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Prenatal exposure to environmental contaminants influences body composition of 8 year old children – data collection and evaluation

Immle DELVAUX
Jolijn VAN CAUWENBERGHE

Promotor: Dr. Isabelle Sioen

Dissertation presented in the 2nd Master year
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Preface

We would like to mention several people who supported us in the process of making this thesis.
First, we would like to thank our promotor dr. Isabelle Sioen for her inexhaustible effort, support and trust in our work. Due to her guidance we were able to finish this thesis successfully.
Next, we would like to thank Nathalie Michels for teaching us how to perform the anthropometric measurements on children.
Finally, we would like to thank our family and friends for their moral support and understanding.

Immle Delvaux
Jolijn Van Cauwenberghe

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Abstract

Background. The aim of our master thesis was first to assess the validity of parental reported anthropometric data compared to measured data in 7 to 9 year old Flemish children especially for use in follow-up studies on prenatal exposure to endocrine disrupting chemicals and obesity later in life. Secondly, we assessed the association between prenatal exposure to endocrine disrupting chemicals (cadmium, PCBs, dioxins, p,p'-DDE and HCB) and anthropometric measures in 7 to 9 year old Flemish children.

Methods. The subjects in the validity-study were 120 Flemish children of a cohort study of participants that were recruited in the first Flemish Environment and Health Study. Data about anthropometric measures (waist circumference (WC), weight and length) were obtained by a postal parental reported questionnaire and during a home visit.

The subjects in the exposure-study were 114 Flemish children from the same population of the validity-study. Cadmium, PCBs, dioxins, p,p'-DDE and HCB were analysed in cord blood. Anthropometric measures obtained during a home visit when the child reached 7-9 years (height, waist circumference, hip circumference, weight and skinfolds) were used for the analyses.

Results. First, the validity-study showed that parents tend to overreport their child's WC and underreport the BMI, especially in children with the largest WC and largest BMI. The mean difference between measured and parental reported WC was 1.8% of the mean measured WC; for BMI, the mean difference was 4.1% of the mean measured BMI. Both for WC and BMI, we observed a good agreement between parental reported values and measured values to classify children in the highest 10% and 20% of the study population. When classifying the children in 'overweight' and 'not overweight', there were less misclassifications when parental reported WC was used compared to parental reported BMI.

Second, the study concerning the effect of prenatal exposure to EDCs, showed a significant negative association between prenatal cadmium exposure and waist circumference and the sum of triceps and subscapular skinfolds in girls (P=0.032 and P=0.004, respectively). This implicates that a higher prenatal cadmium exposure is associated with a decrease in these anthropometric measures. Moreover, a significant positive association was found between prenatal exposure to PCBs and p,p'-DDE and the waist circumference in girls (P=0.033 and 0.021, respectively). Furthermore, a significant positive association was also found between prenatal exposure to p,p'-DDE and the ratio waist/hip in girls (P=0.004) and between prenatal exposure to dioxins and height in boys (P=0.039). This implicates that a higher prenatal

exposure to these environmental pollutants is associated with an increase in waist/hip ratio in girls at the age of 7 to 9 years (indicator of central obesity) and height in boys.

Conclusion. We can conclude that although there is a high agreement between parental reported and measured WC, the parental reported data must be used with reserve. Moreover, this study is the first to suggest that WC is a better indicator compared to BMI when parental reported values are used to classify children. Considering these results, we only used data measured by study nurses for assessing the effect of prenatal exposure to EDCs and anthropometric data in later life. From that study we can conclude that prenatal cadmium exposure is negatively associated with waist circumference and the sum of the tricipital and subscapular skinfolds (the first being an indicator of visceral fat and the latest being an indicator of subcutaneous fat) and that prenatal exposure to PCBs and p,p'-DDE is associated with an increased risk for central obesity in girls. We also found that prenatal exposure to dioxins is associated with increased height in boys.

Samenvatting

Achtergrond. Het doel van onze masterthesis was vooreerst om de validiteit van door ouders gerapporteerde data te onderzoeken in vergelijking met door getrainde onderzoekers gemeten antropometrische data in 7 tot 9 jaar oude Vlaamse kinderen voornamelijk met het oog op gebruik in opvolgstudies rond de effecten van prenatale blootstelling aan endocrien verstorende chemicaliën (EDC's) en obesitas op latere leeftijd. Vervolgens hebben we de associatie tussen prenatale blootstelling aan endocrien verstorende chemicaliën (cadmium, PCB's, dioxines, p,p'-DDE en HCB) en antropometrische data in 7 tot 9 jarige Vlaamse kinderen onderzocht.

Methodologie. In de validiteitsstudie participeerden 120 Vlaamse kinderen uit een cohorte van de FLEHS I (Flemisch Environment and Health Study). Antropometrische data (buikomtrek, gewicht en lengte) werden verzameld door middel van een door de ouders ingevulde vragenlijst en tijdens een huisbezoek.

In de blootstellingsstudie participeerden 114 van de 120 Vlaamse kinderen uit de validiteitsstudie. Cadmium, PCB's, dioxins, p,p'-DDE en HCB werden geanalyseerd in navelstrengbloed. De antropometrische data (lengte, buik- en heupomtrek, gewicht en huidplooimetingen) die verzameld werden tijdens een huisbezoek wanneer deze kinderen de leeftijd van 7 à 9 jaar bereikten, werden gebruikt voor de statische analyses.

Resultaten. Ten eerste wees de validiteitsstudie uit dat ouders neigen tot het overrapporteren van de buikomtrek van hun kinderen maar de BMI van hun kind eerder onderrapporteren, dit is in het bijzonder het geval bij de kinderen met de grootste buikomtrek en de hoogste BMI. Het gemiddelde verschil tussen de gemeten en de door de ouders gerapporteerde buikomtrek was 1.8% van de gemiddeld gemeten buikomtrek, in geval van de BMI was het gemiddelde verschil 4.1% van de gemiddeld gemeten BMI. Voor zowel buikomtrek als BMI werd een goede overeenkomst gevonden tussen gerapporteerde waarden door ouders en de gemeten waarden om kinderen te kunnen classificeren in de hoogste 10% of 20% van de studiepopulatie. Bij de indeling van kinderen in 'overgewicht' en 'geen overgewicht' gebeurden er minder foute classificaties als de door ouders gerapporteerde buikomtrek werd gebruikt in plaats van de door ouders gerapporteerde BMI.

Ten tweede toonde de studie betreffende het effect van prenatale blootstelling aan EDC's een significant negatieve associatie tussen prenatale blootstelling aan cadmium en buikomtrek en de som van de triceps- en subscapularishuidplooien in meisjes (P=0.032 en P=0.004, respectievelijk). Dit wijst erop dat een hogere prenatale blootstelling aan cadmium

geassocieerd is met een vermindering in bovenstaande antropometrische maten. Daarenboven werd een significant positieve associatie gevonden tussen prenatale blootstelling aan PCB's en p,p'-DDE en de buikomtrek in meisjes (P=0.033 en P=0.021, respectievelijk). Verder werd ook een significant positieve associatie gevonden tussen prenatale blootstelling aan p,p'-DDE en de ratio van buik/heupomtrek in meisjes (P=0.004) en tussen prenatale blootstelling aan dioxines en lengte bij jongens (P=0.039). Dit wijst erop dat een hogere prenatale blootstelling aan bovenstaande contaminanten is geassocieerd met een toename van de ratio buik/heupomtrek in meisjes op een leeftijd van 7-9 jaar (indicator van centrale obesitas) en lengte bij jongens.

Conclusie. Ten eerste kunnen we concluderen dat alhoewel er een grote overeenkomst is tussen de door ouders gerapporteerde buikomtrek en de gemeten buikomtrek, de door ouders gerapporteerde data gebruikt moeten worden met voorzichtigheid. Bovendien is dit de eerste studie die suggereert dat buikomtrek een betere indicator is vergeleken met BMI wanneer door ouders gerapporteerde data gebruikt worden om kinderen te classificeren. Deze resultaten in acht genomen, zijn enkel data gemeten door getrainde personen gebruikt voor het effect te bepalen van prenatale blootstelling aan EDC's en antropometrische gegevens op latere leeftijd.

Ten tweede kunnen we concluderen dat prenatale cadmiumblootstelling negatief geassocieerd is met buikomtrek (indicator van visceraal vet) en de som van de triceps- en subscapularishuidplooien (indicator van subcutaan vet) en dat prenatale blootstelling aan PCB's en p,p'-DDE geassocieerd is met een verhoogd risico op centrale obesitas in meisjes. Tenslotte vonden we ook nog een associatie tussen prenatale blootstelling aan dioxines en een toegenomen lengte bij jongens.

Introduction

Obesity is a health problem that is wide spread and has an impact on different aspects of society. In 2005, approximately 10% of the adult population worldwide was obese and the highest prevalence was found in the Western World, approximately 20% (1). Kelly et al. (2008) calculated that if the growing trend in obesity prevalence would continue, 20% of the adults in the world would be obese in 2030 (1). Obesity is not restricted to adults, almost a fifth of children in the Western World is obese (2-4). Therefore, we could state that obesity in children has taken epidemic proportions. On the other hand, it has been reported that there is an overall leveling off of the prevalence of obesity in children and adolescents (5;6). Nevertheless, obesity in children should remain a public health priority since most obese and overweight children grow up to be obese adults (7). Besides the health problems that obesity can cause in adult age, e.g. diabetes mellitus type 2, cardiovascular diseases and certain cancers (8), we should not underestimate the effects of obesity on the child's health and wellbeing. These health problems consist for example of hypertension, diabetes mellitus type 2, obstructive sleep apnea syndrome and asthma (9). Obesity can also influence the psychosocial wellbeing of the child. This effect is illustrated by the link between obesity and low self-esteem and depression in children and adolescents (10). Schwimmer et al. (2003) reported that obese children and adolescents report lower health-related quality of life compared to children with normal weight and the level of quality of life was as low as that in children being treated for cancer (11). Besides the impact on health, we should consider the economic impact on the society of obesity in children. Unfortunately, there is little evidence on the cost analysis of childhood obesity and current evidence is ambiguous (12).

