Blood pressure and arterial stiffness in obese children and adolescents

Effect of weight-reduction

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This PhD thesis is based on the following four papers:

Paper I:
Hvidt KN, Olsen MH, Holm J-C, Ibsen H. Aortic stiffness in obese children and adolescents: Comparison of two distance measures of carotid–femoral pulse wave velocity. Artery Research. 2013; 7:186-193.

Paper II:
Hvidt KN, Olsen MH, Holm J-C, Ibsen H. Obese Children and Adolescents Have Elevated Nighttime Blood Pressure Independent of Insulin Resistance and Arterial Stiffness. American Journal of Hypertension. 2014 Nov; 27(11):1408-15.

Paper III:
Hvidt KN, Olsen MH, Ibsen H, Holm J-C. Weight reduction and aortic stiffness in obese children and adolescents: a 1-year follow-up study. Journal of Human Hypertension. 2015 Jan 15. doi: 10.1038/jhh.2014.127. [Epub ahead of print]

Paper IV:
Hvidt KN, Olsen MH, Ibsen H, Holm J-C. Effect of changes in body mass index and waist circumference on ambulatory blood pressure in obese children and adolescents. Journal of Hypertension. 2014 Jul; 32(7):1470-7.

The papers will be referred in the text as paper I-IV.

References are given for paper II-IV which has been published since submission of the PhD thesis January 30 2014. License to publish this PhD thesis in the Danish Medical Journal has been obtained from the journals.

Abbreviations

ABPM	ambulatory blood pressure monitoring
AC	arm circumference
AIx	augmentation index
AIx@HR75	augmentation index at heart rate 75
BP	blood pressure
BMI	body mass index
cfPWV	carotid-femoral pulse wave velocity
DXA scan	dual energy x-ray absorptiometry scan
HOMA index	homeostatic model assessment index
HR	heart rate
ICC	intraclass correlation coefficient
MAP	mean arterial pressure
PP	pulse pressure
WHR	waist-height ratio

1. OVERALL AIM

The overall aim of this thesis is to investigate arterial stiffness and 24-hour blood pressure (BP) in obese children and adolescents, and evaluate whether these measures are influenced by weight reduction. Such information might bring insight to the pathophysiology of obesity-related elevated BP.

2. INTRODUCTION

2.1 Cardiovascular diseases

Cardiovascular diseases are the primary cause of death Worldwide [1,2]. Obesity, elevated BP and arterial stiffness are risk factors for cardiovascular disease [3–10].

The prevalence of childhood obesity has increased in the past two to three decades [11,12], and a strong relationship exists between obesity and elevated BP in both children and adults [13,14]. Obesity and elevated BP in childhood track into adult life [15–18], and have been strongly associated with premature death [19]. Furthermore, childhood obesity is associated with an increased risk of coronary artery disease in adulthood [20].

Longitudinal studies focusing on cardiovascular risk stratification in children and adolescents need markers of subclinical organ damage [21,22] since non-fatal and fatal cardiovascular events, e.g. acute myocardial infarction, stroke and death, seldom occur in children and adolescents [21,22]. Relevant markers of subclinical organ damage might contribute to a better understanding of

obesity's adverse impact on the cardiovascular system, and ultimately a better prevention and treatment of childhood obesity.

2.2 Obesity

The World Health Organisation has defined overweight and obesity as an abnormal or excessive fat accumulation that may impair health [23]. The fundamental cause of obesity and overweight is an energy imbalance between calorie intake and calorie consumption [23]. Obesity affects multiple organ systems [11], e.g. the cardiovascular system with elevated BP [24–27].

Overweight and obesity are classified according to body mass index (BMI) [23,28], and in adults overweight is a BMI > 25 kg/m2, and obesity a BMI > 30 kg/m2. Growth influences anthropometric measures and normal values over time during childhood [28,29]. Actual measured values of BMI are therefore standardised into so called z scores in respect to a normative reference population with the same gender and age [29]. Hence, BMI z score represents the degree of obesity, where a value of zero correspond to the expected mean of the reference population. Overweight in childhood is defined as a BMI z score above 1, whereas obesity is defined as a BMI z score above 2 [28]. Waist circumference is a surrogate for abdominal fat and can be indexed by height (WHR) representing growth when comparing measurements over time [30–32]. A WHR level below 0.05 has been suggested as a normative cut off point of abdominal fat [32].

Structured treatment of childhood obesity is a relatively new discipline – at least in Denmark. The Children's Obesity Clinic, Department of Paediatrics, Holbæk University Hospital represents a multidisciplinary setting where severe obese paediatric patients undergo lifestyle intervention [33,34].

2.3 Blood pressure

Obesity-related elevated blood pressure (BP) has been linked to insulin resistance in children and adolescents [35–37]. In this respect, insulin resistance may impact the cardiovascular system contributing to the obesity-related elevated BP [25]. Part of insulin resistance's potential adverse effects could be artery wall stiffening (arterial stiffness) [38,39].

Ambulatory BP monitoring (ABPM) is regarded as the most precise measure of the BP-burden [10,40–42], and focus on nighttime BP is growing due to its significant prognostic role [43,44], which has been adopted in paediatrics [41].

Weight reduction has been accompanied with a reduction in clinic BP [45–48]. Weight-loss associated reduction in ambulatory BP has been associated with a reduction in risk factors of cardiovascular disease in adults [49]. Knowledge is lacking on the effect of weight reduction on ambulatory BP in children and adolescents.

2.4 Arterial stiffness

Arterial stiffness (i.e. aortic stiffness) is an independent risk factor for cardiovascular disease [6,8,50,51], and has been suggested as a marker of vascular aging [52]. The main structural changes in the vessel wall leading to arterial stiffness are degradation of elastic fibres and replacement with collagen fibres leading to arteriosclerosis [52,53].

Carotid-femoral pulse wave velocity (cfPWV) is regarded as the gold standard for evaluating arterial stiffness [54,55]. In adults, body fat has been associated with reduced arterial stiffness until middle age [56]. However, divergent associations between obesity and cfPWV exist in children and adolescents [57–60]. CfPWV is a simple velocity measure of the aortic length being the pulse wave travel distance divided by the pulse wave transit time (m/s). Based on an adult MRI study on cfPWV [61], the recommended way to determine the aortic length precisely has changed [54]. Previously the length from the suprasternal notch to the femoral artery minus the length from the suprasternal notch to the carotid artery (subtracted distance) was used [55]. Currently, it is recommended to use 80 % of the direct distance from the carotid artery to the femoral artery (direct distance) (for details see section 4.6) [54,61]. The impact of this change in methodology on measurement of cfPWV is unknown in obese children.

In middle-aged and older adults, weight reduction has been associated with a reduction in arterial stiffness [62,63]. Knowledge is lacking on the effect of weight reduction on arterial stiffness in children and adolescents.

Reflected waves measured by augmentation index (AIx) is regarded as an indirect measure of arterial stiffness [55,64]. AIx is the proportion of the central BP derived from reflected BP waves (for details see section 4.6). The vital organs (i.e. brain, heart, kidney and lungs) are exposed to the central BP, and antihypertensive drugs with equal effect on the brachial BP may have different impact on central BP [65–67]. A better understanding of arterial stiffness and central BP might bring insight to the pathophysiology of obesity-related elevated BP.

2.5 Unanswered questions

- The guideline on cfPWV was revised in 2012 in respect to the distance measure of cfPWV [54]. It is unknown whether this change in methodology impacts the relationship between obesity and arterial stiffness.
- Several studies have shown that obese children have elevated ambulatory BP [37,68–76]. However, knowledge is lacking on whether the presumed higher ambulatory BP in obese children can be related to differences in metabolic factors and arterial stiffness when compared to normal weighted children.
- Weight reduction has led to divergent results on arterial stiffness in adults [62,63,77–79], but the effect is unknown in children and adolescents.
- Weight reduction in children has been associated with a reduction in clinic brachial BP [45–48], but it is unknown whether weight reduction has an impact on ambulatory BP, and it is unknown whether changes in ambulatory BP are more closely related to changes in obesity than changes in clinic brachial BP.

3. SPECIFIC OBJECTIVES

In a cross-sectional design, obese children and adolescents recruited from the Children's Obesity Clinic are compared to a normal weighted control group. The objectives are to investigate whether:

- Increased aortic stiffness is present in obese children and adolescents when previous as well as current recommendations on measurement of cfPWV are employed (paper I).
- Elevated day- and night-time BP exist in obese children and adolescents. Further, it is investigated whether the potential obesity-related ambulatory BP elevation can be related to insulin resistance and arterial stiffness (paper II).

In a longitudinal design, the obese children and adolescents underwent one-year of lifestyle intervention at the Children's Obesity Clinic in purpose of reducing the degree of obesity. The objectives are to investigate the potential impact of weight reduction on:

- Aortic stiffness in the obese patients (paper III).
- Ambulatory BP in the obese patients (paper IV).

4. METHODS

4.1 Study population

Obese patients aged 10-18 years newly referred to the Children's Obesity Clinic, Department of Paediatrics, Holbæk University Hospital [33] were asked to participate in the study. The tertiary obesity clinic receives paediatric patients with a BMI above the 90th percentile (equal to a z score of 1.282) for gender and age according to the Danish BMI charts [29]. Difficulties in communication were the only exclusion criteria. Recruitment period was from January 2011 to January 2012 and continued until 100 obese Caucasian patients were enrolled.

Seventy-one percent of invited patients participated in the study, and these were representative of the patients referred to the clinic (appendix 12.1). Within the same time frame, 50 age and gender matched Caucasian control individuals with an assumed representative normal weight range were recruited from the local area either from hospitals' personals' offspring or school children and adolescents in the region surrounding the Hospital. Clinical and paraclinical measurements in the present study were performed on two consecutive days no later than two months after the patients' first visit in the clinic.

No differences were found in prevalence of smoking (5 (5.4%) obese vs. o control, P=0.12) or use of medication (17 (16%) obese vs. 9 (18%) control, P=0.61). Six obese and four control individuals used medication for asthma or allergy, three obese used medication for gastro-intestinal symptoms, three obese and one control used hormonal supplementation, four obese used birth control medication, one obese used Ritalin, and three obese and five control used other not specified medication. The obese patients did not change medication or smoking status during the study.

