stages. Skin fold measurements will be performed at biceps, triceps, subscapular and suprailiac site with a Harpenden skinfold caliper (Baty international, RH15 9LB England). A portable ultrasound device with a linear probe 7,5 Mhz (Mindray, Diagnostic Ultrasound System, model M 7) will be used to measure
the abdominal subcutaneous fat thickness at the 4 sites of the skinfold measurements. The girths of neck, abdomen and hip will be measured with a flexible Lufkin steel anthropometric tape (Lufkin W606PM). Blood pressure will be measured using a Mindray VS-900. After performing the measurements with the EndoPAT device, Harpender kalliper and ultrasound, the children will be asked to fill out a Faces Pain Scale, validated for children aged 4-16 years old and obtained for use from the International Association for the Study of Pain. (26) The International Standards for Anthropometric Assessments set by the International Society for the Advancement of Kinanthropometry (ISAK) and standard operation procedures will be taken into account to ensure data quality. (27)

**Biochemical data**

A blood sample will be taken by a venipuncture after an overnight of fastening. The serum samples will be stored in appropriate manner during transportation to be processed in a centralized laboratory. All laboratory analyses will be performed by using the laboratory techniques available in the University Hospital of Brussels. Following biochemical parameters will be determined: glucose, insulin, total cholesterol, LDL, HDL, triglycerides, uric acid, IGF-1, adiponectin, leptin, hs-CRP, IL-6, TNF-α and ICAM-1.

A urine sample will be taken to evaluate the albumin to creatinine ratio. This urine sample will be a morning urine portion and we will ask the participants not to engage in physical activity in the preceding 48 hours. This method has been validated by Bartz et al in 2015 and is considered an early marker of endothelial dysfunction. (28)

**Questionnaires data**

Prior to the home visit, the parents will receive a web-based link to fill in the questionnaires. If they don't have a computer or internet available at their home, the investigator will offer them the chance to fill in the questionnaires on a laptop or tablet during the home visit. In order to avoid bias the investigator will not provide them with any additional information. An overview of the used questionnaires is provided in table 2.
ETHICS & DISSEMINATION

- Ethical approval and consent

The study obtained approval from the central Ethics Committee UZ Brussels, which provides final ethical approval (22/02/2017), and the local Ethics Committee UZ Leuven/ KU Leuven. The Faces Pain Scale was added as an amendment, which was approved by the Ethics Committee (15/01/0018). Changes to the study protocol will be submitted to the Ethics Committee and will not be performed before obtaining an approval.

The study will be conducted only after obtaining a prior informed consent of the legal representatives of the children and obtaining an assent of the children according to their age and maturity. The legal representatives of the children will sign an informed consent form and the assent of the children will be noted in the corresponding file. The informed consent form and the assent are reviewed and approved by the Ethics Committee prior to the start of recruitment.

The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data (Directive 95/46/EC and Belgian law of December 8, 1992 on the Protection of the Privacy in relation to the Processing of Personal Data) The data will be coded. The research team will protect the data from disclosure outside the research according to the terms of the research protocol and the informed consent document. The subject’s name or other identifiers will be stored separately from their research data and replaced with a unique code to create a new identity for the subject.

- Safety

The venipuncture for the blood sample can cause transient pain, a syncope, a hematoma or a local skin infection. The peripheral arterial tonometry can cause a tingling feeling in the occluded arm that will disappear after deflation of the cuff. All of these aforementioned adverse events are not considered as serious adverse events. If they occur however, they will be reported according to the rules of Good Clinical Practice (GCP) in the Case Report Form.
• **Publication policy**

All publication rights of unprocessed and processed data belong to the investigators. The data are coded and treated confidential. Participant anonymity is guaranteed during publication of the study results.
TABLES