Obesity is the result of a complex interaction between behavioral (e.g. high calorie intake and low activity), genetic and environmental factors. The endocrine disrupting chemicals (EDCs) are one of these environmental factors. EDCs can interfere with the human endocrine system, which plays a role in the development of obesity (13;14). Humans are mainly exposed to EDCs through ingestion of contaminated food or water, inhalation of polluted air or through dermal exposure (15). Dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans), PCBs (polychlorinated biphenyls), p,p'-DDE (para, para-dichlorodiphenyldichloroethylene), HCB (Hexachlorobenzene) and heavy metals like cadmium are all considered to act as EDCs (13). As these five EDCs are investigated in our master thesis, we shortly introduce them here.

Cadmium (Cd) is a toxic heavy metal that is naturally present in our environment. The concentration of cadmium in our environment is increased due to industrial and agricultural activities. After ingestion, cadmium is stored in the human kidney and can result in kidney tubular damage and eventually renal failure. Another potential toxic effect of cadmium is bone damage and recent data suggests that there is an increased cancer risk in exposed populations (16).

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), summarized as dioxins, and PCBs are highly toxic compounds that are present in our environment. They are lipophilic and accumulate therefore in human fat tissue (17). P,p'-DDE is the primary metabolite of the pesticide DDT in humans. P,p'-DDE accumulates in human adipose tissue and is very stable: p,p'-DDE is thought to persist throughout the life span in the human body. Data suggests that besides the endocrine disrupting effect, p,p'-DDE is associated with certain cancers (18).

HCB is a widespread environmental contaminant that is persistent and toxic. Since the ban on its use as a pesticide, the levels in the environment have decreased greatly. HCB bioaccumulates in human adipose tissue due to its lipophilic properties and it is considered to be a human carcinogen. Besides cancer, HCB exposure is associated with numerous health effects (19).

This master thesis has two major aims. **First**, we aimed to assess the validity of parental reported waist circumference (WC), weight and height compared to measured WC, weight and height in 7 to 9 year old Flemish children, especially for using such data in follow-up studies on prenatal exposure to environmental chemicals and obesity later in life. Indeed, a number of existing prospective studies that follow growth or changes in body composition in relation to prenatal exposure to EDCs make use of self-reporting or parent reporting questionnaires for obtaining anthropometric data (20-22). This is an inexpensive method to collect data in large-scale studies. Whether these data are valid to use in epidemiological or clinical studies needs consideration.

Secondly, we investigated the effect of prenatal exposure to dioxins, PCBs, p,p'-DDE, HCB and cadmium on the weight, height, BMI, skinfold thicknesses, arm circumference, hip circumference, waist, waist to height and waist to hip circumference in 7 to 9 year old Flemish children. Existing data on this subject is contradicting and for some EDCs data is scarce.

Methods

In order to obtain the anthropometric data we carried out 120 home visits. We performed a number of steps to organize these home visits. First, we contacted all the parents who had given their e-mail address by sending them an e-mail in order to find out which days suited them for a home visit. Next, we telephoned all the parents and attempted to make an appointment. We tried to organize these appointments by region in order to work more efficient and to reduce the number of displacements we had to make. There were a number of parents that we were not able to contact due to wrong contact information and some parents were not willing to participate in this study. For other families the suggested data did not fit into their schedule, so we were not able to perform the home visit.

During the home visits we performed different anthropometric measurements: weight, height, arm circumference, waist circumference, hip circumference, triceps skinfold, biceps skinfold, subscapular skinfold and the suprailiac skinfold. All these measurements were performed conform specific rules, which are described further in this master thesis. Carrying out these measurements took about 20 minutes. All the home visits occurred during October and November of 2011. Afterwards all the data about the children were put into a databank and used for assessing the validity of parental reported anthropometric measures and to define the association between prenatal exposure to EDCs and anthropometric measures in later life.

Part I: Validity of parental reported versus measured weight, length and waist in 7 to 9 year old children for use in follow-up studies

1. Abstract

Objective: The aim was to assess the validity of parental reported anthropometric data compared to measured data in 7 to 9 year old Flemish children especially for use in follow-up studies on prenatal exposure to endocrine disrupting chemicals and obesity later in life.

Methods: The subjects were 120 Flemish children of a cohort study of participants that were recruited in the first Flemish Environment and Health Study. Data about anthropometric measures (waist circumference (WC), weight and length) were obtained by a postal parental reported questionnaire and during a home visit.

Results: Our study showed that parents tend to overreport their child's WC and underreport the BMI, especially in children with the largest WC and largest BMI. The mean difference between measured and parental reported WC was 1.8% of the mean measured WC; for BMI the mean difference was 4.1% of the mean measured BMI. Both for WC and BMI, we observed a good agreement between parental reported values and measured values to classify children in the highest 10% and 20% of the study population. When classifying the children in 'overweight' and 'not overweight', there were less misclassifications when parental reported WC was used compared to parental reported BMI.

Conclusions: We can conclude that although there is a high agreement between parental reported and measured WC, the parental reported data must be used with reserve. Moreover, this study is the first to suggest that WC is a better indicator compared to BMI when parental reported values are used to classify children.

2. Introduction

Recent studies have suggested that next to the positive energy balance, there are chemicals in our environment that have a part in the growing obesity problem (1-10). This group of chemicals is called the obesogens, a subgroup among the endocrine disrupting chemicals (EDC's). There is increasing evidence that changes in the prenatal environment is related with a higher risk of developing obesity in later life (4-6;9;10). So far, many prospective studies which follow growth or changes in body composition make use of a self-reporting or parent reporting questionnaire for obtaining anthropometric data (11-14). It is an inexpensive method to collect data in large-scale studies. Whether these data are valid to use in epidemiological or clinical studies needs consideration. A number of studies have shown that adults and adolescents have a tendency to overreport their own height and underreport their weight, leading to an underestimated body mass index (BMI) (15-23). This underestimation is even greater in subjects with a higher BMI (23). Considering children as a study population, researchers often rely on parental reported anthropometric data. However, it is shown that parents tend to overreport their child's height (24-26). This overestimation increases with increasing BMI (25). Only Akinbami et al. (2009) reported an underestimation of the height for children aged 2-11 years (27). But this study compared means instead of individual data. With regard to weight, the data on differences between parental reported and measured weight are not consistent. Two studies found that parents underestimated their child's weight (24;28). Akinbami et al. (2009) also found an overall underestimation, except for the group of 2-3 year old children (27). On the contrary Dubois et al. (2008) detected an overreporting of weight, specifically for boys (29). In the study of Scholtens et al. (2007) and Bekkers et al. (2011) parents of children in the lowest BMI quartiles tended to overreport the body weight, while parents of children in the highest BMI quartiles tended to underreport the weight of their child (25;30). These mixed results on the reporting of children's weight lead to contradictory results on the difference between parental reported and measured BMI. Some studies found an underestimation of BMI (24-25;28), while others reported an overestimation (27;29).

Most studies conclude that the use of parental reported height and weight, and calculated BMI from these measures, must be used with reserve. Measuring height and weight by a trained professional remains the gold standard for the assessment of overweight in children (21;24;27-29). Only one study claims that parental reported measures were reliable to determine childhood obesity (26). Besides, when looking at overweight and obesity in children as an outcome, different indicators can be used. BMI, which is most commonly used,

is a poor measure for the evaluation of central adiposity in children (31-33). Evidence suggests that waist circumference (WC) is a better predictor of trunk fat mass compared to waist-to-hip ratio (WHR) (34;35), conicity index (CI) (35) and skinfold measurements (34). In a longitudinal study in 8-year-olds, WC was found the best predictor of overweight at the age of 12, compared with BMI and the measurement of skinfolds (36). Studies with adults showed that WC is generally underreported (15;23;27-40), this is more distinct in larger waists (15;37;40). On the contrary, Dekkers et al. (2008) described a significant overreporting of WC (19). To our knowledge there is only one study that compared parental reported WC and measured WC in children. This study found that the difference between parental reported and measured WC depended on the WC and BMI of the child (8 years old): the WC of children with a high WC and/or BMI were underreported by their parents while the WC of children with a low WC and/or BMI were overreported by their parents (30).

The aim of this study is to assess the validity of parental reported WC, weight and height compared to measured WC, weight and height in 7 to 9 year old Flemish children, especially for using such data in follow-up studies on prenatal exposure to environmental chemicals and obesity later in life.

3. Methods

3.1. Study population and data collection

The study described in this paper was a part of a human biomonitoring program in Flanders (Belgium), in particular of a cohort of participants that were recruited in the first Flemish Environment and Health Study (FLEHS I 2002–2006) (41). During FLEHS I, 1196 mothers and their newborns were recruited via 25 maternity hospitals between October 2002 and December 2003. The exposure to six traditional pollutants i.e. cadmium, lead, dioxin-like compounds (Calux assay), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and para,para-dichlorodiphenyldichloro-ethylene (p,p'-DDE) was evaluated by measuring concentrations in cord blood. In the summer of 2011, the parents of the children that participated as newborns in 2002-2003 were re-contacted to participate in a follow-up study in order to evaluate the impact of the prenatal exposure to the six contaminants, as measured in FLEHS I, on the health of the 7 to 8-years old children. The parents and children received an invitation letter explaining the aims of the follow-up study, as well as an informed consent and a postal questionnaire. This questionnaire asked the parents to measure their child's length, weight and WC according to the given guidelines. These guidelines stated that the

child should be weighted in the morning with light clothes. For the measuring of the WC, we provided the parents with an identical tape measure and written instructions to perform the measurement at the level of the umbilicus while the child stands calmly upright. To make these instructions more clear, we added a figure of where the measurement of WC should be performed.

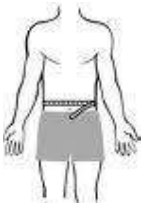

Figure 1. Figure provided to the parents of where the measurement of WC should be performed.

Additionally, the parents were asked permission to be visited by a study nurse to perform anthropometric measurements on the children. In total, 1173 invitation letters for the follow-up study were sent in June 2011. 23 of the 1196 parents had indicated at the baseline study in 2002-2003 that they were not willing to participate in follow-up studies. From the letters send in June 2011, 109 closed envelops came back, because the participants had moved between 2002 and 2011. In total, 281 completed questionnaires came back, leading to a response rate of 26.4%. Not all families gave their permission for a home visit. Therefore and due to practical reasons, we reached 120 families for a home visit. All participating parents provided informed consent for participation. The study protocol was approved by the Ethical Committee of the University of Antwerp (Belgium) and the Ethical Committee of the University of Ghent (Belgium).