The study was declared to ClinicalTrials.gov (NCT01310088), The Danish Data Agency and approved by The Scientific Ethical Committee of Region Zealand. Written informed consent was obtained from parents and individuals aged 18 according to the Helsinki Declaration.

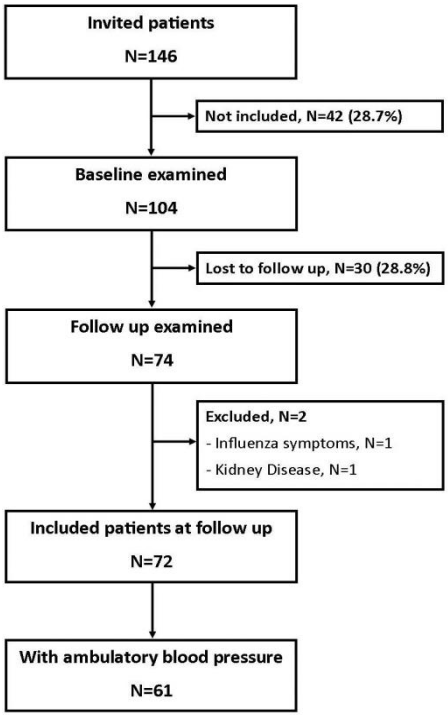
4.2 Design

In a cross-sectional design, the obese patients were compared with the control individuals (paper I and II). In a longitudinal design (figure 1), the obese patients were re-examined from March 2012 to January 2013 after one year of lifestyle intervention (follow up) (paper III and IV). Seventy-four 74 patients (71% of the patients investigated at baseline) were evaluated at follow up one year later. Two patients were excluded from the analyses; one due to onset of influenza symptoms at follow up, and one due to a chronic kidney disease (nephrectomised). None of the remaining patients were diagnosed as having secondary hypertension.

4.3 Anthropometry and obesity measures

Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg wearing light indoor clothes without shoes using an integrated calibrated weight and stadiometer (ADE, Modell MZ10023, Germany). BMI (kg/m2) was calculated into BMI z scores according to a Danish standard population in respect to age and gender [29]. Waist circumference was measured to the nearest 0.1 cm with subjects standing using a stretch-resistant tape at the level of the midpoint between lower margin of the last palpable rib and top of the iliac crest [80]. Waist-height ratio (WHR) was calculated as waist circumference (cm) divided by height (cm).

Figure 1: Flow chart of the obese patients in the study



Total body fat percentage was measured by dual energy x-ray absorptiometry (DXA) scanning (Lunar iDXA, GE Healthcare, enCore version 13.20.033, Madison, USA) (paper III and IV). The DXA scan is included in the treatment protocol at The Children's Obesity Clinic, and patients had these performed close to inclusion in the clinic. Only DXA scans performed less than sixty days before or after examination days were included in the analyses. Eighty-six (83% of the included) obese patients had a DXA scan at baseline, whereas 59 (82% of the followed up) obese patients had a DXA scan at baseline and at follow up. The control individuals had their DXA scan performed on either of the two study days, although three individuals missed their DXA scan.

4.4 Clinic brachial blood pressure

Clinic brachial BP was measured after a rest of minimum 10 minutes in supine position with the oscillometric device Omron 705IT validated in children and adolescents [81]. Upper brachial arm circumference (AC) was measured to the nearest 0.1 cm. An appropriate cuff size; small (AC < 22 cm), medium (AC 22 to 32 cm), and large (AC ≥ 32 cm), was used as recommended by the manufacturer. Mean of the last two out of three BP measurements was reported and calculated into z scores according to an American standard population based on individuals' gender, age and height [82]. Clinic heart rate (HR) was measured during 20 seconds with the SphygmorCor 9.0 device (AtCor Medical).

4.5 Ambulatory blood pressure

Ambulatory BP was measured with the oscillometric device Boso TM-2430 validated in children and adolescents [83]. The device was mounted on the upper brachial arm using an appropriate cuff size; small (AC < 22 cm), medium (AC 22 to 32 cm), and large (AC ≥ 32 cm). The device was programmed to measure with 15 minutes intervals during day (07.00-22.00) and 30 minutes intervals during the night (22.00-07.00). Patients were asked to keep a diary of their sleep time interval to differentiate awake (day-time) from sleep (night-time) in the BP analyses. Mean values of ambulatory systolic and diastolic BP and HR were calculated into z scores according to a German standard population based on gender and height [41,84]. Only patients having a valid ABPM with at least twenty valid BP measurements during day-time, and at least seven during night-time were included in the analyses [10,40].

Dipping status [44] (paper IV) was determined as being the percentage of night-time reduction in BP calculated as (mean day-time systolic BP - mean night-time systolic BP) x 100 / mean day-time systolic BP, and repeated for diastolic BP. Non-dipping was defined as a nocturnal BP reduction of less than 10%, equal to a night-to-day BP ratio (paper II) above 0.90.

Ambulatory BP classification [41] (paper II and IV) was based on cut-off levels of either systolic or diastolic clinic and 24-hour BP; normotension (clinic and 24-hour BP < 95th percentile), white-coat-hypertension (clinic BP ≥ 95th percentile and 24-hour BP < 95th percentile), masked hypertension (clinic BP < 95th percentile and 24-hour BP ≥ 95th percentile), and hypertension (clinic and 24-hour BP ≥ 95th percentile).

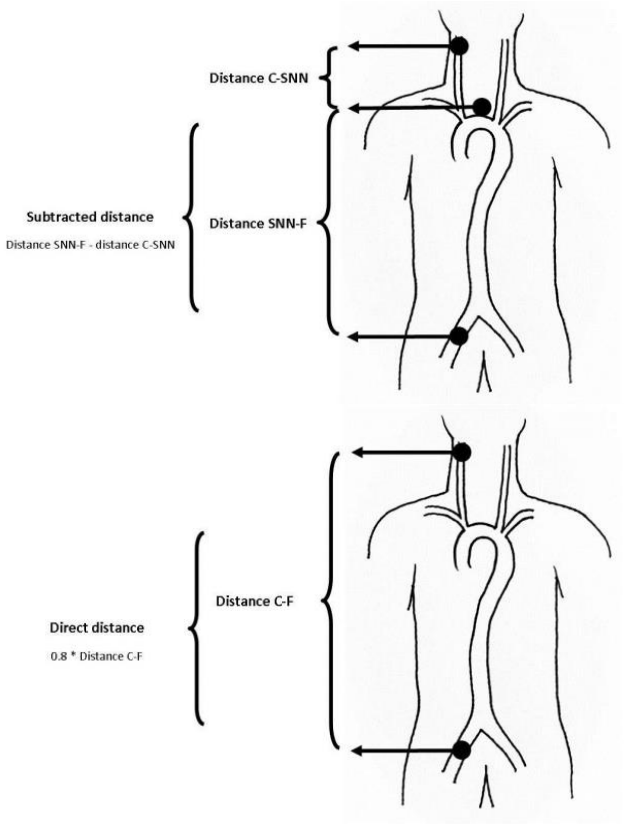
4.6 Arterial stiffness and central blood pressure

CfPWV and AIx were measured non-invasively by applanation tonometry with the SphygmoCor 9.0 device (AtCor Medical, Sydney, Australia) according to recommendations [54,55].

CfPWV was computed as the pulse wave travel distance divided by the transit time. The transit time was determined from the carotid and femoral artery waveforms using the foot-to-foot (intersecting tangent) method to locate the start of the waveforms when recorded consecutively with an ECG gated signal simultaneously recorded. Distances were measured as straight lines between pen's marked anatomical sites with a calliper (infantometer) and determined in two ways (figure 2); the commonly used 'subtracted distance' [55]; the length from the suprasternal notch to the femoral artery minus the length from the suprasternal notch to the carotid artery (paper I), and the newly recommended 'direct distance' [54,61]; 80% of the direct distance from the carotid artery to the femoral artery (paper I, II and III). From the same transit time cfPWV-subtracted and cfPWV-direct were calculated and reported as mean of at least two measurements.

CfPWV-subtracted z scores were calculated by gender and age (cfPWV-subtracted z scoreage), and gender and height (cfPWV-subtracted z scoreheight) in respect to a European standard population using the same subtracted distance [85] (paper I).

Figure 2: Subtracted and direct distance of carotid-femoral pulse wave velocity

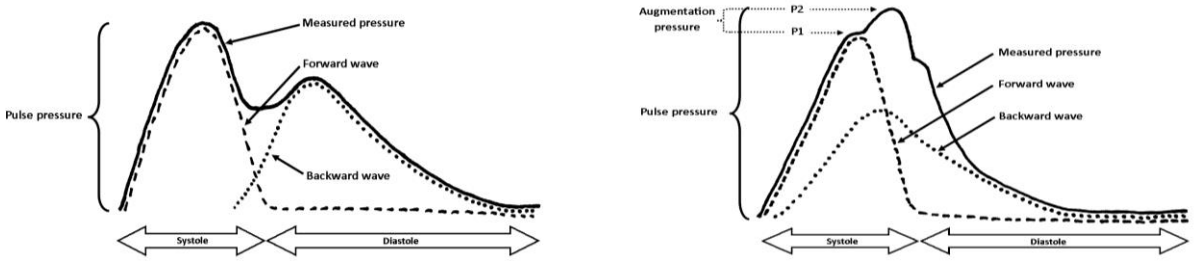


Distance C-SNN: distance between the common carotid and the suprasternal notch. Distance SNN-F: distance between the suprasternal notch and femoral artery. Subtracted distance: Distance SNN-F minus Distance C-SNN. Distance C-F: the direct distance between the common carotid and the femoral artery. Direct distance: 80 % of Distance C-F.

A central BP waveform was collected from the radial artery. AIx is the augmentation pressure expressed as the percentage of the pulse pressure, where augmentation pressure is the difference between the second and first systolic peaks originating from reflected BP waves (figure 3). AIx was corrected for a standard heart rate of 75 bpm (AIx@HR75) by the AtCor software. The central waveform obtained from the radial measurement was calibrated to the clinic brachial systolic and diastolic BP using a generalized transfer function validated in an invasive study on adults [86]. AIx@HR75 was reported as mean of at least two measurements. Due to difficulties in obtaining the measurements one individual had no whereas three individuals had only one radial AIx@HR75 measurement at baseline.