**Table 1:** Overview of original maternal cohorts and characteristics during pregnancy.

<table>
<thead>
<tr>
<th>Maternal pregnancy characteristics</th>
<th>Original cohort</th>
<th>Women in cohort</th>
<th>Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bariatric surgery before pregnancy</td>
<td>PABAS (Pregnancy After Bariatric Surgery) cohort (20)</td>
<td>N = 49</td>
<td>Five Flemish hospitals, Inclusion before 15 weeks of gestation</td>
</tr>
<tr>
<td></td>
<td>AURORA cohort (bAriatric sUrgery Registration in wOmens of Reproductive Age) (22)</td>
<td>Aim 200 (still recruiting)</td>
<td>Eight Flemish hospitals, Inclusion before bariatric surgery</td>
</tr>
<tr>
<td>Normal weight</td>
<td>Bogaerts et al 2013 (18)</td>
<td>N = 156</td>
<td>Three regional Belgian hospitals</td>
</tr>
<tr>
<td>Overweight or obesity during pregnancy</td>
<td>Guelinckx et al 2010 (21)</td>
<td>N = 65</td>
<td>University Hospital of Leuven</td>
</tr>
<tr>
<td></td>
<td>Bogaerts et al 2013 (18)</td>
<td>N = 63</td>
<td>Three regional Belgian hospitals</td>
</tr>
<tr>
<td>Overweight or obesity with lifestyle intervention during pregnancy</td>
<td>DALI cohort (23, 29)</td>
<td>N = 100</td>
<td>University Hospital of Leuven, part of European RCT</td>
</tr>
<tr>
<td></td>
<td>Guelinckx et al 2010 (21)</td>
<td>N = 130</td>
<td>University Hospital of Leuven</td>
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</table>
**Table 2**: Overview of used questionnaires in the data collection process with the purpose.

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Who?</th>
<th>Purpose</th>
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<tr>
<td>Socio-demographic</td>
<td>All</td>
<td>Obtain family composition, education level and employment status of the parents, medical history and school level of the child</td>
</tr>
<tr>
<td>(designed for the study)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch Eating Behaviour (30)</td>
<td>All</td>
<td>Measurement of restrained, emotional and external eating</td>
</tr>
<tr>
<td>Food Frequency (31-33)</td>
<td>All</td>
<td>Evaluation of food intake based on the frequency and portion size of 47 food items.</td>
</tr>
<tr>
<td>Developmental milestones</td>
<td>All</td>
<td>Obtain child’s age at achieving the most obvious developmental milestones</td>
</tr>
<tr>
<td>(designed for the study – short 8-item)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strengths and Difficulties Questionnaire (SDQ) (34-36)</td>
<td>All</td>
<td>Screening tool for mental health problems and common forms of psychopathology</td>
</tr>
<tr>
<td>Developmental Coordination Disorder Questionnaire (37)</td>
<td>≥ 5 years</td>
<td>Detect DCD in children aged 5 to 15 years</td>
</tr>
<tr>
<td>Ages and Stages Questionnaire (38)</td>
<td>≤ 5 years</td>
<td>Screening tool for neurodevelopmental disorders</td>
</tr>
<tr>
<td>Pediatric Quality of Life Inventory (PEDS QL) (39)</td>
<td>≥ 6 years</td>
<td>Measure the health-related quality of life according to the core dimensions of health</td>
</tr>
<tr>
<td>Children’s Behavior Questionnaire (CBQ – very short form) (40)</td>
<td>≤ 6 years</td>
<td>Investigate temperament in preschool children</td>
</tr>
<tr>
<td>Child Behavior Checklist (CBCL)</td>
<td>≥ 6 years</td>
<td>Additional differentiating (eventual) psychopathology</td>
</tr>
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FIGURE LEGENDS

**Figure 1**: This figure provides a graphic overview of the data collection process as it was designed for the EFFECTOR study.

REFERENCES


AUTHORS’ CONTRIBUTIONS

K.V.D.M., I.G. and R.D. contributed to the design of the study. K.V.D.M. wrote the manuscript. I.G. and R.D. did a critical revision of the manuscript. The Funding organizations performed a peer review of the protocol.

COMPETING INTERESTS

All authors have nothing to disclose.

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What is known about this subject

- Obesity and its associated cardiovascular and metabolic diseases impose a considerable challenge for the health care of the future generations.

- Maternal obesity during pregnancy can result in an adverse cardiovascular and metabolic outcome in the offspring.

- Endothelial cell dysfunction is the reversible, preliminary state of atherosclerosis that offers a window of opportunity for a health care intervention.

What this study wants to add

- A better understanding of the long-term effects of certain interventions during pregnancy will provide guidance for future strategies in tackling the epidemic of childhood obesity.