3.2. Anthropometric measurements

In October and November 2011, the children were measured during home visits by two observers and intra- and inter-observer reliability was enhanced by extensive training. The children were measured barefooted in underwear and /or T-shirt. *Weight* was measured with an electronic scale (SECA 815, UK) to the nearest 0.1 kg. *Height* was measured with a telescopic height measuring instrument (SECA 213, UK) to the nearest 0.1 cm. *Waist circumference* (cm) was measured twice with an inelastic tape (Seca 200, precision 0.1 cm, range 0-150 cm) with the subject in a standing position, halfway between the top of the iliac crest and the lower coastal border (10^{th} rib). If the first and second measurement of the

circumference differed more than 2 mm, a third measurement was performed; this was necessary in 58 of the 120 measurements of waist circumference.

3.3. Statistical analyses

The *body mass index* (BMI) z-score was obtained by calculating the BMI (*BMI=weight(kg)/height(m)²*) and adjusted for age and sex using British 1990 growth reference data (42). Overweight was determined by the International Obesity Task Force classification (IOTF) (43), using an IOTF grade larger than 1 as cut-off for overweight. Children were defined as being abdominal overweight by using cut-off values for the WC provided by a Dutch reference population (44).

We calculated the difference between the measured and the parental reported values by subtracting the parental reported value from the measured value. Thus, a negative difference implicates that the parental value was higher than the measured value. In three cases, the difference between measured and parental WC was more than 20.0 cm. These three cases were excluded from the study. Wilcoxon signed-rank tests were performed to assess whether the measured values differed significantly from the parental reported values. Spearman correlation coefficients were calculated to define the associations between measured and reported values. To examine the agreement between parental reported and measured data on an individual level Bland-Altman plots were constructed (45). In these plots the differences between the measured and parental reported values are plotted against the mean of the measured and reported values. We detected the children that were in the highest 10 percent of WC and BMI, both for the measured and for the parental reported value, to study the correspondence between these groups. These analyses were also performed for the highest 20 percent of WC and BMI.

As was hypothesised that over- or underestimation could be determined by the actual BMI or WC, analyses were performed using subgroups. Based on BMI and WC quartiles, the study group was divided into four subgroups and we calculated the mean of the measured values and the differences between the measured and parental reported values per subgroup. Kruskal-Wallis tests showed whether the subgroups differed significantly from each other. Mann-Whitney U tests compared the mean differences between the different quartiles as well as between the subgroup of the highest quartiles (≥P75) with the three other quartile subgroups combined (<P75), both for BMI and WC. Finally, the agreement in classifying overweight was tested using the kappa coefficient (κ coefficient). Values between 0.61 and 0.80 are considered as a good agreement and values between 0.81 and 1 are considered as an almost

perfect agreement (46). We made a comparison between the group of children defined as abdominal overweight using the parental reported WC and using the measured WC. The same was done for 'overall overweight' with BMI.

SPSS Statistic 19.0 for Windows (SPSS Inc, Chicago, IL) was used for all the analyses. A p value <0.05 was considered as significant.

4. Results

The study population consisted of 120 children between 7 and 9 years old (50% boys). The mean age was 8.03 years (SD: 0.42 years). The mean of the measured and parental reported values and the mean differences between measured and parental reported values are given in Table 1. The mean parental reported values were significantly different from the mean of the measured values ($p<0.001$). The mean difference between measured and parental reported WC was negative. This implicates that on average the parents overreported the waist of their child. On the contrary, the mean difference was positive for length, weight and BMI, which shows that these measures were underreported. The mean difference between measured and parental reported WC was -1.07 cm , which is 1.8% of the mean measured WC. For weight, length and BMI, these percentages were 3.9, 0.8 and 4.1, respectively. Thus, the mean difference of WC deviated less from the measured value compared to BMI.

Table 1. Mean of the measured values, parental reported values and mean of the difference between measured and parental reported values.

	Mean measured (SD)	**Mean parental reported (SD)**	**Mean difference (SD) [Range]**
Waist circumference (cm)	58.69 (6.93)	59.72 (7.49)	-1.07 (2.66)* [-10.35 ; 6.13]
Weight (kg)	29.88 (6.55)	28.23 (6.16)	1.69 (1.92)* [-8.60 ; 9.30]
Length (cm)	132.98 (6.72)	131.90 (6.87)	1.08 (2.30)* [-9.10 ; 10.10]
BMI (kg/m²)	16.75 (2.48)	16.09 (2.39)	0.69 (1.20)* [-5.17 ; 6.09]

*P<0.001

Bland-Altman plots demonstrate the agreement between measured and parental reported values on individual level (Figure 2). Figure 2A shows that 95% of the parents reported their child's weight between -2.2 kg and 5.5 kg from the measured weight and their child's length between -3.5 and 5.7 cm from the measured length (Figure 2B). BMI calculated from the parental reported measures differed from -1.7 to 3.1 kg/m² from the measured BMI in 95% of

the cases (Figure 2C). WC was assessed by 95% of the parents from -6.4 below to 4.3 cm above the measured WC (Figure 2D).

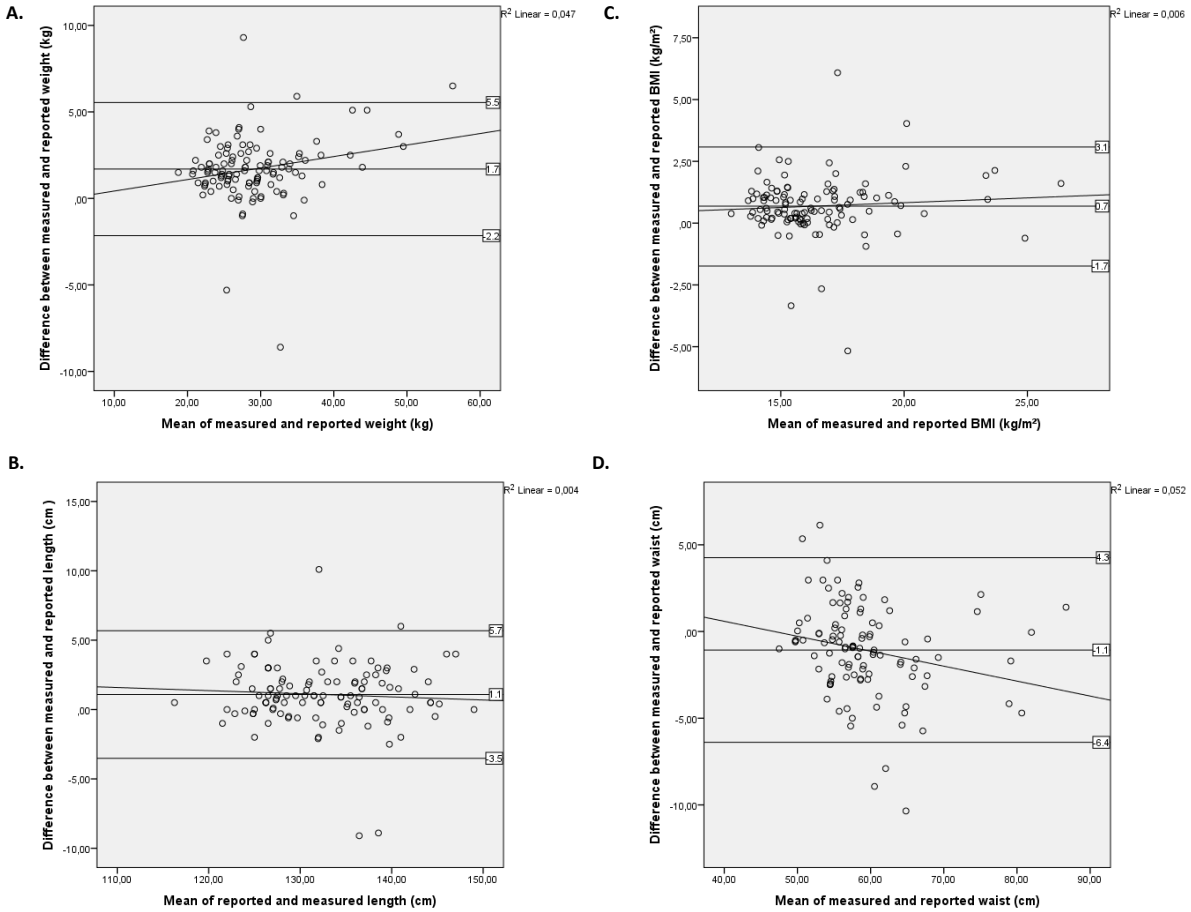

Figure 2. Bland-Altman plots of measured versus reported values. A. measured versus reported weight; B. measured versus reported length; C. measured versus reported BMI; D. measured versus reported waist. The horizontal reference lines represent the upper limit of agreement (mean+2SD), the average difference between the measurements (mean) and the lower limit of agreement (mean-2SD), respectively. The regression lines are also shown in these figures.

All the parental reported measures were strongly correlated to the measured values (P<0.001; length: r=0.942, weight: r=0.925, BMI: r=0.813, WC: r=0.872), indicating that there is a strong relationship between the two measures.

We studied the mean differences between measured and parental reported length, weight, BMI and WC for each subgroup of measured WC quartile and BMI quartile (Table 2). This table shows that the largest difference between reported and measured waist circumference, weight and BMI was situated in the group of children that were classified in the highest WC and BMI quartile. The difference between parental reported and measured WC was not significantly different for the four subgroups of WC quartiles (P=0.21), though it was significant for the four subgroups of BMI quartiles (P=0.04). The difference between parental

reported BMI and measured BMI was significantly different for the four subgroups of WC quartiles (P=0.04) and for BMI quartiles (P=0.02).