Individuals were asked to refrain from smoking at least three hours prior to the central hemodynamic and clinic BP measurements. The corresponding author performed all anthropometric, clinic BP, and central hemodynamic measurements after a training period.

Figure 3: Central blood pressure



A child with elastic arteries **An adult with stiffer arteries**

The measured central BP is the summation of a forward wave, travelling from the heart to the periphery, and a backward (reflected) wave, travelling backward to the heart. The timing of the backward wave is dependent on age and arterial stiffness. In children with elastic arteries, the backward wave returns in the diastole. In adults with stiffer arteries, the backward wave returns in the systole, and superimpose the forward wave. This leads to a higher systolic BP and pulse pressure as well as an increased load on the heart [53]. P1: first systolic peak of the forward wave. P2: second systolic peak of the reflected wave. PP: pulse pressure. Augmentation pressure: P2-P1. Augmentation index: Augmentation pressure/PP. Modified from Laurent & Cockcroft [53].

4.7 Repeatability of arterial stiffness

The daily variation in the central hemodynamic measurements was evaluated in 25 representative obese patients (35% of the followed up patients) (paper III and appendix 12.3).

CfPWV-direct: The mean difference with limits of agreement (mean difference ± 1.95*SD) the two days in between was 0.03 m/s (-0.68; 0.74, P=0.64), it did not depend on the magnitude of the measurement (figure 4), whereas the intra class correlation coefficient (ICC) was 0.80 (P<0.0001). The time difference was 24.2 ± 1.6 hours between measurements of cfPWV-direct.

AIx@HR75: The mean difference was -2.5 %-point (-16.6; 11.6, P=0.11), not dependent on the magnitude of the AIx@HR75 measurement (figure 5), and had an ICC of 0.68 (P=0.0002) the two days in between. The time difference was 24.2 ± 1.5 hours between measurements of AIx@HR75.

Figure 4: Bland Altman plot of cfPWV-direct

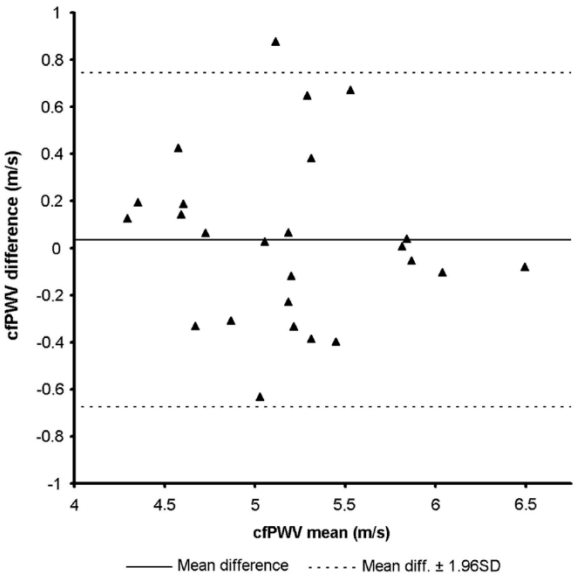
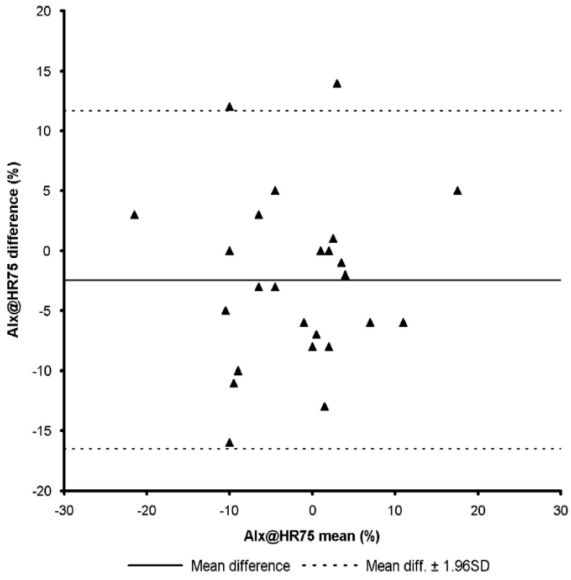


Figure 5: Bland Altman plot of AIx@HR75



4.8 Biochemical measures

Venous blood samples were drawn early morning after overnight fasting. Biochemical plasma concentrations of glucose and insulin were measured with Cobas 6000 (Roche Diagnostics, Switzerland). However, plasma insulin in six of the obese blood samples was measured with the former laboratory method (Immulite 2000, Siemens, Germany). Insulin resistance was determined as the homeostatic model assessment (HOMA) index calculated as glucose (mmol/l) multiplied by insulin (mmol/l) and divided by 135 [87] (paper II). Due to either haemolysis of blood samples, no attendance or visit delay exceeding 60 days, the total number of blood samples were 79 (86%) in the obese and 47 (96%) in the control group (paper II).

4.9 Statistical analysis

Statistical analyses were performed using SAS software (version 9.2, SAS Institute, USA). The significance level was set as a p value below 0.05 on 2-sided tests. Results were reported as mean ± standard deviation (SD) or median (interquartile range (IQR)) dependent on whether data were normally distributed.

Potential differences in measures between the obese and the control group as well as genders at baseline were calculated with unpaired Student's t-tests for normally distributed continuous variables, otherwise Wilcoxon rank sum tests, Chi-squared tests for categorical variables or Fischer's exact test when appropriate. Cochran-Armitage trend test was used to test for a potential difference in the BP classification between the obese and the control group.

In the cross-sectional design (paper I and II), relationships between obesity measures and hemodynamic variables were investigated separately for the obese and the control group using linear regression analyses. Potential gender differences in these potential relationships were investigated in multiple regression analyses. In pooled multiple regression analyses, the relationship between a group variable (obese vs. control individuals) and arterial stiffness and day- and night-time BP's were investigated when adjusting for relevant confounders. Due to the design of the recruitment, the group variable encompasses the differences between the two groups in obesity measures (BMI z score and WHR). In order to avoid over-adjustment, these measures were not included in the analyses. The regression models were tested for possible interactions between the group and gender variables with the other explanatory variables in order to pool data, and repeated when excluding smokers and individuals receiving medication.

In the longitudinal design (paper III and IV), differences in measurements between baseline and follow up were investigated with paired Student's t-tests or Wilcoxon signed rank test dependent on whether differences were normally distributed. We investigated whether changes in arterial stiffness and BP's were related to changes in obesity measures using linear and multiple regression analyses when adjusting for relevant confounders. In order to pool data from the two genders, we tested for a possible interaction of gender with the explanatory variable of interest (the change in the obesity measure).

In linear regression analyses (paper III), we investigated whether measures of aortic stiffness were related to age at baseline and follow up. In mixed model analyses, we tested whether the linear regression equations between cfPWV and age at baseline and follow up differed, in order to evaluate whether a potential difference in the level of cfPWV at follow up was merely ascribed to the higher age, or whether gender and hemodynamic differences (mean arterial pressure and heart rate) contributed.

Reproducibility (paper I) and repeatability (paper III) of arterial stiffness measures were investigated with paired (one sample) Student's t-tests for possible systematic differences, Bland Altman plots for possible differences in the magnitude of the measurements, and intraclass correlation coefficients (ICC) as indexes of reliability. ICC was calculated as Pearson's correlation coefficient when no systematic difference was found (paper III), otherwise with a formula taking the systematic difference into account (paper I) [88].

5. RESULTS

5.1 Results of the cross-sectional design

5.1.1 The study design: obesity

In paper I and II, the obese and the control group were matched for age, gender and height (table 1). As expected due to the design of the recruitment, the obese group had higher weight, BMI, BMI z score, waist circumference, WHR, and DXA total body fat percent. In the obese group, fasting glucose was lower whereas insulin and HOMA index were higher when compared to the control group.

No gender differences were found in anthropometric measures in the followed up obese patients at baseline (paper III and appendix 12.3). However, obese girls had a higher DXA total body fat percent (girls 45.4 (43.0-49.0)% vs. boys 42.6 (38.2-47.0)%, P=0.02) when compared to obese boys.

Table 1: Body composition and metabolic factors

	Obese Group N=104	Control Group N=50	
Variable	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P Value
Male/Female (N/N)	50/54	23/27	0.81
Age (years)	12.6 (11.4-15.0)	13.2 (11.7-14.9)	0.44
Height (cm)	159.9 ± 11.9	163.2 ± 12.1	0.11
Weight (kg)	66.9 (57.7-90.9)	50.7 (41.3-58.4)	<0.0001
BMI (kg/m2)	27.63 (24.1-32.4)	18.76 (16.7-20.1)	<0.0001
BMI z score	2.76 ± 0.68	0.08 ± 0.84	<0.0001
Waist circumference (cm)	94.8 (85.3-107.5)	66.4 (62.7-69.6)	<0.0001
WHR	0.60 (0.56-0.64)	0.40 (0.38-0.42)	<0.0001
DXA total body fat (%)	44.6 (41.1-48.5)	25.5 (22.0-30.9)	<0.0001
Fasting glucose (mmol/l)	5.3 ± 0.6	5.6 ± 0.6	0.025
Insulin (mmol/l)	95.3 (57.2-155.9)	60.1 (42.5-84.4)	0.0001
HOMA index	3.7 (2.3-6.0)	2.6 (1.8-3.4)	0.002

The study design: Comparing the obese and the control group (paper I). The total number of dual energy x-ray absorptiometry (DXA) scans was 86 in the obese and 47 in the control group. The total number of blood samples was 79 in the obese and 47 in the control group (paper II). BMI: body mass index. WHR: waist-height ratio. HOMA index: homeostatic model assessment index.

LogHOMA index was related to BMI z score (β=0.25, 95% CI 0.13-0.37, P<0.0001) and WHR (β=2.52, 95% CI 1.39-3.65, P<0.0001) in the obese group, whereas these relationships were not found in the control group. No significant gender differences were found in these relationships (paper II).