- Characterization of children with an increased risk for endothelial cell dysfunction could guide clinicians in identification of children who could benefit from early screening.
INTRODUCTION

According to the World Health Organization tackling childhood obesity is one of the major global public health challenges of this century. (1) Because of a consequently rising incidence of associated diseases e.g. cardiovascular diseases and diabetes, overweight and obesity are seen to be an origin of a continuously intergenerational challenge in the health care of future generations.

The research field on maternal obesity is of broad and current interest since there are known adverse outcomes for the offspring both in short- and long-term. There is an increased risk of large for gestational age or macrosomia, delivery by caesarean section, admission to a neonatal care unit and stillbirth. (2, 3) Several hypotheses have been formulated trying to identify the in utero origin of long-term adverse metabolic effects on the offspring, including a tendency to insulin resistance, adverse body composition and lipid profile and chronic state of low-grade inflammation. (4-9)

In order to influence these in utero programming processes, the pre-conception period and early pregnancy are considered as a window of opportunity for health care providers. Consensus on the long-term effects of a lifestyle intervention during pregnancy in the offspring is conflicting since most studies use a different methodology making it difficult to draw unambiguous conclusions. (10) Scarce data might point in the direction of less macrosomia and less obesity at childhood age. (3, 11, 12) Furthermore, bariatric surgery is widely used when the desired weight loss cannot be obtained through a lifestyle intervention. The current evidence indicates favorable neonatal and long-term outcomes with less macrosomia and large for gestational age, less childhood obesity, favorable lipid profile and less chronic inflammation. (13-15) Nevertheless further research is needed to confirm these observed trends.
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METHODS & ANALYSIS

- Study outcomes

The primary aim of the study is to compare the endothelial function (measured by peripheral arterial tonometry) in the offspring of mothers who underwent bariatric surgery before pregnancy to the endothelial function in the offspring of mothers with obesity during pregnancy.

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The study is designed as a cross-sectional cohort study and the subjects are children of women that were recruited previously in different studies and have a maximum age of 11 years at the start of our study. The children are assigned to a specific subgroup based on the characteristics of their mother, but all subgroups undergo the same prospective data collection procedure. There is no randomization or blinding procedure.
• **Patient and Public Involvement**

Patients were not directly involved in the design of the study.

• **Study population and recruitment**

The EFFECTOR-study population will consist of children whose mothers were recruited in previous Flemish studies, supervised by the Ethical Committee of the University Hospital of Leuven. All of these mothers were well informed about possible follow-up studies at the time of the original study. For reasons of clarification the children are arbitrarily assigned to a subgroup according to the characteristics of the original maternal cohort, identified as can be seen in table 1.

The mothers will be contacted by letter or e-mail, including an information brochure. Afterwards, they will be contacted by phone by an investigator to inform them thoroughly and answer all their questions. The inclusion process will start with the eldest children, going gradually down to the youngest ones.

• **Power calculation and statistics**

The main primary outcome for the endothelial cell function is the reactive hyperemia index (RHI) measured by the EndoPAT device: a continuous variable, possibly not normally distributed. The power calculation in based on the existing literature and the subgroups where we expect the largest difference. (9, 24) Assuming a mean of 1.6 in the offspring of obese mothers without intervention compared to 1.9 in the normal weight group, with a standard deviation of 0.05, assuming a participation rate of one in three (35%) i.e. 45 children of obese mothers without intervention and 58 children in the control group, we have a power of 0.82 to demonstrate a statistical significant difference between the two groups (two-sided significance level < 0.05, Mann-Whitney test). If the actual difference in RHI would be larger, for example 0.5 (1.4 versus 1.9), than the power of the test increases to 0.99.
A participation rate of 35% is feasible, based on reported response rates in literature and a high response rate (almost 50%) in a follow-up study that is actually ongoing on the children of one of the original cohorts. With a participation rate of 35%, we subsequently have 80 children of obese mothers with lifestyle intervention and 87 children of mothers after bariatric surgery, so in total 270 children. Comparing the other subgroups will be exploratory, no formal hypotheses are available in the literature.