Table 2. Mean differences between measured and parental reported anthropometrics and SD by waist circumference quartiles and by BMI quartiles

	Measured WC quartiles								
Range (cm)	**1** ≤ 54.76		**2** 54.76- 57.20		**3** 57.20 – 60.23		**4** ≥ 60.23		
Difference between measured and reported	N	Mean±SD	N	Mean±SD	N	Mean	N	Mean	P[1]
Length (cm)	28	1.20±3.12	28	0.98±2.43	29	1.13±1.64	28	1.00±1.93	0.87
Weight (kg)	28	1.55±2.23[a]	27	1.24±2.26[a]	29	1.40±1.29[a]	28	2.66±1.63[b]	0.01
BMI (kg/m²)	28	0.68±1.55[a]	27	0.51±1.41[a]	29	0.50±0.65[a]	28	1.11±0.98[B]	0.04
Waist circumference (cm)	26	-1.26±2.34	27	-0.29±2.89	29	-1.13±2.97	26	-1.61±2.30	0.21
	Measured BMI quartiles								
Range (kg/m²)	**1** ≤ 15.13		**2** 15.13 – 16.0		**3** 16.0 – 17.71		**4** ≥ 17.71		
Difference between measured and reported	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	P[1]
Length (cm)	28	1.06±1.60	28	1.38±3.17	29	0.71±2.40	28	1.18±1.82	0.65
Weight (kg)	27	1.07±1.55 [a]	28	1.17±2.24 [a]	29	1.66±1.26 [a]	28	2.94±2.08 [b]	0.001
BMI (kg/m²)	27	0.43±0.94 [a]	28	0.33±1.42 [a]	29	0.77±0.74 [a,b]	28	1.26±1.40 [b]	0.02
Waist circumference (cm)	26	-0.61±1.62 [a]	28	-0.38±3.12 [a]	28	-1.10±2.95 [a,b]	26	-2.22±2.38 [b]	0.04

[1]Kruskal-Wallis test; a,b: Data of quartiles with different letters in superscript are significantly different between each other based on a one to one Mann-Whitney U-test

Table 3 shows that the mean difference between measured and parental reported BMI and WC was twice as high in the subgroup of the highest WC quartile (≥P75) compared to the three other quartile subgroups combined (<P75). The comparison of these means was significant for BMI (P=0.008) but not for WC (P=0.18). Table 3 also shows the same comparison for BMI quartiles. The mean difference between measured and parental reported WC and BMI was more than twice as high in the subgroup of the highest BMI quartile (≥P75) compared to the three other quartile subgroups combined (<P75). The differences between these means were significant both for WC (P=0.005) and for BMI (P=0.004). Thus, parents of children in the highest WC and BMI quartile underreported their child's BMI significantly and parents of the children in the highest BMI quartile overreported their child's waist circumference significantly.

Table 3. Mean differences (± SD) between measured and parental reported length, weight, BMI and WC in the highest WC/BMI quartile (≥P75) and in the three other WC/BMI quartiles combined (<P75)

Difference between measured and reported	Subgroups of WC quartiles				
	Other WC subgroups (<P75)		Highest WC subgroup (≥P75)		
	N	Mean±SD	N	Mean±SD	P[1]
Length (cm)	85	1.10±2.43	28	1.00±1.93	0.43
Weight (kg)	84	1.40±1.95	28	2.66±1.63	0.001
BMI (kg/m²)	84	0.56±1.25	28	1.11±0.98	0.008
Waist circumference (cm)	82	-0.89±2.76	26	-1.61±2.32	0.18
Difference between measured and reported	Subgroups of BMI quartiles				
	Other WC subgroups (<P75)		Highest BMI subgroup (≥P75)		
	N	Mean±SD	N	Mean±SD	P[1]
Length (cm)	85	1.05±2.46	28	1.18±1.82	0.85
Weight (kg)	84	1.30±1.73	28	2.94±2.08	<0.001
BMI (kg/m²)	84	0.51±1.08	28	1.26±1.40	0.004
Waist circumference (cm)	82	-0.70±2.66	26	-2.23±2.38	0.005

[1]Mann-Whitney U test

The κ-coefficient was calculated to study whether parental reported measures identified the same children in the highest 10% compared to measured value. For WC and BMI the κ-coefficient was 0.67 and 0.78, respectively. There was also a good agreement between parental reported WC and measured WC and between parental reported BMI and measured BMI for identifying the children that were in the highest 20% (WC: κ=0.67; BMI: κ=0.78).

We compared the children that were defined as abdominal overweight based on the parental reported WC with the children that were defined as abdominal overweight based on the measured WC (Table 4) (44). Of the 85 children that were classified as 'not abdominal overweight' based on measured WC there were 2 children misclassified as being 'abdominal overweight' when parental reported WC was used. There were no children misclassified as 'not abdominal overweight'. In total, there was 1.9% (2/107) of the children misclassified when parental reported WC was used in comparison to measured WC. When parental reported values for BMI were used, we found that there were 8 children misclassified as being 'not overweight' and 2 children that were misclassified as being 'overweight'. In total, there were 10 children (8.7%) that were misclassified when parental reported BMI was used compared to measured BMI (Table 4). The prevalence of abdominal overweight children was 22.4% when parental reported WC was used and 20.6% with measured WC. The use of parental reported WC thus leads to an overestimated prevalence of abdominal overweight. For BMI, the prevalence of overweight was underestimated when parental reported measures were used in comparison to measured values (11.3% vs. 16.5%).

The κ-coefficient was also calculated to study the concordance between parental reported values and measured values for classifying the children as 'not abdominal overweight' or 'abdominal overweight'. For WC and BMI the κ-coefficient was 0.95 and 0.64, respectively.

Table 4. Classification of children into (abdominal) overweight or not (abdominal) overweight based on measured and parental reported WC/BMI

		Parental reported WC		
		Not abdominal overweight	Abdominal overweight	Total
Measured WC	Not abdominal overweight	83	2	85
	Abdominal overweight	0	22	22
Total		83	24	107
		Parental reported BMI		
		Not overweight	Overweight	Total
Measured BMI	Not overweight	94	2	96
	Overweight	8	11	19
Total		102	13	115

Overweight was determined by the International Obesity Task Force classification (IOTF), using an IOTF grade larger than 1 as cut-off for overweight. Children were defined as being abdominal overweight by using cut-off values for the WC provided by a Dutch reference population.

5. Discussion

Our study shows that parents tend to overreport their child's waist and underreport the BMI, this is most distinct in parents of children with the largest waist circumference and largest BMI. The mean difference between measured and parental reported WC was 1.8% of the mean measured WC; for BMI the mean difference was 4.1% of the mean measured BMI. Both for WC and BMI there was a good agreement between parental reported measures and measured values to identify children in the highest 10% and 20% of the study population. When classifying the children in 'overweight' and 'not overweight', there were less misclassifications when WC was used compared to BMI. The prevalence of overweight was overestimated when parental reported WC was used and underestimated when parental reported BMI was used.

We compared the results of this study with the results obtained from the study by Bekkers et al. (2011), the only other study examining parental report of children's WC (30). The results regarding the prevalence of overweight (overestimation with parental reported WC and underestimated with parental reported BMI) correspond with the results found by Bekkers et al. (2011) (30). But not all our results show agreement with the study performed by Bekkers et al. (2011). First of all, Bekkers et al. (2011) observed a higher percentage of children being misclassified compared to this study (11.5% vs. 1.9%) when parental reported WC was compared with measured WC. It is important to note that Bekkers et al. (2011) divided the children into three groups: normal weight, moderate abdominal overweight and abdominal

obesity, which can explain the higher percentage of misclassification (30). We could not make this division, due to our smaller study group. Secondly, while this study shows an overreporting of the waist circumference in all groups of children regardless of their waist circumference, Bekkers et al. (2011) noted that parents of children with a high waist tend to underreport their child's waist (30).

Studies with adults showed that WC is generally underreported (23;38-40), this is more distinct in larger waists (15;37;40). On the contrary, Dekkers et al. (2008) described a significant overreporting of WC (19). Concerning length, our results correspond well with previous studies: parents tend to overreport their child's length (24-26;30). The underestimation of weight and BMI, detected in this study, matches with results of previous studies (24;27;28;30). In our study, the prevalence of overweight was underestimated when parental reported length and weight were used to calculate BMI compared to measured values. This result was also seen in previous studies (24;25;30). Huybrechts et al. (2006) calculated similar Spearman correlation coefficients between measured and reported weight and height (0.91 and 0.90, respectively). For the correlation between measured and reported BMI, Huybrechts et al. (2006) found a lower Spearman correlation coefficient than this study (0.59 vs. 0.81). The agreement between parental reported BMI and measured BMI for assessing overweight, calculated as the κ coefficient, was also lower in the study of Huybrechts et al. (2006), 0.43 vs. 0.64 (24).

Finally, we want to emphasize the importance of providing clear instructions to the parents on how to perform the measurements. Especially WC is not a measurement that parents are familiar with. Clear instructions, adding a figure and providing a tape measure increases the accuracy of the parental reported values.

5.1. Limitations and strengths of the follow-up study

A major strength of this study are the standardized measurements of not only weight and length, but also of WC. The measurements were performed by only two trained observers, as such enhancing the intra- and inter-observer reliability. Moreover, the pressure on answering in a socially acceptable way was reduced by using a written questionnaire with clear instructions instead of a face-to-face questionnaire.

Nevertheless, this study has some limitations. First, the size of the studied sample was quite low as the response rate of this follow-up study was only 26.4%. The main reason is that this follow-up study was not announced to the participants during the baseline survey. Moreover, the only contact information that we had was the family's address at baseline. Possibly, a lot

of families moved between 2002 and 2011, which decreased the participation rate. In the future we will be able to use the number of the National Register if approved by the parents, in order to be able to trace back participants who moved. As not all families were agreeing with a home visit and since some families could not be visited due to practical reasons (e.g. sickness, distance between home and research center, etc…), we only reached 120 families for a home visit. In order to study the difference in characteristics between the participants and non-participants, a non-responder analysis was conducted. There was no difference in sex between the participants and non-participants groups (p=0.13). The prenatal exposure to PCBs and p,p'-DDE was significant higher in the non-participant group compared to the participants (89.9 versus 81.8 ng/g fat (p=0.052) for PCBs and 185.2 versus 157.3 ng/g fat (p=0.008) for p,p'-DDE). For the other contaminants (lead, cadmium, dioxin-like compounds and HCB) no significant differences were found. The age of the mothers at birth of the participants was slightly but significantly higher compared to the non-participants (30.1 versus 29.4 years, p=0.026), their education level was also higher (p<0.001) and the percentage of mothers who had ever smoked was lower in the participant group compared to the non-participants (30.6% versus 37.4%, p=0.039).