5.1.2 Arterial stiffness and central blood pressure

Both clinic brachial and central systolic and diastolic BP were higher in the obese compared to the control group (table 2 and paper I). Likewise, mean arterial pressure (MAP) was higher in the obese group (obese 76.8 ± 6.3mmHg vs. control 74.4 ± 5.5 mmHg, P=0.02). No group differences were found for clinic brachial and central pulse pressures, HR or AIx@HR75.

CfPWV-subtracted was higher in the obese compared to the control group, whereas cfPWV-direct was lower in the obese compared to the control group. Likewise, cfPWV-subtracted z scores were significantly higher in the obese compared to the control group (cfPWV-subtracted z scoreage: obese -0.60 ± 0.80 vs. control -0.96 ± 0.89, P=0.013, cfPWV-subtracted z scoreheight: obese -0.76 ± 0.79 vs. control -1.22 ± 0.87, P=0.0014). Both groups had cfPWV-subtracted z scores below zero, which is in the lower normal range (below 1.645, i.e. below the 95th percentile).

Arterial stiffness measures did not differ between genders in the two groups (paper I). Further, clinic brachial and central BP did not differ between genders in the obese followed up patients, (paper III and appendix 12.3). However, obese girls had a higher clinic brachial diastolic BP (girls 63.6 ± 5.7 mmHg vs. boys 60.5 ± 5.3 mmHg, P=0.02) and central diastolic BP (girls 64.6 ± 6.2 mmHg vs. boys 61.6 ± 5.3 mmHg, P=0.03), while a lower central pulse pressure (girls 28.7 ± 5.4 mmHg vs. boys 31.4 ± 5.6 mmHg, P=0.04) when compared to obese boys.

Table 2: Arterial stiffness and clinic brachial and central blood pressure

	Obese Group N=104	Control Group N=50	
Variable	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P Value
Clinic brachial SBP (mmHg)	110.9 ± 8.51	107.7 ± 8.0	0.03
Clinic brachial DBP (mmHg)	61.8 ± 5.7	59.1 ± 5.3	0.004
Clinic brachial PP (mmHg)	49.0 ± 7.5	48.6 ± 8.4	0.75
Central SBP (mmHg)	93.3 ± 7.3	90.4 ± 6.8	0.02
Central DBP (mmHg)	62.8 ± 5.9	60.1 ± 5.4	0.009
Central PP (mmHg)	30.5 ± 5.3	30.4 ± 6.1	0.86
Heart rate (bpm)	66.6 ± 9.5	63.4 ± 10.0	0.06
CfPWV-subtracted (m/s)	4.52 ± 0.52	4.32 ± 0.53	0.03
CfPWV-direct (m/s)	4.83 ± 0.57	5.10 ± 0.65	0.008
AIx@HR75 (%)	-0.11 ± 10.2	-1.30 ± 10.9	0.51

AIx@HR75: augmentation index at heart rate 75. CfPWV: carotid-femoral pulse wave velocity. HR: heart rate. SBP: systolic BP. DBP: diastolic BP. PP: pulse pressure.

The components of the cfPWV measures are listed in table 3. Despite a higher pulse wave transit time, the higher cfPWV-subtracted velocity in the obese group was related to a higher subtracted distance due to a shorter carotid to suprasternal notch distance and a longer suprasternal notch to femoral distance in obese patients. The lower cfPWV-direct velocity in the obese group was related to an equal direct distance and a higher pulse wave transit time in the obese patients.

Table 3: The components of cfPWV-subtracted and cfPWV-direct

	Obese Group N=104	Control Group N=50	
Variable	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P Value
Distance C-SNN (mm)	74.5 ± 13.0	93.1 ± 15.7	<0.0001
Distance SNN-F (mm)	493.1 ± 51.1	470.0 ± 40.5	0.006
Subtracted distance (mm)	418.6 ± 49.3	376.8 ± 30.5	<0.0001
Distance C-F (mm)	558.6 ± 55.7	557.3 ± 54.4	0.89
Direct distance (mm)	446.9 ± 44.5	445.8 ± 43.5	0.89
Transit time (ms)	88.5 ± 12.3	82.6 ± 12.1	0.006

Distance C-SNN: distance between the common carotid and the suprasternal notch. Distance SNN-F: distance between the suprasternal notch and femoral artery. Subtracted distance: distance SNN-F minus distance C-SNN. Distance C-F: the direct distance between the common carotid and the femoral artery. Direct distance: 80 % of distance C-F.

In both groups, the reproducibility of the two corresponding distance (subtracted distance and direct distance) and velocity (cfPWV-subtracted and cfPWV-direct) measures showed systematic differences, but the magnitude of the corresponding measurements did not differ. In this respect, Bland-Altman plots (not shown) showed a shifted level above zero with a random scatter. Taking into account these systematic differences the ICC of the distance measures were 0.75 for the obese and 0.30 for the control group, whereas the ICC of the cfPWV's were 0.77 for the obese and 0.49 for the control group.

Figure 6 and 7 show the distance measures plotted against height. Figure 6 show different slopes for the subtracted distance across groups (height*group interaction estimate P=0.006). Whereas figure 7 show almost superimposed with close to identical slopes for the direct measure (height*group interaction estimate P=0.74). The common carotid to suprasternal notch distance was also different across groups (height*group interaction estimate P=0.001). Whereas suprasternal notch to femoral artery distance was merely different (height*group interaction estimate P=0.06).

Figure 6: The subtracted distance as a function of height in the obese and the control group

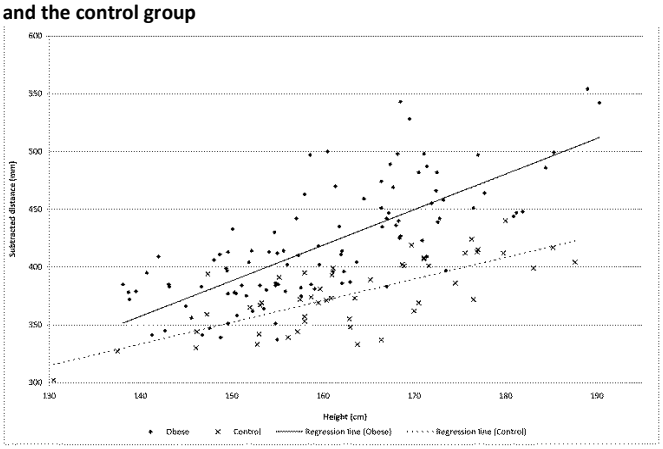
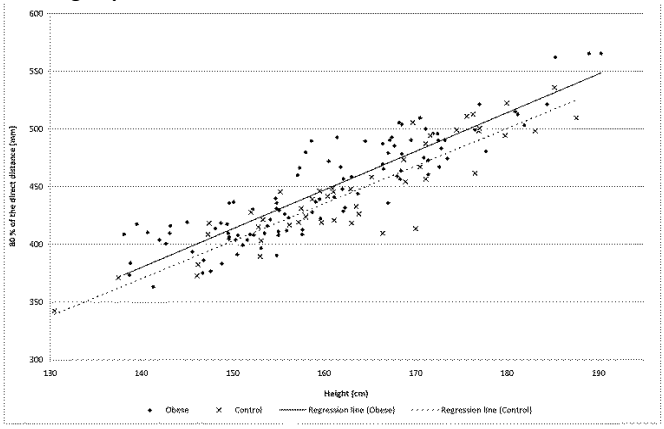


Figure 7: The direct distance as a function of height in the obese and the control group



5.1.3 Relationship between obesity and arterial stiffness

In the obese group, cfPWV-subtracted was related to BMI z score (β=0.202, 95% CI: 0.054 to 0.349, P=0.008), whereas no relationship was found between BMI z score and cfPWV-direct (β=0.039, 95% CI: -0.125 to 0.203, P=0.64). In the control group, no relationships were found between BMI z score and cfPWV-subtracted or cfPWV-direct (paper I).

CfPWV-direct was not related to logHOMA index in the obese or the control group, and no gender differences were found herein (paper II).

In pooled multiple regression analyses (paper I) a positive, but insignificant relationship was found between obese group status and cfPWV-subtracted (β=0.13, 95% CI -0.04-0.29, P=0.13), whereas it was significantly negative for cfPWV-direct (β=-0.34,

95% CI -0.529 to -0.154, P=0.0004) when adjusted for age, gender, MAP and HR.

Findings of arterial stiffness were reproducible when individuals using medication and smokers were excluded, but the unadjusted group comparison of PWV-subtracted became insignificant (P=0.11), as well as the linear relationship between BMI z score and cfPWV-subtracted in the obese group (P=0.11).

CfPWV-direct is used in the remaining part of the results section, and referred merely as cfPWV.

5.1.4 Ambulatory blood pressure

Twenty-four-hour, day- and night-time systolic BP, pulse pressure, and heart rate were consistently higher in the obese as compared to the control group (table 4 and paper II). No difference was found in 24-hour or day-time diastolic BP, while night-time diastolic BP was higher in the obese group. Twenty-four-hour MAP was higher in the obese group and apparently driven by a higher night-time MAP, since no difference was found in day-time MAP. Differences in BP z scores between the obese and the control group are found in appendix 12.2, and do not differ from differences in BP's in mmHg.

No differences in ambulatory BP in mmHg or z scores were found between genders at baseline in the followed obese patients (paper IV and appendix 12.4).

