

# Protocol Clinical Trial

## Study synopsis

Study overview	
Title of clinical trial	Low grade inflammation in childhood obesity : an independent risk factor for endothelial dysfunction ?
Sponsor name	University Hospital of Brussels
Principal Investigator	prof. dr. Jean De Schepper / Inge Gies
Medical condition or disease under investigation	Obesity and overweight, cardiovascular risk factors
Purpose of clinical trial	To assess whether low grade inflammation in childhood obesity contributes to endothelial dysfunction
Primary objective	To investigate if the reactive hyperemia index by peripheral arterial tonometry is higher in adolescents with a history of childhood obesity and presenting a low grade inflammation in comparison with those without low grade inflammation ( hSCRp < or > 1 mg/L)
Secondary objective (s)	To analyze the changes in hCRP and adiposity in obese children and adolescents after 5 to 10 years
Trial Design	Observational cohort study
Endpoints	RH-PAT scores, measured by peripheral artery tonometry . hSCRp, measured by automated immunoturbidometry BMI z-score and % body fat by skinfold thickness
Sample Size	54 ( $\alpha = 0,05$ – power 80%)
Summary of eligibility criteria	Overweight and obese adolescents, aged 12 to 21 years, with a known level of hs-CRP at initial evaluation for metabolic syndrome
Maximum duration of intervention	Single visit
Version and date of final protocol	Protocol version 1, date 18/08/2018
Version and date of protocol amendments	Not applicable

## Study Schedule

### Administrative information

#### 1. Sponsor

The study is sponsored by the division of pediatric endocrinology of the University Hospital of Brussels (UZ Brussel).

#### 2. (Principal) Investigator(s)

Prof. dr. J. De Schepper  
Dr. K. Stabenow  
Prof. Dr. Inge Gies  
Dr. Karolien Van De Maele  
Dr. Jesse Vanbesien

#### 3. Departments involved

Department of Pediatrics  
Division of Pediatric Endocrinology  
University Hospital of Brussels  
Laarbeeklaan 101, 1090 Brussel

### Ethical and regulatory information

#### 1. Study registration

The study is registered at ClinicalTrials.gov and has a unique number: ###.

#### 2. Study type

The study is an observational cohort study

#### 3. Competent Ethics Committee

Central Ethics Committee:  
Ethical Committee University Hospital of Brussels  
Phone: 32 2 474 94 15  
Email: Centraal\_Studieloket@uzbrussel.be

#### 4. Ethical conduct of the study

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (current version), the principles of Good Clinical Practice (GCP) and in accordance with all

applicable regulatory requirements. This protocol and related documents will be submitted for review to Ethics Committee before start of the trial.

#### **5. Declaration of conflict of interest**

The investigators of this study declare that they don't have any competing interests.

#### **6. Patient information and informed consent**

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 will be reviewed by the Ethics Committee prior to the start of recruitment.

#### **7. Participant privacy and confidentiality**

The investigators shall treat all information and data relating to the study disclosed to Participating Site and/or Investigator in this Study as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the study. 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) as well as the General Data Protection of April 2018.

The data will be coded. There continues to be a link between the data and the individual who provided it. The research team is obligated to 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 are stored separately from their research data and replaced with a unique code to create a new identity for the subject.

#### **8. Early termination of the study**

Not applicable.

#### **9. Protocol amendments**

Changes to the study protocol will be submitted to the Ethics Committee and will not be performed before obtaining an approval.

#### **10. Reward**

Participant will not receive a compensation for their participation in the study. If needed, an absence certificate will be provided for the participant and/or the accompanying adult at the time of the study visit.

### **Background and rationale**

Obesity and overweight have become an important health burden in children and adolescents, with 19% of all children between 5 and 18 years being either obese or overweight in Belgium<sup>(1,2)</sup>. Obesity and especially visceral adiposity early in life may contribute to the development of cardiovascular disease at older age, as it shows tracking into adulthood and is be associated with cardiovascular risk

factors such as dyslipidemia, insulin resistance, arterial hypertension and low grade inflammation, in a variable percentage (4) (5)

Low grade inflammation, as assessed by hSCRp, was found to be present in 20.6 % and 19.8 % of overweight children and adolescents (6). In adults, hSCRp levels between 1 and 3 mg/L and above 3 mg/L are considered coronary disease risk factors (7).