Secondly, the parental reported values were collected during the summer of 2011 and the children were measured from October till November of that same year. The maximum interval between the parental reported and the measured value was 4 months (median: 3 months). This interval could be responsible for a part of the difference between parental reported and measured values. Finally, for the measurement of the waist circumference a different tape measure was used by the study nurses compared to the tape measure used by the parents, which could partially explain the difference between measured and parental reported WC.

We can conclude that although there is a high agreement between parental reported WC and measured WC, the data obtained by questionnaire from parents must be used with reserve. In addition, this study is the first to suggest that waist circumference is a better indicator compared to BMI when parental reported values are used in research concerning overweight in children if good instructions are given. More studies handling this subject must be performed.

6. Acknowledgment including declarations

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Part II: Prenatal exposure to environmental contaminants and body composition at age 7-9 years

1. Abstract

Background. The aim of this study was to assess the association between prenatal exposure to endocrine disrupting chemicals (cadmium, PCBs, dioxins, p,p'-DDE and HCB) and anthropometric measures in 7 to 9 year old Flemish children.

Methods. The subjects were 114 Flemish children (50% boys) that were recruited in the first Flemish Environment and Health Study (FLEHS I 2002–2006). Cadmium, PCBs, dioxins, p,p'-DDE and HCB were analysed in cord blood. When the child reached 7-9 years, anthropometric measures (height, waist circumference, hip circumference, weight and skinfolds) were obtained during a home visit.

Results. A significant negative association was found between prenatal cadmium exposure and waist circumference and the sum of triceps and subscapular skinfolds in girls (P=0.032 and P=0.004, respectively). This implicates that a higher prenatal cadmium exposure is associated with a decrease in these anthropometric measures. Moreover, a significant positive association was found between prenatal exposure to PCBs and p,p'-DDE and the waist circumference in girls (P=0.033 and 0.021, respectively). Next, a significant positive association was also found between prenatal exposure to p,p'-DDE and the ratio waist/hip in girls (P=0.004) and between prenatal exposure to dioxins and height in boys (P=0.039). This implicates that a higher prenatal exposure to these environmental pollutants is associated with an increase in waist/hip ratio in girls at the age of 7 to 9 years (indicator of central obesity) and height in boys.

Conclusions. Prospective studies are important to study long-term health effects in regard to prenatal exposure to environmental contaminants. Recent studies suggest an effect on body composition with the current exposure level, so it seems important to reduce the degree of prenatal exposure to environmental contaminants.

2. Introduction

Obesity in children is an important health problem since most obese and overweight children grow up to be obese adults (1). Recent studies suggest a stabilization of the obesity epidemic in children and adolescents (2;3). Nevertheless, prevention of overweight and obesity in children should remain a priority because they are linked to a number of severe health problems such as diabetes mellitus type 2, cardiovascular diseases and certain cancers (4). Besides genetic, behavioral and dietary factors, also environmental factors, e.g. exposure to endocrine disrupting chemicals (EDCs), may be risk factors for developing obesity. EDCs can interfere with the human endocrine system, playing a role in the development of obesity (5;6). Humans are exposed to EDCs through ingestion of contaminated food or water, inhalation of polluted air or through dermal exposure (7). Polychlorinated biphenyls (PCBs), dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans), para,para-dichlorodiphenyldichloroethylene (p,p'-DDE), hexachlorobenzene (HCB) and heavy metals like cadmium are all considered to act as EDCs. Studies have indicated that dioxins can bind the aryl hydrocarbon receptor, induce the cytochrome P450 1A enzyme and have an antiestrogenic action, while PCBs induce the pregnane X receptor and the constitutive androstane receptor and induce thyroid hormone disruption (8). HCB and p,p'-DDE are thought to have an antiandrogenic function (8). For cadmium, studies have shown that it can affect the secretory patterns of pituitary hormones and the synthesis of progesterone (9).
Recent epidemiological studies demonstrate that exposure to specific EDCs during the critical period of fetal development is associated with overweight and obesity later in life (5).

Table 1 comprises a summary of data available on the effects of prenatal exposure to EDCs later in life.

Table 1. Current data on the effect of prenatal exposure to EDCs and anthropometric measures in later life

EDC	Study	Age (years)	Number	Anthropometric measure	Result	Significant (S)/ Non-significant (NS
Cd	Lin et al. (2011)	0 - 3	402	Height	Decrease	S
		0 - 3	402	Weight	Decrease	S
		0 - 3	402	Head circumference	Decrease	S
	Tian et al. (2009)	4.5	106	Height	Decrease	S
		4.5	106	Weight	Decrease	NS
Dioxins	Su et al. (2010)	2	136	Height	Increase	S[1]
		2	136	Weight	Increase	S[1]
		2	136	BMI	Increase	NS
		5	149	Height	Increase	S
		5	149	Weight	Increase	NS
		5	149	BMI	Increase	NS
	Verhulst et al. (2009)	0-3	138	BMI	No association	
PCB	Jacobsen et al. (1990)	4	123	Weight	Decrease	S
	Verhulst et al (2009)	0 - 3	138	BMI	Increase	S
	Valvi et al. (2012)	6.5	344	BMI	Increase	S[2]
	Gladen et al. (2000)	10-15	594	Weight adjusted for height	Increase	S[3]
		10-15	594	Height	Increase	NS
	Karmaus et al. (2009)	20-50	176 (only females)	Height	Decrease	NS
		20-50	176 (only females)	Weight	Increase	NS
		20-50	176 (only females)	BMI	Increase	NS
	Karmaus et al. (2002)	0-10	212-313	Height	No association	
p,p'-DDE	Verhulst et al. (2009)	0 - 3	138	BMI	Increase	S
	Valvi et al (2012)	6.5	343	BMI	Increase	S[4]
	Gladen et al. (2000)	10-15	594	Weight adjusted for height	Increase	S[5]
		10-15	594	Height	Increase	NS
	Karmaus et al. (2009)	20-50	176 (only females)	Height	Increase	NS
		20-50	176 (only females)	Weight	Increase	S
		20-50	176 (only females)	BMI	Increase	S
	Ribas-Fitó (2006)	1	1540	Height	Decrease	NS
		4	1289	Height	Decrease	S
		7	1371	Height	Decrease	S
	Karmaus et al. (2002)	0-8	212	Height	Decrease	S[1]
		10	212-313	Height	Decrease	NS
	Garced et al. (2012)	0-1	253	Height, weight	No association	
	Jusko et al.	0-5	399	Growth (9 measures)	No	

	(2006)				association	
	Gladen et al. (2004)	10-20	304 (only males)	Height, weight, BMI, skinfolds	No association	
HCB	Verhulst et al. (2009)	0-3	138	BMI	No association	
	Smink et al. (2008)	6.5	405	Height	No association	
		6.5	405	Weight	Increase	NS
		6.5	405	BMI	Increase	S

[1]Significant for girls only; [2]Significant for third tertile, but not for second tertile; [3]Significant for white girls only; [4]Significant for second tertile, but not third tertile; [5]Significant for boys only

There have been multiple studies that investigated the effect of cadmium exposure during the pregnancy on the developing child but current evidence is inconsistent. Some studies found that cadmium had a significant adverse effect on the weight (10) and head circumference (11) at birth. Other studies also found this negative effect on weight (12;13) and length at birth and a positive effect on chest circumference and head circumference (13) at birth but these effects were not significant. A number of studies found no association between prenatal exposure to cadmium and birth weight (11;14;15) and height (11). Whether cadmium exposure during pregnancy has an effect on the growth of the child later in life has hardly been investigated. Lin et al. (2011) found that an increase of cadmium in cord blood was associated with a significant decrease in height, weight and head circumference up to 3 years of age (11). The adverse effect of cadmium in cord blood on length was also reported in 4.5 year olds, but this study found no significant decrease in weight (16).

Concerning prenatal exposure to dioxins, the results of the effect on the developing child are inconsistent. A number of studies reported that prenatal dioxin exposure has a significant negative effect on length (17) and weight (18;19) at birth. Nishijo et al. (2009) also found a decrease in birth weight and length with increasing exposure to dioxins but there was no significant relationship (20). These associations between exposure to dioxins and birth weight (17;20) and length (21) were not found in other studies. Similarly, for chest circumference (17;20) and head circumference (21) at birth there were also no associations found with prenatal dioxin exposure. Some studies found a negative correlation between 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCCD), which is considered the most toxic dioxin, and head circumference at birth (17;20) or birth weight (19), while others did not find an association with TCCD and birth weight (22). There are only a few studies that investigated the effect of prenatal exposure to dioxins on anthropometric measures in later life. Su et al. (2010) found that girls with a high prenatal dioxin exposure were significantly taller and heavier at 2 years

of age, while the increase in BMI was not statistically significant (23). At 5 years, Su et al. (2010) reported that a higher prenatal exposure to dioxins resulted in an increase in height, weight and BMI in all children, however this result was only significant for height (23). Verhulst et al. (2009) reported that there was no association between prenatal dioxin exposure and BMI from birth to 3 years of age (24).

Like dioxins, the data on the effects of prenatal exposure to PCBs are inconsistent. Some studies report that PCBs exposure during pregnancy has a significant negative influence on birth weight (24-27) and birth length (24). This negative influence on birth length has also been reported in other studies though there was no statistical significance (26;28). Concerning head circumference at birth, Hert-Picciotto et al. (2005) found that prenatal exposure to PCBs leads to a significant decrease in head circumference in boys (26), while others found no association (28;29). A number of studies reported that there was no association between PCBs exposure and birth weight (19;28;29). Jacobson et al. (1990) reported that prenatal PCB exposure leads to a significant decrease in weight at 4 years (30). However, other studies found an association between PCBs and an increase in weight in adults (31) and an increase in weight adjusted for height in adolescents, with a significant result for white girls (32). For BMI, all studies reported that prenatal PCBs exposure was associated with a significant increase in BMI between 1 and 3 years (24), at 6.5 years in girls (33) and a non-significant increase in BMI in female adults (31). For height, data are inconsistent: one study reported a non-significant increase in height with higher exposure to PCBs (32) and another study a non-significant decrease (31), while Karmaus et al. (2002) reported that there was no association between prenatal exposure to PCBs and height (34).