Table 4: Ambulatory blood pressure

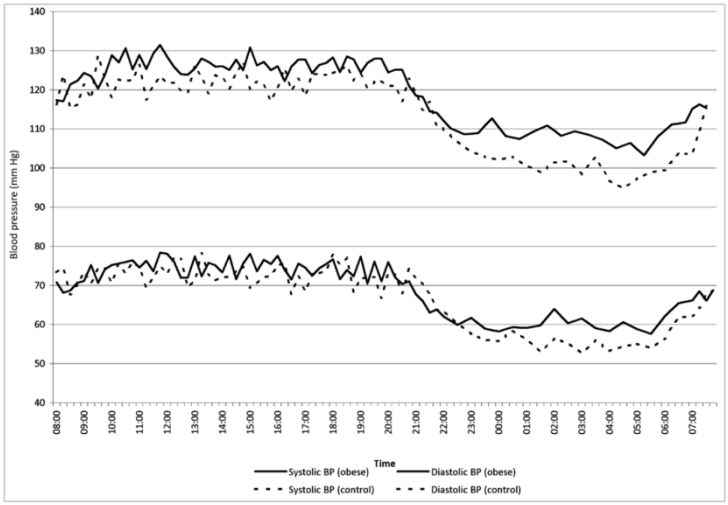
	Obese Group N=92	Control Group N=49	
Variable	Mean ± SD	Mean ± SD	P Value
24-hour systolic BP (mmHg)	121.2 ± 7.8	116.6 ± 8.9	0.002
24-hour diastolic BP (mmHg)	70.3 ± 4.8	68.9 ± 5.5	0.11
24-hour MAP (mmHg)	87.3 ± 5.4	84.7 ± 6.2	0.01
24-hour PP (mmHg)	51.0 ± 5.5	47.7 ± 6.1	0.002
24-hour HR (bpm)	79.6 ± 7.6	75.0 ± 9.3	0.002
Day-time systolic BP (mmHg)	124.8 ± 8.3	121.3 ± 10.1	0.03
Day-time diastolic BP (mmHg)	73.1 ± 5.9	72.6 ± 6.8	0.67
Day-time MAP (mmHg)	90.3 ± 6.3	88.9 ± 7.4	0.22
Day-time PP (mmHg)	51.7 ± 5.6	48.7 ± 6.5	0.005
Day-time HR (bpm)	82.0 ± 7.9	78.3 ± 9.7	0.02
Night-time systolic BP (mmHg)	108.4 ± 10.7	101.5 ± 8.2	0.0001
Night-time diastolic BP (mmHg)	60.0 ± 6.6	56.8 ± 4.8	0.001
Night-time MAP (mmHg)	76.1 ± 7.4	71.7 ± 5.6	<0.0001
Night-time PP (mmHg)	48.3 ± 7.2	44.8 ± 6.0	0.004
Night-time HR (bpm)	70.4 ± 9.0	64.1 ± 9.4	0.0002

MAP: arterial pressure. PP: pulse pressure. HR: heart rate.

The variation of systolic and diastolic BP throughout the day in the two groups is plotted in figure 8 (paper II). The figure displays the relatively higher night- than day-time BP in the obese group when compared to the control group, also demonstrated by higher night-to-day BP ratios in the obese group (systolic: 0.864 ± 0.074 obese vs. 0.835 ± 0.062 control, P=0.02, and diastolic: 0.820 ± 0.103 obese vs. 0.781 ± 0.082 control, P=0.02).

Twenty-four-hour BP was consistently higher than clinic brachial BP in the obese (Δsystolic BP: 10.1 ± 8.0 mmHg and Δdiastolic BP: 8.4 ± 6.2 mmHg, P values <0.0001) and the control group (Δsystolic BP: 9.3 ± 10.7 mmHg and Δdiastolic BP: 9.8 ± 6.6 mmHg, P values <0.0001). In this respect, 20 (22%) obese vs. 12 (24%) control individuals were white-coat hypertensive. Although no difference was found in the BP classification (P=0.18), respectively, 15 (16%) obese vs. 3 (6%) control individuals were hypertensive, 10 (11%) vs. 6 (12%) masked hypertensive, and 47 (51%) vs. 28 (57%) normotensive.

Figure 8: Circadian variation of the ambulatory blood pressure



Mean values of ambulatory systolic and diastolic BP for a given time plotted throughout the day in the obese and the control group. Time interval between BP readings was every fifteen minutes during the daytime (07:00-22:00) and every half an hour during night-time.

5.1.5 Relationship between obesity and ambulatory blood pressure

In the obese group, no relationship was found between BMI z score or WHR and day-time systolic or diastolic BP (paper II). Night-time systolic BP was related to BMI z score (β=6.0, 95% CI 2.9-9.1, P=0.0002) and WHR (β=36.7, 95% CI 5.6-67.9, P=0.02). Further, night-time diastolic BP was related to BMI z score (β=2.4, 95% CI 0.3-4.4 P=0.02) although not to WHR in the obese group.

In the control group, no significant relationships were found between BMI z score or WHR and day-time systolic or diastolic BP.

Night-time systolic BP in the obese group was 7.9 mmHg higher compared to the control group independent of cfPWV, logHOMA, and relevant confounders (table 5). At the same time, night-time systolic BP was related to cfPWV, and tended to be related to logHOMA (P=0.056).

The night-time diastolic BP was 2.9 mmHg higher in the obese when compared to the control group, while not related to logHOMA index or cfPWV when adjusted for relevant confounders.

Table 5: Multiple regression models of night-time systolic and diastolic blood pressure

	Night-time systolic BP		Night-time diastolic BP	
	β	95% CI	β	95% CI
Group (obese vs. control)	7.9***	4.1-11.6	2.9*	0.4-5.4
Age (years)	-0.7	-1.9-0.4	0.1	-0.7-0.8
Height (cm)	0.3*	0.06-0.5	0.01	-0.1-0.1
Gender (male vs. female)	4.8**	1.4-8.1	3.3**	0.4-5.4
Period dependent HR (bpm)	0.1	-0.1-0.3	0.2**	0.07-0.3
CfPWV (m/s)	3.8*	0.7-6.8	1.5	-0.5-3.5
LogHOMA index	5.5†	-0.1-11.1	0.8	-2.9-4.5
Model (r square)	***	0.355	***	0.236

β: beta coefficients. 95% CI: 95% confidence interval. Level of significance is denoted by *P<0.05, **P<0.01, and ***P<0.001, whereas †P<0.10. CfPWV: carotid-femoral pulse wave velocity. HOMA index: homeostatic model assessment index. HR: heart rate. The number of individuals (N=115) was reduced in the models due to missing blood sample values. No interactions existed between group and gender with the other explanatory variables.

The analysis of day-time systolic BP was restricted to the obese group (N=74) due to interactions of the group variable with other explanatory variables: group*day-time HR (P=0.02) and group*gender (P=0.04). Here, day-time systolic BP was related to logHOMA (β=8.0, 95% CI 2.6-13.5, P=0.004) and tended to be related to cfPWV (β=3.3, 95% CI -0.05-6.7, P=0.053) when adjusted for relevant confounders (model: R2=0.288, P=0.0007, no interactions).

The day-time diastolic BP model was also restricted to the obese group due to an interaction of group*heart rate (P=0.02) in pooled analysis. However, the day-time diastolic BP model including only the obese group was inconclusive (R2=0.07, P=0.52). The number of individuals was reduced in the models due to missing blood sample values.

Ambulatory BP differences between the two groups were reproducible when restricted to non-smokers and individuals not receiving medication. However, the relationship between logHOMA (P=0.21), gender (P=0.11), and height (P=0.057) with night-time systolic BP became insignificant.

5.2 Results of the longitudinal design

5.2.1 The study design: changes in obesity measures

Seventy-two obese patients (girls: 37 (51%)) were followed up after one year of lifestyle intervention. The followed up patients had a median age of 12.5 (IQR: 11.2-14.6) years at baseline and a follow up time of 364 (363-371) days (paper III).

Fifty-three patients (74% of the 72 followed up patients) experienced a reduction in their BMI z score (responders) with no significant difference between genders (girls 24 (64%) vs. boys 29 (83%), P=0.08) (paper III). BMI z score, WHR, and DXA total body fat percent were significantly lower at follow up despite an increased height and weight, and no change in BMI, waist circumference, or AC (table 6) (paper III). No differences in age and anthropometric measures were found between the followed up obese patients with (N=61) and without (N=11) an ABPM at baseline (paper IV).

In linear regression analyses (paper IV), changes in anthropometric measures were strongly inter-related; ΔAC were related to ΔBMI z score (β=2.6, 95% CI 1.7-3.5, R2=0.369, P<0.0001) and ΔWHR (β=24.6, 95% CI 15.8-33.5, R2=0.343, P<0.0001), and ΔWHR related to ΔBMI z score (β=0.07, 95% CI 0.05-0.09, R2=0.446, P<0.0001).

5.2.2 Changes in arterial stiffness and central blood pressure

CfPWV was higher at follow up, whereas no significant change was found for AIx@HR75 (table 7 and paper III). In the 25 patients included in the repeatability sub study, the year-to-year difference of cfPWV was higher than the day-to-day difference (0.25 ± 0.46 m/s, P=0.01), whereas no difference was found in AIx@HR75 between the year-to-year difference and the numeric day-to-day difference (-2.07 ± 13.31 %, P=0.45).

No significant differences were found in clinic brachial and central systolic or diastolic BP's and HR at follow up (table 7), but clinic brachial systolic BP z score was higher at follow up (Δ 0.33 ± 0.82, baseline: 1.50 ± 1.13 vs. follow up: 1.84 ± 1.00, P=0.0009). No difference was found in clinic brachial diastolic BP z score (Δ 0.004 ± 0.49, baseline: 0.44 ± 0.61 vs. 0.44 ± 0.66, P=0.94). Clinic brachial and central pulse pressures were higher at follow up.

Table 6: Anthropometrics and obesity measures at baseline and follow up

	Baseline N=72	Follow up N=72	Difference (Δ)	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P Value
Height (cm)	159.5 ± 11.6	164.5 ± 10.2	5.0 ± 3.5	<0.0001
Weight (kg)	66.7 (57.1-89.7)	72.1 (61.9-61.9)	4.1 ± 5.9	<0.0001
BMI (kg/m2)	27.0 (24.0-31.7)	26.5 (22.8-32.0)	-0.08 ± 2.06	0.74
BMI z score	2.75 ± 0.62	2.51 ± 0.87	-0.24 ± 0.45	<0.0001
Arm circumference (cm)	30.1 ± 4.4	30.1 ± 4.7	0.03 ± 1.9	0.88
Waist circumference (cm)	96.9 ± 15.0	97.2 ± 17.0	0.38 ± 6.61	0.63
DXA total body fat (%)	44.2 ± 4.7	41.0 ± 7.3	-3.3 ± 4.3	<0.0001

Twelve (17%) patients at baseline and one (1%) patient at follow up lacked a dual energy x-ray absorptiometry scan (DXA) scan, why comparison of DXA total body fat percent is based on 59 (82%) patients having a DXA scan at both baseline and follow up.