The statistical analysis will be performed by using an intention-to-treat principle. The statistical software package IBM SPSS version 24 will be used for the analyses. Descriptive statistics will be used for the description of the study population at baseline. RHI will be assessed as continuous variable. Linear regression will be used if the Gauss-Markov assumptions can be met. Otherwise, quantile regression will be used. The association between continuous explanatory variables and the outcome RHI will be assessed using splines or loess smoothers. Explanatory variables are at least the four groups (obese no intervention, obese lifestyle intervention, bariatric and control), children’s age, children’s BMI, children’s metabolic profile, children’s eating behavior, socio-demographic characteristics.

- **Data collection process**

1. **Secondary data analysis**

After obtaining a written informed consent, this section of data collection will ensue by collecting available data from the previous studies. The collection of these data took place in a prospective manner at the time of the previous studies. The collection of these data will give us the opportunity to set a point of origin for all the children that will participate in our study.

The secondary data consists of the following data: maternal pre-pregnancy weight, pregnancy weight gain, pregnancy- and birth outcomes such as pregnancy duration and birth weight, relevant medical (and obstetric) history (previous bariatric surgery, identification of any pregnancy lifestyle intervention, preeclampsia); biochemical data (white blood cell count, glucose metabolism screening and CRP at time of delivery).
2. Prospective data collection

Data will be obtained by performing a single home visit for approximately 90 minutes to gather additional data independently from the age of the child at that time (depends on the year when the mother was included in the previous study). An overview of the study intervention can be seen in figure 1. This intervention is identical for all the subgroups of the cohort and will contain following parts:

**Peripheral arterial tonometry**

Pulse wave amplitude will be measured by using a finger plethysmograph (peripheral arterial tonometry) by using the EndoPAT device from Itamar Medical Ltd. Briefly explained, both index fingers of the patient are placed in the pneumatic probes. First the device performs 5 minutes of baseline measurement and thereafter a blood pressure cuff occludes the arterial flow of the arm for 5 minutes. After a rapid deflation of the cuff a reactive hyperemia takes place, which is a measure for the arterial endothelial function. There is a concomitant software package. Children on growth hormone treatment will be excluded for the test with the EndoPAT, since this drug influences the test result (25). If there is an infection ongoing, the examination will be postponed for 2 weeks. Since taking a blood sample can influence the test results as well, the blood sample will be done after the test with the EndoPAT device. (25)

**Anthropometric data**

The anthropometric data will consist of weight and height, four site skinfold measurement, neck-, waist- and hip circumference, body composition by BIA, ultrasound and blood pressure. Standing height will be measured using a portable stadiometer with a movable headboard and vertical backboard (seca 217, SECA, Hamburg, Germany). The body weight and total body fat percentage will be assessed by using a BIA (Tanita MC-780U). BMI will be calculated from measured weight and height and expressed as SDS according to the Flemish growth charts. Pubertal development will be assessed according to Tanner stages. Skin fold measurements will be performed at biceps, triceps, subscapular and suprailiac site with a Harpenden skinfold caliper (Baty international, RH15 9LB England). A portable ultrasound device with a linear
probe 7.5 Mhz (Mindray, Diagnostic Ultrasound System, model M 7) will be used to measure
the abdominal subcutaneous fat thickness at the 4 sites of the skinfold measurements. The
girths of neck, abdomen and hip will be measured with a flexible Lufkin steel anthropometric
tape (Lufkin W606PM). Blood pressure will be measured using a Mindray VS-900. After
performing the measurements with the EndoPAT device, Harpender kalliper and ultrasound,
the children will be asked to fill out a Faces Pain Scale, validated for children aged 4-16 years
old and obtained for use from the International Association for the Study of Pain. (26) The
International Standards for Anthropometric Assesments set by the International Society for the
Advancement of Kinanthropometry (ISAK) and standard operation procedures will be taken
into account to ensure data quality. (27)

**Biochemical data**

A blood sample will be taken by a venipuncture after an overnight of fasting. The serum
samples will be stored in appropriate manner during transportation to be processed in a
centralized laboratory. All laboratory analyses will be performed by using the laboratory
techniques available in the University Hospital of Brussels. Following biochemical parameters
will be determined: glucose, insulin, total cholesterol, LDL, HDL, triglycerides, uric acid, IGF-
1, adiponectin, leptin, hs-CRP, IL-6, TNF-α and ICAM-1.