Next, Wojtyniak et al. (2010) reported a significant association between prenatal exposure to p,p'-DDE and decreased birth weight (35). This negative association, although not statistically significant, was also reported by Govarts et al. (2012) (25). On the other hand, other studies report no association between prenatal exposure to p,p'-DDE and birth weight (24;29;36;37), length (24;36) or head circumference (29;36;37) at birth. Ribas-Fitó et al. (2002) reported that exposure to p,p'-DDE had a negative influence on birth weight, but this effect was not significant (37). There have also been a number of studies that investigated the effect of prenatal exposure to p,p'-DDE on the body measures later in life. Karmaus et al. (2009) found that female adults who had a higher prenatal p,p'-DDE exposure were significantly heavier and non-significantly taller (31). Similarly, Gladen et al. (2000) reported

a significant increase of weight adjusted for height at puberty among boys and a non-significant increase in height in adolescents with prenatal exposure to p,p'-DDE (32). On the other hand, two studies reported a significant reduced height in children exposed to p,p'-DDE: Karmaus et al. (2002) reported this effect in 1 to 8 year old girls, while Ribas-Fito et al. (2006) reported a significant reduced height in children age 4 and 7 years (34;37). For BMI, a number of studies found that prenatal p,p'-DDE exposure lead to a significant higher BMI in later life (24;31;33). Other studies state that there was no association between prenatal exposure to p,p'-DDE and growth during the first years of life (36;38) or in adolescent males (39).

In contrast to p,p'-DDE, the effects of prenatal exposure to HCB on the developing child have not been extensively investigated. Eggesbo et al. (2009) reported a negative association between HCB and birth weight, length and head circumference among past and current smoking mothers (40). Similarly, Schade et al. (1998) found that higher HCB levels were associated with lower birth weights, especially in female infants (41) and Ribas-Fitó et al. (2002) reported a negative association between HCB exposure and length at birth, but this study did not find an association between HCB exposure and birth weight nor head circumference (28). There are only two studies that investigated the effect of prenatal exposure to HCB on anthropometric measures later in life. Smink et al. (2008) reported that prenatal exposure to HCB is associated with a significant increase in BMI and a non-significant increase in weight at age 6.5 and this effect is stronger in mothers who smoked during the pregnancy (42). There was no association found between prenatal exposure to HCB and height at age 6.5 (42) or BMI from birth to 3 years of age (24).

Here we test the hypothesis that prenatal exposure to low levels of five EDCs influences body composition of Flemish children when being 7 to 9 years old and increases the risk on obesity. In this cohort, earlier results indicate that prenatal exposure to EDCs influences their birth weight (25) and body composition on the age of 3 (24). We investigated the effect of prenatal exposure to cadmium, PCBs, dioxins, p,p'-DDE and HCB on a set of different anthropometric parameters: height, weight, BMI, waist and hip circumference, waist/hip and waist/height ratio and the sum of four skinfolds (triceps, biceps, supra-iliac and subscapular) and the sum of the tricipital and subscapular skinfolds. To the authors' knowledge, this is the first study using circumferences and skinfolds, besides more common anthropometric measures e.g.

weight and height, measured by trained professionals as body composition parameters in this kind of studies.

3. Methods

3.1. Study population and data collection

The study described in this paper was part of a human biomonitoring program (HBM) in Flanders (Belgium), in particular of a cohort study of participants that were recruited in the first Flemish Environment and Health Study (FLEHS I 2002–2006) (43). Within FLEHS I, 1196 mothers and their newborns were recruited through 25 maternity hospitals between October 2002 and December 2003. These hospitals were spread over eight different areas with characteristic environmental exposures. Inclusion criteria were living for at least five years in the area of interest, being able to fill out Dutch questionnaires and giving informed consent. Cord blood was sampled at the moment of delivery and the exposure to five environmental pollutants i.e. cadmium, PCBs, dioxins, p,p'-DDE and HCB was assessed.

More information about the study design of FLEHS I is described by Koppen et al. (2009) (44). Birth weight was registered shortly after delivery.

In the summer of 2011, the parents of the children that participated as newborns in 2002-2003 were re-contacted to participate in a follow-up study in order to evaluate the impact of the prenatal exposure to the five contaminants -as measured in FLEHS I - on the health of the 7 to 9 -year old children. The parents and children received an invitation letter explaining the aims of the follow-up study, as well as an informed consent and a postal questionnaire. Additionally, they were asked whether they were willing to be visited by a study nurse to measure length, weight, waist and hip circumference and four skinfolds of the children.

In total, 1173 invitation letters for the follow-up study were sent in June 2011, since 23 of the 1196 parents indicated at the baseline study in 2002-2003 that they were not willing to participate in follow-up studies. However, 109 closed envelops came back, because the participants had moved between 2002 and 2011. In total, 281 completed questionnaires came back, leading to a response rate of 26.4%. Not all families gave their permission for a home visit. Therefore and due to practical reasons, we reached 120 families for a home visit. In this paper, we only used the data from 114 of these children due to incorrect or missing data. All participating parents provided informed consent for participation. The study protocol was approved by the Ethical Committee of the University of Antwerp (Belgium) and the Ghent University (Belgium).

3.2. Prenatal exposure

Cord blood was aliquoted and plasma was separated by centrifugation within one day in either the maternity or blood bank laboratories. The aliquoted samples were kept in the refrigerator for maximal one week. Since we only measured persistent chemicals (cadmium and persistent chlorinated compounds) that do not degrade, this method is in line with quality standards. Afterwards they were put at -20°C until analysis.

In cord blood, cadmium was analysed using High Resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS). For more information see Schroijen et al. (2008) (45). The limit of detection (LOD) for cadmium in whole blood samples was 0.09 µg/L.

It was possible to assess cadmium concentrations in 106 of the 114 cord blood samples. In 40 of the 106 cases, the concentration was below the LOD and replaced by 0.05 µg/L (LOD/2). PCBs and p,p'-DDE were assessed in respectively 108 and 110 of the 114 samples, none of the concentrations were below the LOD. HCB concentration was assessed in 108 of the 114 samples, with 32 of the concentrations (29.6%) below the LOD and replaced by 0.01 µg/L (LOD/2). Dioxins were assessed in 89 of the 114 samples, none of the concentrations were below the LOD. Due to low volume of the blood samples (< 5ml serum) and technical reasons in the laboratory not all contaminants could be tested on each sample.

In cord plasma, marker polychlorinated biphenyls (PCB 138, 153 and 180), PCB 118 and 170 - which may have more neurotoxic potentials -, dioxin-like compounds (CALUX® assay) and chlorinated pesticides (HCB and p,p'-DDE (being a metabolite of DDT)) were analyzed. The PCBs and chlorinated pesticides were analyzed by gas chromatography - electron capture detection (GC/ECD) using the method of Gomara et al. (2002) - (46). The analyses were performed by two labs. Both laboratories participated in the AMAP proficiency testing scheme (Institute National de Santé Publique, Quebec, Canada). The limit of detection (LOD) for all chlorinated compounds was 0.02 µg/L. Routinely measured cholesterol and triglycerides were used to calculate total serum lipid content (47), which was used to express the results on a lipid weight basis.

Exposure to dioxin-like compounds was assessed via the CALUX® assay, based on in vitro activation of the aryl-hydrocarbonreceptor (AhR) of cultured H4IIE rat hepatoma cells by the dioxin-like compounds present in 5 mL cord plasma (BioDetection Systems BV, Amsterdam, The Netherlands). The extraction and clean-up procedures were performed as described in Koppen et al.(2001) (48).

The limit of detection was 0.03 pg CALUX-TEQ/mL or 14 pg CALUX-TEQ/g lipids for 5 mL plasma, with a lipid content of 200 mg/dL (44).

3.3. Follow-up questionnaire

In 2011, the parents completed a general questionnaire to collect information on the current lifestyle (food consumption and physical activity habits) of the children and on general characteristics of the family (weight and height as well as educational level of both parents). Educational level was determined by the highest level of education from either mother or father and used as a proxy for socio-economic status. BMI (kg/m^2) of both parents was calculated by dividing the weight (kg) by the length^2 (m^2). Information on food consumption was collected with a short 18-items food frequency questionnaire (FFQ). Based on that FFQ, a score was calculated indicating the frequency (times per week) sugar- and/or fat-rich foods (confectionary, chocolate, soft drinks, chips, fried potato products and fast food) the children ate weekly at age 7-9 years. A physical activity-score was obtained by grading the frequency of time children spend on sport or outdoor playing in their spare time and by grading the way they went to school (e.g. high score for cycling and walking,…).

3.4. Anthropometric data measured by the study nurses

In October and November 2011, the children were measured during home visits by two observers, intra- and inter-observer reliability was enhanced by extensive training. The children were measured barefooted in underwear and /or T-shirt. *Weight* was measured once with an electronic scale (SECA 815, UK) to the nearest 0.1 kg. *Height* was measured once with a telescopic height measuring instrument (SECA 213, UK) to the nearest 0.1 cm. The body mass index (BMI) z-score was obtained by calculating the BMI ($BMI=weight(kg)/height(m)^2$) and adjusted for age and sex using British 1990 growth reference data (49).

Skinfold thicknesses (mm) were measured twice on the right side of the body to the nearest 0.2 mm with a skinfold calliper (Holtain, UK, range 0-40 mm) according to the international standards for anthropometric assessment (50) and the mean of both measurements was calculated. The triceps and biceps skinfold were taken halfway between the acromion process and the olecranon process at the back side of the arm for the triceps and at the front side of the arm for the biceps. The subscapular skinfold was measured 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body. The suprailiac skinfold measuring point was identified 2 cm above the iliac crest and 2 cm towards the medial line. It was

aligned inferiomedially at 45° to the horizontal. The sum of all four skinfold thicknesses and the sum of the tricipital and subscapular skinfold thickness were calculated as an indicator of subcutaneous fat.

Circumferences (cm) were measured twice with an inelastic tape (Seca 200, precision 0.1 cm, range 0-150 cm) with the subject in a standing position with arms to the sides. Circumference measurements were performed at the following two sites: 1) waist (indicator of visceral fat), horizontally halfway between the top of the iliac crest and the lower coastal border (10^{th} rib); 2) hip (indicator of subcutaneous fat), horizontally at the maximum extension of the buttocks. Waist-to-hip ratio was calculated as an indicator of central obesity and waist-to-height ratio was used as a normalized index of body fat distribution.

If the first and second measurement of the skinfolds and/or circumferences differed more than 2 mm, a third measurement was performed and the mean of all three measurements was calculated.