Table 7: Arterial stiffness and clinic brachial and central blood pressure at baseline and follow up

	Baseline N=72	Follow up N=72	Difference (Δ)	
	Mean ± SD	Mean ± SD	Mean ± SD	P Value
Clinic brachial SBP (mmHg)	110.7 ± 8.9	112.5 ± 8.1	1.9 ± 8.3	0.06
Clinic brachial DBP (mmHg)	62.1 ± 5.7	61.3 ± 6.2	-0.8 ± 5.5	0.25
Clinic brachial PP (mmHg)	48.6 ± 7.9	51.2 ± 7.9	2.6 ± 6.5	0.001
Central SBP (mmHg)	93.2 ± 7.6	94.5 ± 6.6	1.3 ± 7.6	0.14
Central DBP (mmHg)	63.1 ± 5.9	62.7 ± 6.1	-0.5 ± 5.8	0.51
Central PP (mmHg)	30.0 ± 5.6	31.8 ± 5.8	1.8 ± 4.7	0.002
Heart rate (bpm)	67.1 ± 9.7	65.3 ± 10.0	-1.8 ± 8.4	0.08
CfPWV (m/s)	4.84 ± 0.57	5.11 ± 0.60	0.27 ± 0.47	<0.0001
AIx@HR75	-1.03 ± 10.39	1.07 ± 9.15	2.10 ± 9.73	0.07

AIx@HR75: augmentation index at heart rate 75. CfPWV: carotid-femoral pulse wave velocity. SBP: systolic BP. DBP: diastolic BP. PP: Pulse pressure.

In linear regression, cfPWV was equally related to age at baseline (β=0.13 m/s per year, 95% CI 0.08-0.18, R2=0.252, P<0.0001) and follow up (β=0.13 m/s per year, 95% CI 0.07-0.19, R2=0.237, P<0.0001) (figure 9), and the regression slopes did not differ in mixed model analysis (P=0.97). In further mixed model analysis, cfPWV was still strongly related to age (β=0.12 m/s per year, 95% CI 0.07-0.17, P<0.0001) but the values were higher at follow up (β=0.17 m/s higher at follow up, 95% CI 0.28-0.05, P=0.005) when adjusted for heart rate (β=0.01 m/s per bpm, 95 CI 0.004-0.02, P=0.005), mean arterial pressure (β=0.01 m/s per mmHg, 95% CI 0.0009-0.03, P=0.04), and gender (β=0.06 m/s higher in girls, 95% CI -0.15-0.27, P=0.60). In this respect, the higher cfPWV at follow up was explained by the increase in age and partly by changes in BP and heart rate.

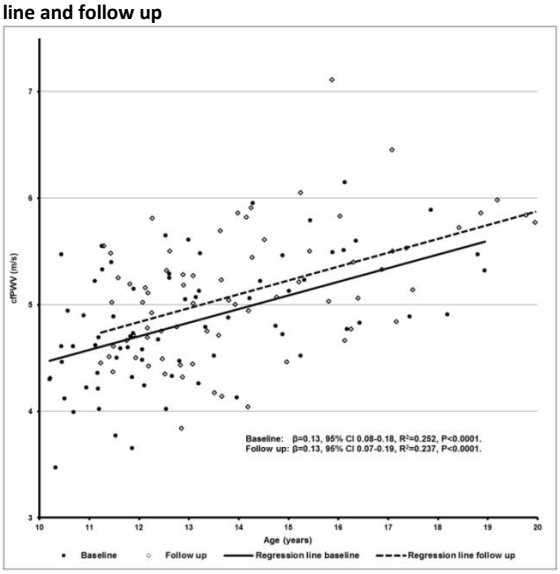
AIx@HR75 was not related to age at baseline (β=-0.62, 95% CI: -1.71-0.47, R2=0.018, P=0.26) or at follow up (β=-0.02, 95% CI: -0.99-0.95, R2=0.00002, P=0.97).

5.2.3 Relationship between changes in obesity measures and changes in arterial stiffness

In linear regression analyses, changes in cfPWV were not related to changes in obesity measures (models: R2≤0.006, P≥0.52). This was reproducible in multiple regression analyses adjusting for baseline confounders (models: R2≤0.048, P≥0.064).

Changes in AIx@HR75 tended to be linear related to changes in BMI z score (β=4.32, 95% CI -0.71-9.35, R2=0.040, P=0.09), WHR (β=48.01, 95% CI -2.94-98.96, R2=0.048, P=0.06), and DXA total fat percent (β=0.57, 95% CI -0.04-1.17, R2=0.058, P=0.07). In multiple regression analyses, changes in AIx@HR75 were related to changes in WHR (β=50.32, 95% CI 6.67-93.97, P=0.02, model: R2=0.430, P<0.0001) but not significantly to changes in BMI or DXA fat percent (models: R2≥0.392, P<0.0001). None of the multiple regression models had an interaction with gender and change in the obesity measure of interest.

Figure 9: Carotid-femoral pulse wave velocity in relation to age at baseline and follow up



5.2.4 Changes in ambulatory blood pressures

In the 61 followed up patients with valid ABPM's (paper IV), no significant differences were found between ambulatory BP's in mmHg and dipping status at baseline and follow up. When calculating ambulatory BP z scores, a reduction was found in day-time systolic, diastolic, and MAP BP z scores and a trend in 24-hour diastolic BP z score reduction at follow up, but no difference was found in night-time BP z scores (table 8).

BP classification status at follow up; 19 (31%) patients were normotensive, 23 (38%) were white-coat hypertensive, 5 (8%) masked hypertensive and 14 (23%) were hypertensive. Thirty-six (59%) patients had a normal 24-hour BP (below the 95th percentile) at baseline and follow up, whereas five (8%) patients were hypertensive at both baseline and follow up.

Table 8: Ambulatory blood pressure z scores at baseline and follow up

	Baseline N=61	Follow up N=61	Difference (Δ)	
	Mean ± SD	Mean ± SD	Mean ± SD	P Value
24-hour systolic BP z score	1.14 ± 0.97	0.82 ± 1.25	-0.33 ± 1.22	0.04
24-hour diastolic BP z score	0.60 ± 0.88	0.32 ± 1.18	-0.28 ± 1.11	0.05
24-hour MAP z score	1.01 ± 1.00	0.73 ± 1.30	-0.29 ± 1.27	0.08
24-hour HR z score	-0.22 ± 0.95	-0.08 ± 1.02	0.13 ± 0.870	0.23
Day-time systolic BP z score	0.95 ± 0.97	0.59 ± 1.15	-0.36 ± 1.12	0.01
Day-time diastolic BP z score	0.22 ± 1.09	-0.11 ± 1.18	-0.32 ± 1.13	0.03
Day-time MAP z score	0.77 ± 1.23	0.43 ± 1.19	-0.34 ± 1.23	0.04
Day-time HR z score	-0.78 ± 0.97	-0.63 ± 0.95	0.15 ± 0.91	0.19
Night-time systolic BP z score	0.72 ± 1.11	0.64 ± 1.44	-0.08 ± 1.41	0.65
Night-time diastolic BP z score	0.70 ± 1.01	0.69 ± 1.28	-0.01 ± 1.46	0.95
Night-time MAP z score	0.78 ± 1.16	0.81 ± 1.64	0.04 ± 1.73	0.87
Night-time HR z score	0.15 ± 0.98	0.05 ± 1.01	-0.10 ± 0.82	0.35

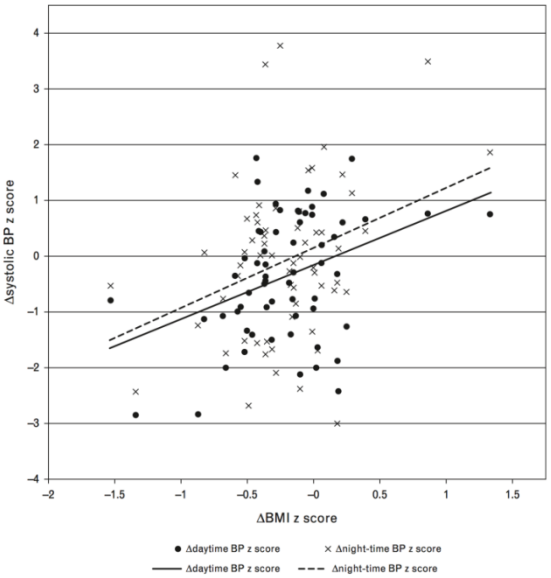
Mean ambulatory BP values were calculated into BP z scores in respect to gender and height [84]. HR: heart rate. MAP: mean arterial pressure.

5.2.5 Relationship between changes in obesity measures and changes blood pressures

Changes in ambulatory systolic and diastolic BP's in mmHg and z scores (24-hour, day- and night-time) were related to changes in BMI z scores in both unadjusted and adjusted analyses (table 9 and table 4 in paper IV). There was only a trend for the unadjusted relation of changes in night-time diastolic BP z score and the adjusted relation of changes in night-time diastolic BP in mmHg. Figure 10 displays the unadjusted relations of changes in day- and night-time systolic BP z scores with changes in BMI z scores. Furthermore, unadjusted and adjusted relations were found for changes in ambulatory BP's in mmHg and z scores with changes in WHR.

Contrary, no significant relationship was found between changes in clinic brachial systolic or diastolic BP in mmHg or z scores and changes in BMI z score or WHR in linear or multiple regression analyses (table 9 and table 4 in paper IV). Also, no relationship was found between changes in either clinic brachial or ambulatory BP's in mmHg or z scores and changes in DXA total body fat percent for the 51 patients having a DXA scan.

Figure 10: The relationship between changes in BMI z score and changes in day- and night-time systolic BP z scores



B coefficients of plotted linear regressions are listed in table 9.