A urine sample will be taken to evaluate the albumin to creatinine ratio. This urine sample will
be a morning urine portion and we will ask the participants not to engage in physical activity in
the preceding 48 hours. This method has been validated by Bartz et al in 2015 and is
considered an early marker of endothelial dysfunction.(28)

**Questionnaires data**

Prior to the home visit, the parents will receive a web-based link to fill in the questionnaires. If
they don’t have a computer or internet available at their home, the investigator will offer them
the chance to fill in the questionnaires on a laptop or tablet during the home visit. In order to
avoid bias the investigator will not provide them with any additional information. An overview
of the used questionnaires is provided in table 2.
ETHICS & DISSEMINATION

- Ethical approval and consent

The study obtained approval from the central Ethics Committee UZ Brussels, which provides final ethical approval (22/02/2017), and the local Ethics Committee UZ Leuven/ KU Leuven. The Faces Pain Scale was added as an amendment, which was approved by the Ethics Committee (15/01/0018). Changes to the study protocol will be submitted to the Ethics Committee and will not be performed before obtaining an approval.

The study will be conducted only after obtaining a prior informed consent of the legal representatives of the children and obtaining an assent of the children according to their age and maturity. The legal representatives of the children will sign an informed consent form and the assent of the children will be noted in the corresponding file. The informed consent form and the assent are reviewed and approved by the Ethics Committee prior to the start of recruitment.

The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data (Directive 95/46/EC and Belgian law of December 8, 1992 on the Protection of the Privacy in relation to the Processing of Personal Data) The data will be coded. The research team will protect the data from disclosure outside the research according to the terms of the research protocol and the informed consent document. The subject’s name or other identifiers will be stored separately from their research data and replaced with a unique code to create a new identity for the subject.

- Safety

The venipuncture for the blood sample can cause transient pain, a syncope, a hematoma or a local skin infection. The peripheral arterial tonometry can cause a tingling feeling in the occluded arm that will disappear after deflation of the cuff. All of these aforementioned adverse events are not considered as serious adverse events. If they occur however, they will be reported according to the rules of Good Clinical Practice (GCP) in the Case Report Form.
• Publication policy

All publication rights of unprocessed and processed data belong to the investigators. The data are coded and treated confidential. Participant anonymity is guaranteed during publication of the study results.
### TABLES

**Table 1:** Overview of original maternal cohorts and characteristics during pregnancy.

<table>
<thead>
<tr>
<th>Maternal pregnancy characteristics</th>
<th>Original cohort</th>
<th>Women in cohort</th>
<th>Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bariatric surgery before pregnancy</strong></td>
<td>PABAS (Pregnancy After Bariatric Surgery) cohort (20)</td>
<td>N = 49</td>
<td>Five Flemish hospitals Inclusion before 15 weeks of gestation</td>
</tr>
<tr>
<td></td>
<td>AURORA cohort (bAriatric sUrgery Registration in wOmen of Reproductive Age) (22)</td>
<td>Aim 200 (still recruiting)</td>
<td>Eight Flemish hospitals Inclusion before bariatric surgery</td>
</tr>
<tr>
<td>Normal weight</td>
<td>Bogaerts et al 2013 (18)</td>
<td>N = 156</td>
<td>Three regional Belgian hospitals</td>
</tr>
<tr>
<td>Overweight or obesity during pregnancy</td>
<td>Guelinckx et al 2010 (21)</td>
<td>N = 65</td>
<td>University Hospital of Leuven</td>
</tr>
<tr>
<td></td>
<td>Bogaerts et al 2013 (18)</td>
<td>N = 63</td>
<td>Three regional Belgian hospitals</td>
</tr>
<tr>
<td>Overweight or obesity with lifestyle intervention during pregnancy</td>
<td>DALI cohort (23, 29)</td>
<td>N = 100</td>
<td>University Hospital of Leuven, part of European RCT</td>
</tr>
<tr>
<td></td>
<td>Guelinckx et al 2010 (21)</td>
<td>N = 130</td>
<td>University Hospital of Leuven</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>Who?</td>
<td>Purpose</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>--------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Soco-demographic (designed for the study)</td>
<td>All</td>
<td>Obtain family composition, education level and employment status of the parents, medical history and school level of the child</td>
<td></td>
</tr>
<tr>
<td>Dutch Eating Behaviour (30)</td>
<td>All</td>
<td>Measurement of restrained, emotional and external eating</td>
<td></td>
</tr>
<tr>
<td>Food Frequency (31-33)</td>
<td>All</td>
<td>Evaluation of food intake based on the frequency and portion size of 47 food items.</td>
<td></td>
</tr>
<tr>
<td>Developmental milestones (designed for the study – short 8-item)</td>
<td>All</td>
<td>Obtain child’s age at achieving the most obvious developmental milestones</td>
<td></td>
</tr>
<tr>
<td>Strengths and Difficulties Questionnaire (SDQ) (34-36)</td>
<td>All</td>
<td>Screening tool for mental health problems and common forms of psychopathology</td>
<td></td>
</tr>
<tr>
<td>Developmental Coordination Disorder Questionnaire (37)</td>
<td>≥ 5 years</td>
<td>Detect DCD in children aged 5 to 15 years</td>
<td></td>
</tr>
<tr>
<td>Ages and Stages Questionnaire (38)</td>
<td>≤ 5 years</td>
<td>Screening tool for neurodevelopmental disorders</td>
<td></td>
</tr>
<tr>
<td>Pediatric Quality of Life Inventory (PEDS QL) (39)</td>
<td>≥ 6 years</td>
<td>Measure the health-related quality of life according to the core dimensions of health</td>
<td></td>
</tr>
<tr>
<td>Children’s Behavior Questionnaire (CBQ – very short form) (40)</td>
<td>≤ 6 years</td>
<td>Investigate temperament in preschool children</td>
<td></td>
</tr>
<tr>
<td>Child Behavior Checklist (CBCL)</td>
<td>≥ 6 years</td>
<td>Additional differentiating (eventual) psychopathology</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1: This figure provides a graphic overview of the data collection process as it was designed for the EFFECTOR – study.