For circumferences and skinfolds the mean value of the two or three measurements was calculated per child. We performed all statistical analyses with these mean values.

3.5. Statistical analyses

Statistical analyses were performed using the SPSS for Windows software program (version 19.0). The normality of each outcome variable was examined by performing the Shapiro-Wilk test. Differences in markers of body composition between boys and girls were assessed with an independent samples T-test for the normal distributed markers and with a Mann-Whitney U-test for the non-normal markers. For the markers that differed significantly (p-value < 0.05) (height: P=0.002, waist: P=0.048, ratio of waist/hip: P=0.006, sum of triceps and subscapular skinfolds: P=0.045) between boys and girls, regression analyses were stratified for gender.

The associations between prenatal exposure and body composition markers at the age of 7 to 9 years were assessed with linear regression analysis. For each of the five EDCs, a separate regression model was used, since these EDCs influence the endocrine system via another mechanism (8). A p-value < 0.05 was considered significant. Some outcome variables (waist circumference, hip circumference, weight, the waist/height ratio, the sum of all 4 skinfolds and the sum of the tricipital and subscapular skinfold) were log-transformed to fulfill the conditions for linear regression.

All regression analyses were adjusted for age of the child, BMI of the mother/father, gender of the child, birth weight of the child, the weekly frequency of sugar- and/or fat-rich food consumption and the highest level of education of both parents. Only when height was the

outcome-variable, we decided to adjust for height of both parents instead of BMI of both parents. The calculated score for physical activity was not significantly correlated with the different outcome parameters and therefore not included in the regression analyses.

To avoid loss of the subjects in the multiple regression analyses, missing values on maternal and paternal BMI (n=1 and 12, respectively), the weekly frequency of sugar- and/or fat-rich food consumption (n=1), and the highest level of education of both parents (n=1) were imputed, using the median value.

4. Results

Our study population consisted of 114 children between 7 and 9 years old (± 50% boys). The mean age was 8.43 years (SD: 0.34). Table 1 provides an overview of the characteristics of the study population. The highest education level of both parents was high school in 20% of participants, college in 50% of the cases and university in another 30%. The frequency of sugar- and/or fat-rich foods varied from 9 to 27 times a week (+/- 1 to 4 times a day), with a median of 16 times a week (+/- 2.5 times a day).

Table 1. Descriptive and anthropometric data of the study population

	Total			Boys			Girls		
	N	**mean ± SD**	**range**	**N**	**mean ± SD**	**range**	**N**	**mean ± SD**	**range**
Age child (y)	114	8.43 ± 0.34	(7.65 – 8.98)	57	8.45 ± 0.35	(7.65 – 8.96)	57	8.41 ± 033	(7.75 – 8.98)
Birthweight child (kg)	114	3.42 ± 0.51	(2 – 5.58)	57	3.56 ± 0.57	(2 – 5.58)	57	3.29 ± 0.40	(2.5 – 4.25)
Heigth (cm)	114	133.0 ± 6.7	(116.5 - 149)	57	135 ± 6.9	(121.5 - 149)	57	131.0 ± 5.9	(116.5 - 149)
Weight (kg)	114	29.9 ± 6.6	(19.5 - 59.5)	57	31.1 ± 7.5	(21.6 - 59.5)	57	28.6 ± 5.3	(19.5 - 45.1)
BMI child (kg/m²)	114	16.8 ± 2.5	(13.2 - 27.2)	57	16.9 ± 2.7	(13.2 - 27.2)	57	16.6 ± 2.3	(13.7 - 24.7)
Waist circumference (cm)	112	58.79 ± 6.96	(47.00 – 87.40)	56	59.98 ± 7.60	(47 – 87.40)	56	57.59 ± 6.09	(49.40 – 78.30)
Hip circumference (cm)	112	68.63 ± 6.46	(57.17 - 92.1)	56	69.14 ± 7.22	(59.67 - 92.1)	56	68.12 ± 5.61	(57.17 - 84)
Waist/heigth	112	0.44 ± 0.04	(0.36 - 0.59)	56	0.44 ± 0.04	(0.36 - 0.59)	56	0.44 ± 0.04	(0.39 - 0.58)
Waist/hip	112	0.86 ± 0.05	(0.76 - 0.96)	56	0.87 ± 0.05	(0.76 - 0.96)	56	0.85 ± 0.04	(0.78 - 0.94)
Skinfold biceps (mm)	112	7.91 ± 4.29	(3 - 25.2)	56	7.65 ± 4.52	(3 - 25.2)	56	8.17 ± 4.07	(3.2 - 20.73)
Skinfold triceps (mm)	112	10.73 ± 4.45	(5.1 - 30.3)	56	10.23 ± 4.78	(5.1 - 30.3)	56	11.23 ± 4.07	(5.5 - 22.67)
Skinfold subscapular (mm)	111	7.3 ± 4.8	(3.9 - 32.1)	55	7.4 ± 5.3	(4 - 32.1)	56	7.3 ± 4.2	(3.9 - 27.8)

Skinfold suprailiacal (mm)	111	8.28 ± 6.04	(3.2 - 32.53)	55	8.17 ± 6.15	(3.2 - 32.5)	56	8.39 ± 5.99	(3.3 - 32.53)
Sum of 4 skinfolds* (mm)	112	34.13 ± 18.85	(9 – 120.1)	56	33.15 ± 20.23	(9 – 120.1)	56	35.11 ± 17.49	(16.4 – 98.73)
Sum of tricipital and subscapular skinfolds (mm)	112	18.0 ± 8.9	(6 – 62.4)	56	17.5 ± 9.9	(6 – 62.4)	56	18.5 ± 7.9	(9.4 – 50.2)
BMI mother (kg/m²)	114	24.01 ± 3.99	(17.58 – 36.57)	57	24.83 ± 4.11	(17.58 – 36.05)	56	23.19 ± 3.72	(18.17 – 36.57)
BMI father (kg/m²)	114	25.86 ± 2.91	(20.15 – 34.50)	50	25.85 ± 2.51	(20.15 – 32.27)	52	25.86 ± 3.28	(20.20 – 34.50)

*sum of triceps, biceps, subscapular and suprailiacal skinfolds

Table 2 shows the descriptive data on the prenatal exposure to the five considered EDCs.

Table 2. Contaminants levels in cord blood or plasma

	N			Median [P25;P75]		P-value for difference in exposure between boys and girls
	Boys	**Girls**	**Total**	**Boys**	**Girls**	
PCB (ng/g fat) [a]	53	55	108	71.43 [38.79; 112.06]	74.47 [45.48; 119.62]	0.761
Dioxins (CALUX) (pg CALUX-TEQ /g fat) [a]	50	39	89	27.12 [13.16; 43.02]	20.12 [9.44; 33.36]	0.044
HCB (ng/g fat) [a]	53	55	108	24.44 [11.45; 34.79]	23.40 [10.47; 33.96]	0.815
P,p'-DDE (ng/g fat) [a]	54	56	110	130.83 [71.33; 193.43]	112.16 [57.97; 204.41]	0.745
Cadmium (µg/L) [b]	54	52	106	0.17 [0.05; 0.59]	0.21 [0.05; 0.74]	0.404

[a]: measured in plasma; [b]: measured in whole blood
PCB= sum of PCB 138, 153 and 180; HCB= hexachlorobenzene; p,p'-DDE= para,para-dichlorodiphenyldichloroethylene

Table 3 shows the results of the unadjusted linear regression analyses. A significant negative association was found between prenatal exposure to cadmium and height as well as waist circumference and the sum of tricipital and subscapular skinfolds in girls (P=0.017, 0.011 and 0.003, respectively) and between cadmium and hip circumference in the combined population of boys and girls (P=0.045). A significant positive association was found between prenatal

exposure to p,p'-DDE and waist circumference and waist/hip ratio in girls (P=0.010 and 0.007, respectively) and between prenatal exposure to HCB and the waist/hip ratio in girls (P=0.027).

Table 3. P-value (P) and regression coefficient (B) for the linear regression to investigate the association between prenatal contaminant exposure and body composition at 7-9 years without correction for confounders

		PCB (ng/g fat)	Dioxin (pg CALUX-TEQ /g fat)	HCB (ng/g fat)	P,p'-DDE (ng/g fat)	Cadmium (µg/L)
Height (cm)	*Boys*	P=0.535	P=0.264	P=0.488	P=0.547	P=0.795
		B=0.775	B=1.672	B=0.729	B=0.701	B=-0.191
	Girls	P=0.696	P=0.547	P=0.588	P=0.784	**P=0.017**
		B=0.353	B=-0.830	B=-0.434	B=0.210	**B=-1.384**
Weight (kg)		P=0.543	P=0.580	P=0.255	P=0.097	P=0.058
		B=0.015	B=-0.017	B=0.010	B=0.015	B=-0.012
BMI (kg/m²)		P=0.610	P=0.278	P=0.177	P=0.063	P=0.291
		B=0.066	B=-0.182	B=0.151	B=0.210	B=-0.083
Waist circumference (cm)	*Boys*	P=0.717	P=0.937	P=0.892	P=0.928	P=0.824
		B=-0.008	B=-0.002	B=0.002	B=-0.002	B=-0.003
	Girls	P=0.094	P=0.489	P=0.065	**P=0.01**	**P=0.011**
		B=0.012	B=-0.018	B=0.012	**B=0.015**	**B=-0.011**
Hip circumference (cm)		P=0.499	P=0.494	P=0.217	P=0.164	**P=0.045**
		B=0.007	B=-0.01	B=0.005	B=0.006	**B=-0.006**
Waist/height		P=0.550	P=0.375	P=0.136	P=0.081	P=0.223
		B=0.007	B=-0.013	B=0.014	B=0.017	B=-0.008
Waist/hip	*Boys*	P=0.727	P=0.752	P=0.407	P=0.493	P=0.561
		B=-0.003	B=-0.003	B=-0.006	B=-0.006	B=-0.003
	Girls	P=0.197	P=0.694	**P=0.027**	**P=0.007**	P=0.742
		B=0.008	B=0.004	**B=0.013**	**B=0.014**	B=0.001
Sum of 4 skinfolds* (mm)		P=0.613	P=0.345	P=0.213	P=0.263	P=0.079
		B=0.027	B=-0.069	B=0.057	B=0.052	B=-0.057

Sum of triceps and subscapular skinfold (mm)	*Boys*	P=0.736	P=0.949	P=0.446	P=0.604	P=0.891
		B=-0.026	B=0.006	B=0.049	B=0.037	B=-0.006
	Girls	P=0.414	P=0.399	P=0.263	P=0.228	**P=0.003**
		B=0.048	B=-0.072	B=0.059	B=0.060	**B=-0.104**

**sum of biceps, triceps, subscapular and suprailiacal skinfolds*

For the markers that differed significantly (between boys and girl) regression analyses were stratified for gender.