Endothelial dysfunction, known to precede the formation of atherosclerotic plaque, can be assessed in a non-invasive manner in children by peripheral artery tonometry. (8) Only few studies have been performed in obese children with this bed-side technique, showing either normal or a disturbed function, reflected by a lower reactive hyperemia index (9, 10,11) The association with low grade inflammation however was assessed in only one study. We hypothesize that in adolescents and young adults with a history of childhood obesity a more disturbed endothelial function will be present in those with and /or current elevated hSCRp value.

## Study Objectives

To investigate whether obese and overweight adolescents with an elevated hs-CRP are more at risk for the development of endothelial dysfunction assessed peripheral artery tonometry, compared to those with a normal hs-CRP at their initial evaluation for obesity-associated metabolic syndrome

To assess the degree of endothelial dysfunction, assessed by the reactive hyperemia index by peripheral artery tonometry in adolescents and young adults with a history of childhood obesity in relation to their current hS CRP value

To investigate the changes in hs-CRP and adiposity in adolescence and young adulthood of initially obese children and adolescents with either normal or elevated hs-CRP levels

## Study outcomes

### **1. Primary outcome**

Reactive hyperemia index (RHI) measured by the EndoPAT device

### **2. Secondary outcome**

hs CRP value , BMI z-score and body fat percentage assessed by skinfold thickness

## Study design

### **1. Overall design**

This is an observational cohort study

### **2. Randomization/blinding procedure.**

Not applicable.

### **3. Statistical methodology**

The statistical analysis will be done with the SPSS software.

The difference in THI index between the population with and without low grade inflammation will be evaluated by the Mann Whitney U test and Multivariate analysis of variance, taking current hCRP, age, BMI z-score, and systolic blood pressure into account. The change in hS

CRP in the population with and without low grade inflammation will be analyzed by the Kruskal Wallance test.

The power calculation was done at [clincalc.com/stats/samplesize](http://clincalc.com/stats/samplesize) software. Assuming a mean RHI score of 1.51 in overweight children with a standard deviation of 0.2 (9), assuming an equal distribution between the study groups (elevated vs. normal hs-CRP), we have a power of 0.80 to demonstrate a difference of 20 % between the two groups with 27 patients enrolled in each group.

## Study population

### **1. Recruitment**

Eligible candidates will be recruited within the children with overweight and obesity having a metabolic risk evaluation before treatment in the period 2006 and 2010 at the pediatric clinic of the UZ Brussel. In total 60 participants will be investigated. Firstly, 30 participating patients with initially elevated hsCRP will be selected at random ( following the date of their initial investigation) and afterwards matched for age, BMI z-score, and blood pressure with the same number of patients without initially elevated hCRP values

### **2. Recruitment procedure**

Eligible candidates, or their parents if underage, will be personally contacted by telephone and/or e-mail by their initial pediatric endocrinologist and will be invited to partake in the study. All written information about the study will be provided beforehand.

### **3. Eligibility criteria**

#### ➤ Inclusion Criteria:

- Age 12-21 years at the moment of evaluation
- BMI > 1.3 SDS at the initial evaluation
- Hs-CRP available at initial evaluation

#### ➤ Exclusion Criteria

- Acute or chronic Infection at the time of the study visit
- Be or have been a smoker of tobacco

## Study intervention

### **1. Study overview**

A single visit is planned. During this visit a standard questionnaire on current health status, life style will be filled in a selfscore of pubertal development using standardized pictures will be done. Standard anthropometrics and blood-pressure measurements , a blooddrawn for hSCRp and a peripheral artery tonometry will be performed.

### **2. Identification of intervention**

#### ➤ **Anthropometry:**

Anthropometric measurement sites are defined according to the international standards. (10)

- Weight will be measured using a calibrated digital scale (SECA, Hamburg, Germany) and will be rounded up to the nearest 0,05 kg. Participants will be wearing light clothing (underwear).
- Standing height will be measured using a stadiometer with a movable headboard and vertical backboard and will be rounded up to the nearest 0,1 cm.
- Waist circumference will be measured in the middle of the lowest rib (10th rib) and the iliac crest at the end of a normal expiration. The girths will be measured with a flexible Lufkin steel anthropometric tape (Lufkin W606PM).
- Tricipital and subscapular skinfold thickness will be measured by a Harpenden caliper using the Slaughter guidelines.
- The BMI z-score will be calculated using the Vlaamse groeistudie 2004 references
- The Slaughter formula will be used to calculate the body fat percentage.