REFERENCES


AUTHORS’ CONTRIBUTIONS

K.V.D.M., I.G. and R.D. contributed to the design of the study. K.V.D.M. wrote the manuscript. I.G. and R.D. did a critical revision of the manuscript. The Funding organizations performed a peer review of the protocol.

COMPETING INTERESTS

All authors have nothing to disclose.

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Confidential: For Review Only

Study population

Offspring of mothers with...

1. Normal weight before and during pregnancy
2. Obesity before and during pregnancy
3. Obesity before pregnancy and a lifestyle intervention during pregnancy
4. Bariatric surgery before pregnancy

Content Study visit (once)

Questionnaires
- Age<6 years old
  - Socio-economic + general + questionnaire
  - Nutrient intake and eating behaviour:
    - Dutch Eating Behaviour Questionnaire
    - Food Frequency Questionnaire (vd. Houtbrechts)
  - Psychomotor development and behaviour:
    - Ages and Stages Questionnaire (ASQ)
    - Strengths and Difficulties Questionnaire (SDQ)
    - Questionnaire on developmental milestones
    - Children's Behavior Questionnaire (CBQ – very short form)

- Age>6 years old
  - Socio-economic + general + questionnaire
  - Pediatric Quality of Life Inventory (PedsQL)
  - Nutrient intake and eating behaviour:
    - Dutch Eating Behaviour Questionnaire
    - Food Frequency Questionnaire (vd. Houtbrechts)
  - Psychomotor development and behaviour:
    - Child behavior Checklist (CBC)
    - Strengths and Difficulties Questionnaire (SDQ)
    - Questionnaire on developmental milestones
    - Developmental Coordination Disorder Questionnaire

Anthropometry
- Actual Height and weight
- Calculated BMI
- Potential development (Tanner stage)
- Circumference of neck, waist and hip
- Skinfold measurement 4 site (Biceps, Triceps, Subscapular and Suprailiac)
- Blood pressure
- Subcutaneous fat by ultrasound
- BIA

Blood & urine sample
- Blood sample after an overnight fastening with determination of following parameters:
  - glucose, insulin, total cholesterol, LDL, HDL, triglycerides, uric acid, IGF-1, adiponectin,
  - leptin, hs-CRP, IL-6, TNF-α and (CIMT-1)
- Urine sample to evaluate albumin to creatinine ratio

Endothelial function
- Measurement of pulse wave amplitude by using the EndoPAT device, a finger plethysmograph
  (peripheral arterial tonometry)

564x809mm (72 x 72 DPI)