Table 4 shows the results of the regression analyses after correction for the fixed set of earlier mentioned confounders.

A negative association was found between prenatal cadmium exposure and waist circumference in girls (P=0.032) and between cadmium and the sum of tricipital and subscapular skinfolds in girls (P=0.004). This implicates that a higher prenatal cadmium exposure is associated with a decrease in the above mentioned anthropometric measurements. The association between prenatal cadmium exposure and height and hip circumference in girls (P=0.164 and P=0.055, respectively) was no longer significant after adjustment for the confounders.

Moreover, a significant positive association was found between prenatal exposure to PCBs and p,p'-DDE and waist circumference in girls (P=0.033 and 0.021, respectively). Next, a significant positive association was also found between prenatal exposure to p,p'-DDE and the ratio waist/hip in girls (P=0.004). In boys, a significant positive association was found between prenatal exposure to dioxins and height (P=0.039). This implicates that a higher prenatal exposure to these environmental pollutants is associated with an increase in anthropometric measures of abdominal fat (indicator of central obesity). The association between prenatal HCB exposure and the ratio waist/hip in girls (P=0.057) was no longer significant after adjustment for the confounders. On the other hand, the association between prenatal PCBs exposure and waist circumference in girls became positively significant (P=0.033) after adjustment for the confounders.

Table 4. Regression coefficient (B) and p-value (P) for the multiple regression to investigate the association between prenatal contaminant exposure and body composition at 7-8 years after correction for age, gender and birth weight of the child, BMI of mother and father, education level of the parents and consumption frequency of sugar- and/or fat-rich foods, and, with regard to height of the child, for height of the parents

		PCB (ng/g fat)	Dioxin (pg CALUX-TEQ /g fat)	HCB (ng/g fat)	P,p'-DDE (ng/g fat)	Cadmium (µg/L)
Height (cm)	*Boys*	P=0.775	**P=0.039**	P=0.964	P=0.809	P=0.999
		B=-0.328	**B=2.510**	B=-0.039	B=-0.241	B=-0.001
	Girls	P=0.542	P=0.244	P=0.752	P=0.769	P=0.164
		B=0.426	B=-1.115	B=-0.198	B=-0.166	B=-0.650
Weight (kg)		P=0.550	P=0.685	P=0.576	P=0.672	P=0.066
		B=0.006	B=-0.005	B=-0.005	B=0.004	B=-0.011
BMI (kg/m²)		P=0.579	P=0.422	P=0.964	P=0.258	P=0.196
		B=0.068	B=-0.127	B=-0.005	B=0.126	B=-0.101
Waist circumference (cm)	*Boys*	P=0.486	P=0.754	P=0.180	P=0.389	P=0.887
		B=-0.006	B=-0.003	B=-0.010	B=-0.007	B=-0.001
	Girls	**P=0.033**	P=0.843	P=0.194	**P=0.021**	**P=0.032**
		B=0.014	B=-0.002	B=0.008	**B=0.013**	**B=-0.009**
Hip circumference (cm)		P=0.441	P=0.653	P=0.798	P=0.616	P=0.055
		B=0.003	B=-0.003	B=-0.001	B=0.002	B=-0.005
Waist/height		P=0.537	P=0.645	P=0.868	P=0.149	P=0.159
		B=0.006	B=-0.006	B=0.002	B=0.013	B=-0.009
Waist/hip	*Boys*	P=0.418	P=0.559	P=0.136	P=0.269	P=0.535
		B=-0.007	B=-0.006	B=-0.011	B=-0.009	B=-0.003
	Girls	P=0.080	P=0.283	P=0.057	**P=0.004**	P=0.771
		B=0.012	B=0.012	B=0.012	**B=0.015**	B=0.001
Sum of 4 skinfolds* (mm)		P=0.531	P=0.526	P=0.932	P=0.536	P=0.073
		B=0.032	B=-0.044	B=-0.004	B=0.029	B=-0.059
Sum of triceps and subscapular skinfold (mm)	*Boys*	P=0.672	P=0.973	P=0.630	P=0.846	P=0.981
		B=-0.031	B=-0.003	B=-0.031	B=0.014	B=0.001
	Girls	P=0.231	P=0.873	P=0.561	P=0.477	**P=0.004**
		B=0.064	B=-0.013	B=0.029	B=0.032	**B=-0.099**

**sum of biceps, triceps, subscapular and suprailiacal skinfolds* For the markers that differed significantly (between boys and girl) regression analyses were stratified for gender.

5. Discussion

This study showed a significant negative association between prenatal cadmium exposure and waist circumference and the sum of tricipital and subscapular skinfolds in girls, whereas a positive association was found for PCBs and p,p'-DDE in regard to markers of central obesity (waist circumference and waist/hip ratio), only in girls. In boys, our analyses showed a significant positive association between prenatal dioxin exposure and height.

Previous studies reported an association between prenatal exposure to cadmium and height and weight in later life (11;16), while this study could not confirm this association. Because this is the first study using circumferences and skinfolds, comparison with previous results on these parameters is not possible.

In this cohort of Flemish children, earlier results indicated that prenatal exposure to EDCs influences their birth weight (25) and their body composition on the age of 3 (24). Concerning PCBs, Govarts et al. (2012) reported that birth weight was influenced negatively by prenatal exposure to PCBs (25) and Verhulst et al. (2009) reported that prenatal PCB exposure leads to an increase in BMI at the age of 3 (24). This study did not find an association with BMI, but prenatal PCBs exposure was significantly associated with markers of central obesity in 7 to 9 year old girls, which is complementary with the results at the age of 3 reported by Verhulst et al. (2009) (24).

Similarly, for p,p'-DDE exposure in this cohort, Govarts et al. (2012) did not find a significant relationship between prenatal exposure and birth weight (25), although Verhulst et al. (2009) did report a significant increase in BMI at the age of 3 (24). Likewise, we reported that prenatal exposure to p,p'-DDE leads to an increase in markers of central obesity in 7 to 9 year old girls.

At the age of 3, prenatal exposure to dioxins had no association with BMI in this cohort (24) but our analyses showed a significant positive association between prenatal dioxin exposure and height in 7 to 9 year old boys. This result has also been reported in another cohort at the age of 2 and 5 years (23). In contrast to the association between prenatal dioxins exposure and weight reported by Su et al. (2010) (23), we did not find such an association.

Last, in regard to prenatal HCB exposure, no statistically significant associations with anthropometrics were found in our study, only a trend toward a higher waist/hip ratio in girls, nor in the previous results at the age of 3 (24). Smink et al. (2008) showed that a higher

prenatal exposure to HCB leads to a higher BMI in 6.5 year-old children (42), however this could not be confirmed in our study.

5.1. Strengths and limitations of this follow-up study

A first strength is the longitudinal design of this study. Prenatal exposure data were collected at birth and the effect of this exposure on birth weight was studied by Govarts et al. (2012) (25). After 3 years, another assessment of the effects of prenatal exposure to EDCs and anthropometric measures was performed by Verhulst et al. (2009) (24). At the age of 7 to 9, the children were revisited to collect anthropometric data and parents were asked to complete a questionnaire.

A second strength includes the use of anthropometric data that were collected in a standardized way by two trained study nurses. This kind of data has a higher validity compared to self-reported anthropometric data. Moreover, it was possible to consider a set of different anthropometric parameters, not only height and weight but also circumferences and skinfolds, which made it possible to distinguish markers of subcutaneous fat from markers of visceral fat.

However, the limited sample size is the largest limitation of our study. Only 114 children of the original set of 1196 newborns were included in the study. The main reason for this is that the follow-up study conducted in 2011 was not announced to the participants during the baseline survey. Moreover, the only information that we had to contact the participants was their address at baseline. Possibly, a lot of families moved between 2002 and 2011, which decreased the participation rate. In future cohort studies, it is the aim to ask the number of the National Register, in order to be able to trace back participants who have moved. An analysis was conducted in order to study the difference between the participants that received a home visit (n=114) and the non-participants (n=1053). There was no difference in sex between the group of participants and non-participants ($p=0.655$). Concerning the mothers, there was no difference in the percentage of mothers who had ever smoked ($p=0.186$), nor was there a difference in the pre-pregnancy BMI of the mothers between the participants and the non-participants ($p=0.475$), however the education of the mothers was higher in the group of the participants ($p<0.001$) and the age of the mothers at birth who participated was a little higher compared to the non-participants (30.5 versus 29.5 years, respectively, $p=0.015$). There were no significant differences between the participants and the non-participants concerning prenatal exposure to cadmium, HCB, p,p'-DDE, PCB or dioxins.

Another limitation of this study is the lack of exposure data for the postnatal period up to 8 years. It is probable that postnatal exposure to some of these contaminants is also correlated to the body composition of the children. However, it was not possible to collect new blood samples from the children.

6. Conclusion

To the authors' knowledge, this is the first study investigating the effect of prenatal EDCs exposure on the body composition at the age of 7 to 9 years old, making use of a variety of anthropometric parameters: weight, height, BMI, waist and hip circumference, waist/hip ratio, waist/height ratio, sum of four as well as two skinfolds. Prenatal exposure to prenatal chlorinated EDCs (PDBs and p,p'-DDE) was positively associated with markers of central obesity (waist circumference and waist/hip ratio) and this was only the case in girls. Prenatal cadmium exposure was negatively associated with waist circumference (indicator of visceral fat) and with the sum of tricipital and subscapular skinfolds (indicator of subcutaneous fat) only in girls. Only in boys, prenatal exposure to dioxins was positively associated with increased height.

This study helped in creating evidence in the role of prenatal ECDs exposure on the development of obesity later in life. However, more studies with larger sample sizes using a variety of anthropometric parameters are needed in the future to further investigate this topic.

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