Table 9: Relationship between changes in obesity measures and changes in blood pressure z scores

	Δ BMI z score		Δ Waist/height ratio	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Δ clinic brachial SBP z score	-0.16	-0.12	-1.16	0.82
Δ clinic brachial DBP z score	0.16	0.21	1.72	2.41†
Δ 24-hour SBP z score	1.09**	1.12**	8.79*	9.63**
Δ 24-hour DBP z score	0.85**	0.90*	6.65*	7.47*
Δ Day-time SBP z score	0.97**	0.98**	7.57*	8.35**
Δ Day-time DBP z score	0.77*	0.81*	4.65	5.80†
Δ Night-time SBP z score	1.08**	1.22**	9.41*	9.94*
Δ Night-time DBP z score	0.84†	0.85*	10.71*	9.44*

Results are β coefficients of regression analyses of Δ BP z score (outcome) in relation to Δ obesity measure (explanatory variable), i.e. Δ BMI z score or Δ waist-height ratio. Unadjusted analyses are linear regression. Adjusted analyses are multiple regression analyses adjusted for baseline measures of arm circumference, cuff size, the specific BP variable as well as the corresponding obesity measure, i.e. the baseline measure of either BMI z score or waist-height ratio. Additionally, changes in ambulatory BP z scores were adjusted for baseline age. All multiple regression models had a P<0.05 and a minimum r square of 0.196.
SBP: systolic BP. DBP: diastolic BP. Level of significance: †P<0.10, *P<0.05, **P<0.01.

6. DISCUSSION

6.1 Main findings

6.1.1 The study design: obesity

In the cross-sectional design (paper I and II), the obese and the control group were matched for age, gender and height, and the obese group had higher measures of obesity when compared to the control group. In the longitudinal design (paper III and IV), 74% of the 72 followed up obese patients experienced a significant weight reduction close to earlier published treatment results of the Children's Obesity Clinic although still severe obese at follow up [33].

6.1.2 Arterial stiffness

CfPWV was dependent on the method used to measure the length of the aorta (paper 1). This finding is critical for the interpretation of whether obese patients have increased aortic stiffness or not. CfPWV-subtracted using the previously used subtracted distance method was not consistent in its relation to height in the two groups and was increased in the obese group – although not statistically significant in a model adjusted for relevant confounders. CfPWV-direct using the newly recommended direct distance method was consistent in its relation to height in the two groups and was reduced in the obese group after adjustment for known confounders.

The present study suggests that weight reduction across one year did not have an impact on aortic stiffness assessed as cfPWV in severe obese children and adolescents (paper III). In fact, cfPWV was higher at follow up. This was explained by the increased age and partly by changes in BP and heart rate, as cfPWV was equally related to age at baseline and follow up.

No difference in AIx@HR75 was found between the obese and the control group (paper I), but changes in AIx@HR75 after one year follow up were related to changes in WHR in the obese patients (paper III). However, the interpretation of this relationship might be questioned by the variation in AIx@HR75.

6.1.3 Ambulatory blood pressure

The obese group had a relatively higher night- than day-time BP when compared to the control group (paper II). The obesity-related elevated night-time BP was independent of insulin resistance and arterial stiffness. Although night-time systolic BP was related to arterial stiffness and tended to be related to insulin resistance, insulin resistance and arterial stiffness were not related.

Changes in anthropometric obesity measures from baseline to follow up were associated with changes in 24-hour, day- and night-time BP, and associations were significant when adjusted for relevant confounders at baseline (paper IV). At the same time, no association was found between changes in anthropometric obesity measures and changes in clinic brachial BP.

6.2 Methodological considerations

6.2.1 The study design

There are limitations to the cross-sectional design (paper I and II). The obese group represents a selected population of severe obese patients having a BMI z score above 2 for gender and age, whereas the control group had a BMI in the normal weight range. The range between normal weight and severe obesity was covered limited, and therefore it is uncertain whether the hemodynamic findings of the present study are applicable in this more moderate overweight range.

It was difficult to recruit control individuals from the same social class as the obese group, since overweight is more often seen in lower socioeconomic groups. Unintended, questions regarding socioeconomic status were omitted in the version of the questionnaire send to the control individuals, why this was not analysed in detail.

A statistical limitation was that the degree of obesity (BMI z score) could not be evaluated when hemodynamic measures were compared between the obese and the control group, because the obese patients were included on the basis of their BMI z score. Therefore, the hemodynamic outcome variables of interest, i.e. cfPWV (paper I) and day- and night-time BP (paper II), were related to the degree of obesity separately in the two groups. Hence, the statistical power in these analyses was reduced.

Inference on causative relationships is not possible in cross-sectional designs. Although a longitudinal design was included in the study protocol, we found that not all associations could lead to a clear inference on biological mechanisms, e.g. it is uncertain whether the relationship between obesity measures and changes in ambulatory BP's are independent of changes in AC (paper IV). Further, the drop out of approximately 30% of the obese patients limits detection of small differences in hemodynamic measures between baseline and follow up.

Ideally, the longitudinal design (paper III and IV) would have been a blinded randomised trial with a group receiving treatment at the Children's Obesity Clinic and a group receiving the usual care. However, this was unfeasible in the present study design, as the obese patients were included at time of referral to the Children's Obesity Clinic, and some had already begun their treatment in the Clinic.

6.2.2 Arterial stiffness

Carotid-femoral pulse wave velocity

There are methodological considerations when assessing arterial stiffness non-invasively by cfPWV as no validation studies exist in children and adolescents. The anatomical reference sites of the distance measure have impact on the resulting cfPWV compromising comparisons of exact values from methodological different studies [89]. In adults, the distance measure of cfPWV is validated in an MRI study [61]. The exact reference sites for a precise distance are unknown in children and adolescents. However, an empirical assumption must be that the length of the vascular tree is related to height independently of weight status. In paper I, the obese patients had a shorter neck and longer torso length when compared to the control individuals. This seems merely due to the nature of their fat distribution, not reflecting increased aorta length, explaining the higher subtracted distance despite similar height. These issues challenges the use of the subtracted distance measure while the newly recommended, direct distance measure, seems more suitable. Therefore, the direct distance was used in paper II and III. In the absence of a gold standard measurement, however, we cannot conclude with certainty which of the distance measures is the "true" one.

The use of calliper instead of tape for the distance measurement should be emphasized as a bias with tape method may be introduced due to overestimating the distance because of abdominal obesity [90–92]. Possibly, a significant weight reduction can lead to a smaller distance and hence a decreased cfPWV.

Another consideration is the day-to-day variation of cfPWV vs. the expected long term effect of weight reduction on the parameter (paper III). Although weight reduction did not lead to a reduction in cfPWV in paper III, cfPWV was reproducible and age-related, as also found in adults [93,94].

Augmentation index

The general transfer function used in the computation of AIx from the clinic brachial BP is validated in adults in an invasive study [86]. Invasive validation of central BP and AIx in children is difficult due to ethical considerations [22]. In healthy adults, the week-to-week variation of AIx@HR75 is acceptable [95], as we found for the day-to-day variation (paper III). However, this day-to-day variation of AIx@HR75 did not differ from the year-to-year variation. We found a relationship between changes in WHR and changes in AIx@HR75 in our follow up period but this might be questioned by the variation in AIx@HR75 (paper III).

Other methodological considerations of AIx is the simplicity of its interpretation as it shift from negative to positive values in children [96,97]. It is dependent on the clinic brachial BP for its calibration [98], which might compromise comparisons between studies using different calibration techniques. Carotid tonometry does not require a transfer function and might be preferable [55]. However, non-consistent results between radial and carotid tonometry have been found in the obese children and adolescents of the present study [99]. An alternative to the reflected waves' theory on vascular aging is a more direct mechanism, which suggests that the higher systolic BP found with aging is due to increased aortic stiffness by loss of aortic compliance [100].

6.2.3 Ambulatory blood pressure

Cuff size and AC have an impact on BP measurements [101–103] - and the recognition is probably underestimated [104] - why adjustments for baseline measures of these were made in paper IV. Changes in anthropometric and obesity measures were very strongly inter-related - being collinear in statistical terms. Hence, it is difficult to evaluate how changes in obesity measures affect ambulatory BP's independent of changes in AC. Our primary treatment endpoint was difference in BMI z score, and not ΔAC. Recommended cuff sizes and methodologies were used and we adjusted for AC and cuff size. This implies that changes in anthro-

pometric obesity measures are biologically associated to changes in ambulatory BP.

A limitation to the present study was no data on physical activity were collected, and intervention programmes incorporating exercise may also have a better effect on the BP [46]. Further, the quality of sleep at baseline and follow up might have been different. In this respect, only valid ABPM using individual sleep time intervals were included.

6.2.4 Growth

A limitation to the present study is that no puberty measures were collected, and these can potentially influence arterial stiffness and BP [105,106]. However, in the cross-sectional design (paper I and II), no difference in gender, age or height between the obese and the control group was established suggesting that development between groups were similar.

Normative reference materials exist for arterial stiffness in adults [107,108], whereas those for children account for growth [85,109,110]. The material on cfPWV by Reusz et al [85] is based on tonometric measurements using the subtracted distance. In paper I, cfPWV-subtracted was compared to this European reference material [85]. As no longitudinal study exists for a young population linking elevated cfPWV with a cardiovascular outcome, no upper risk limit could be predicted. However, in paediatrics often the 95th percentile (equals z score 1.645) is used as this level by convention. CfPWV-subtracted z score values would be expected to be very close to zero in our control group if identical methods were used in our study and the reference paper. To the contrary, negative cfPWV-subtracted z scores were found for both the obese and the control group, although the latter had significantly lower values than the obese group. Opposed to present study, the reference material used surface tape for travel distance measures, and as previously described a bias related to tape distance measure would be expected [90–92].

Difficulties when dealing with growth when evaluating BP over time in obese children and adolescents are acknowledged in a meta-analysis investigating weight reduction's impact on cardiovascular risk factors in obese children and adolescents [111].

BP z scores are the basis for the BP classification, and we found differences between findings of BP's in mmHg and z scores: the baseline clinic brachial systolic BP in mmHg was higher in the obese group (paper I and II), but the clinic brachial systolic BP z scores did not differ between the obese and the control group (appendix 12.2). Furthermore, and contrary to anticipated [48,112], clinic brachial systolic BP z scores in the obese patients were higher at follow up despite that no significant difference in clinic brachial systolic BP in mmHg was found between baseline and follow up (paper IV). Hence, the worse distribution of the BP categories at follow up is likely attributed to the higher clinic brachial systolic BP z scores, as the 24-hour BP z score level did not rise at follow up (paper IV).