#### ➤ **Blood pressure (mean of 3 measurements)**

Blood pressure will be measured using an oscillometric, electronic device (Mindray). The blood pressure will be measured 3 times; the first measurement will not be taken into account and the mean of the second and third measurement will be calculated.

#### ➤ **Peripheral arterial tonometry**

The pulse wave amplitude will be measured by a commercial finger plethysmograph EndoPAT, Itamar Medical Ltd, Caesarea, Isreal.)

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 which calculates the Reactive Hyperemia Index (RHI) and peak dilatation.

Participants will be asked not to engage in strenuous sport activities the 24 hours preceding the test. (12)



#### **hS-CRP**

Will be measured at the Chemical Chemistry Laboratory of the UZ Brussel using by immunoturbimetry by the Vitros 4600 / Ortho Clinical Diagnostics analyzer.

### **Safety**

#### **1. Insurance**

All damage or injuries resulting from the study participation will be covered by the no fault insurance available at Ethias through the University Hospital of Brussels.

#### **2. Anticipated possible adverse events**

The peripheral arterial tonometry can cause a tingling feeling in the occluded arm that will disappear after deflation of the cuff.

Drawing blood may lead to discomfort, pain or a hematoma at the puncture site and syncope.

### **Quality assurance**

EndoPAT device measurements will be done by the same person after training by the technicians. Calibrated instrumented will be used and blood will be drawn according to standard procedures in the University Hospital of Brussel (UZ Brussel). The CRP analysis has a CV of 4.8 % for 1.03 mg/L value.

### **Privacy policy**

The medical file data will be collected after agreement of the responsible treating paediatric endocrinologist. The questionnaire data and medical file data will be merged into an Excel document, which will be protected by a password. All the obtained data will be handled in a confidential way. All names, birth dates and residence data will be erased from the final datafile, which will be stored on a protected server of the UZ Brussel by Prof dr Jean De Schepper, who is the responsible for the data collection and storage. Collected data will be stored for 5 years. The Data Protection Officer of the UZ Brussel ( Audrey Van Scharen (dpo@vub.be) ) is responsible for further information on data protection. Participants will be informed about the DPO and their right to correct or withdraw collected data.

### **Publication policy**

All publication rights of unprocessed and processed data belong to the investigators.

## References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-81.
2. Wetenschappelijk Instituut Volksgezondheid. Gezondheidsenquête 2013.
3. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States. *JAMA*. 2014; 311(8):806-14.
4. Park MK. Park's pediatric cardiology – sixth edition. Philadelphia: Elsevier Saunders; 2014. 33, Dyslipidemia and other cardiovascular risk factors; p. 520-552.
5. Franks PW et al. Childhood obesity, other cardiovascular risk factors and premature death. *N Engl J Med*. 2010;362(6):485-93.
6. Visser M et al. Low-grade systemic inflammation in overweight children. *Pediatrics*. 2001 Jan;107(1):E13.
7. Pearson TA et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-51
8. Skrzypczyk P, Panczyk-Tomaszewska M. Methods to evaluate arterial structure and function in children- State-of-the- art knowledge. *Advances in Medical Sciences* 62; 280-
9. Pareyn A, Allegaert K, Verhamme P, Vinckx J, Casteels K. Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry. *Pediatric Diabetes*. 2015; 16: 98-103.
10. Tryggestad JB, Thompson DM, Copeland KC, Short KR. Obese children have higher arterial elasticity without a difference in endothelial function: the role of body composition. *Obesity*: 20: 165-171.
11. Tomsa A et al. Endothelial function in youth. A biomarker modulated by adiposity-related insulin resistance. *Pediatric*: 178: 171-177..
12. Bruyndonckx L, Radtke T, Eser P, Vrints CJ, Ramet J, Wilhelm M, et al. Methodological considerations and practical recommendations for the application of peripheral arterial tonometry in children and adolescents. *Int J Cardiol*. 2013;168(4):3183-90.