The differences between clinic systolic BP in mmHg and z scores might be explained by differences in methodology of the clinic BP measurements in respect to the normative reference material [82] from where the clinic BP z scores are calculated. In the present study, clinic BP was measured in supine position after at least 10 minutes of rest with an oscillometric device [81], and calculated into z scores. In the Fourth Report on diagnosis and treatment of high BP in children and adolescents by an American working group [82], clinic BP is measured in sitting position after 5 minutes of rest with an auscultatory mercury sphygmomanometer.

6.3 Clinical findings

6.3.1 Obesity

The present study (paper III and IV) confirms that it is possible to treat and achieve a significant weight reduction in obese children and adolescents [33,45,112–114].

6.3.2 Arterial stiffness

Carotid-femoral pulse wave velocity

The 'direct distance' measure of cfPWV gave no bias when comparing the obese and the control group (paper I). The consequence for the interpretation and in agreement with others [56,57] is that young obese patients have a lower central arterial stiffness when compared to a control group.

CfPWV was not related to insulin resistance (paper II). In some studies, increased cfPWV has been related to insulin resistance [38,39] but not in all [58]. However, both scientific groups [38,115] find opposite to us higher cfPWV in obese and even higher cfPWV in obese type 2 diabetics compared to non-obese control individuals. This fundamental difference could be due to differences in age, ethnicity, the methodology of cfPWV [116], and the fact that we had no apparent type 2 diabetics in our obese group. Despite the lower cfPWV in the obese patients in the present study, we found as expected from other studies [56,57] a positive relationship between cfPWV and age, BP and heart rate. Altogether, our findings indicate that the central vasculature has not 'yet' been damaged in the obese group.

The mechanism behind the lower cfPWV in the obese group is unclear, and we speculate that our findings may be a compensatory mechanism to a hyperkinetic circulation in obese children and adolescents with a supposed higher stroke volume, cardiac output and a higher circulating blood volume [117,118].

The present study suggests that weight reduction across one year did not have an impact on aortic stiffness assessed as cfPWV in severe obese children and adolescents. In fact, cfPWV was higher at follow up. This was explained by the increased age and partly by changes in BP and heart rate, as cfPWV was equally related to age at baseline and follow up. In adults aged 21-46 years, weight reduction after 6 and 12 months of lifestyle intervention was accompanied with a reduction in cfPWV [77,78], but the changes in cfPWV were not related to changes in weight [78]. In these studies, the distance measure of cfPWV was measured with tape by a subtracted technique. This might lead to bias [90,91,116]. Possibly, a significant weight reduction, and less abdominal fat, can lead to a shorter subtracted distance and therefore a decreased cfPWV.

Augmentation index

No difference was found in AIx between the obese and the control group in the cross-sectional design (paper I). This was opposite to findings by Urbina et al [58], but in agreement with others [57,59]. These opposite findings may be due to differences in study populations as previously described, and in a field with methodological concerns the present study supports the view that obesity in children does not relate to arterial stiffness.

Although it might be questioned by methodological considerations and the variation in AIx@HR75, a relationship existed between changes in WHR and changes in AIx (paper IV). A potential mechanism for the association between changes in waist circumference and changes in AIx@HR75 could be the linkage between

abdominal obesity and resistance artery function [119–121], which is a determinant of peripheral pulse wave reflection and AIx.

6.3.3 Ambulatory blood pressure

In the cross-sectional design (paper II), the obesity-related elevated BP was primarily driven by the elevated night-time BP as also found by Aguilar et al [70]. This was supported by our findings of increased night-to-day BP ratios, and the relationship between the degree of obesity (BMI z score) and night-time BP in the obese group.

The 24-hour BP was markedly higher than the clinic brachial BP. Although obesity might increase the chances of masked hypertension [41], we also found higher out-of-office BP in the control group. The observed higher out-of-office BP pattern perhaps was merely due to the young age of the participants [122]. It is likely that BP measured after 10 minutes rest in supine position is the most relaxing moment during a day for active children, and it might ease comparison with the night-time BP. Part of it might be due to other differences in methodology (e.g. oscillometric algorithms or cuff bladder sizes). The method used was chosen to ensure the best suitable brachial BP measure for non-invasive central hemodynamic measures.

Night-time systolic BP has been related to insulin resistance independent of BMI z score in a study by Lurbe et al [35]. In our study, night-time systolic BP only tended to be related to insulin resistance when adjusted for obesity status. This could be due to a type-two error following limited sample size. However, in other studies using multiple regression, systolic night-time BP was related to BMI z score but not HOMA index or other metabolic measures [70,73]. In adults, it has been shown that the relationship between insulin resistance and BP is largely, if not entirely, explained by waist circumference [123]. In our study design, the group variable (obese vs. control) encompasses the differences in obesity measures, why we could not include BMI z score or WHR in the multiple regression models.

We can only speculate on possible mechanisms involved in the higher night-time BP in the obese group, such as inferior sleep quality due to snoring or obstructive sleep apnoea [124], a changed autonomic function [69], or an impaired ability to excrete sodium [125].

Ambulatory and clinic brachial BP's in mmHg were not reduced at follow up although the obese patients experienced a weight reduction when evaluated on a group level (paper IV). Not all patients were responders, and the mixture of patients reducing and gaining weight possibly influenced the BP differently. When changes in ambulatory BP's in mmHg were related to changes in obesity measures, i.e. evaluated as a continuum, patients loosing or gaining weight by one BMI z score had a corresponding decrease or increase in e.g. 24-hour systolic BP of 6.5 mmHg. BP's in mmHg are clinically interpretative, however, z scores account for growth, and the relationship was also found for changes in ambulatory BP z scores. The lack of a relationship between changes in DXA total body fat percent and changes in ambulatory BP might be explained by a lower statistical power.

No relationship was found between changes in obesity measures and changes in clinic BP in mmHg or z scores. This negative finding may be due to the limited number of clinical BP readings compared to the 24-hour BP measurement. However, clinical oscillometric BP devices are observer-independent. Karatzi et al [126] have found that out-of office BP, measured as home BP, was more closely related to the degree of obesity than clinic BP. They used the same clinical oscillometric BP device as in the present study [126]. In agreement, the findings in our study suggest that out-of-office BP seems superior to clinic BP when detecting associations between weight reduction and BP changes.

7. CONCLUSIONS

7.1 Arterial stiffness

The previously used cfPWV-subtracted used a distance measure method which seemed to overestimate cfPWV in obese children and adolescents as the relationship between the subtracted distance and height was different in the obese when compared to the control group (paper I). This was not the case for the new method of cfPWV-direct, and it ascertains the appropriateness of this distance measure. These results suggest that obesity in children is not 'yet' associated with structural changes in aorta.

Further, no clear effect of weight reduction was found on measures of arterial stiffness in obese children and adolescents after one year of lifestyle intervention. The higher arterial stiffness at follow up was due to the increased age and partly due to changes in BP and heart rate (paper III). A relationship between changes in abdominal obesity and changes in AIx was found, although the significance of this relationship might be questioned by the large variation in AIx.

7.2 Ambulatory blood pressure

The obese children and adolescents had a relatively higher night- than day-time BP when compared to the control group (paper II). The obesity-related elevated night-time BP was independent of insulin resistance and arterial stiffness.

Changes in BMI z score and WHR were related to changes in ambulatory BP in severe obese children and adolescents after one-year of lifestyle intervention (paper IV). Associations were consistent when ambulatory BP was evaluated in standardised values that accounted for growth. No relationship was found between changes obesity measures and changes in clinic BP.

8. PERSPECTIVES

8.1 Clinical perspectives

In perspective of known BP tracking [16–18], the adverse night-time BP pattern of the obese children (paper II) might contribute in the future to an adverse cardiovascular risk profile of adult obese patients [19,20]. It is reassuring that the higher BP of young obese patients is not 'yet' associated with subclinical vascular damage as assessed by measurements of arterial stiffness (paper I). In this respect, no clear relationship was found between changes in obesity and changes in arterial stiffness (paper III).

The study suggests that changes in ambulatory BP are more closely related to changes in the degree of obesity as compared to changes in clinic brachial BP (paper IV). The findings emphasises the use of 24-hour ambulatory BP measurements in children and adolescents. Furthermore, it is reassuring that weight changes is accompanied with a change in 24-hour BP as ambulatory BP is the most precise measure to evaluate the BP burden [10,40–42].

It is important to recognise, that obese children who recover their normal weight before adulthood will have a similar cardiovascular risk as those who were never obese [45]. Hence, early treatment and prevention of childhood obesity is important because it may prevent irreversible damage to the cardiovascular system [45].

8.2 Perspectives for future research and methodology

An MRI validation study of the distance measure of cfPWV conducted in adults [61] could clarify the 'true' distance of the cfPWV in children and adolescents. Further, vascular MRI could measure the aortic arch pulse wave velocity as also performed in obese adults [56].

Ambulatory BP devices exist for estimating 24-hour central BP and AIx [127,128]. Although no differences were found in central BP and AIx in the present study, quantification of 24-hour central BP and AIx may represent a more precise evaluation of these central hemodynamic measures in parallel with ABPM measurements being the most precise measure of the BP [10,40–42]. Validation studies are warranted when using these methods.

There are data in the present study that have not been analysed. Metabolic status have been found to impact the ability to achieve weight reduction [129], but knowledge is lacking on whether metabolic status at baseline and changes herein impacts the changes in 24-hour BP during weight reduction. The size of BP cuffs influences the BP measurement [101–103], an issue that is quite neglected despite the fact that it deserves serious consideration. We are currently analysing how this issue potentially influences found BP differences between the obese and the control group. Further, autonomic function evaluated with heart rate variability, echocardiographic measures, as well as stored blood samples have been collected with the goal of a better insight of the pathophysiology of obesity-related elevated BP.

9. SUMMARY

Obesity, elevated blood pressure (BP) and arterial stiffness are risk factors for cardiovascular disease. A strong relationship exists between obesity and elevated BP in both children and adults. Obesity and elevated BP in childhood track into adult life increasing the risk of cardiovascular disease in adulthood. Ambulatory BP is the most precise measure to evaluate the BP burden, whereas carotid-femoral pulse wave velocity (cfPWV) is regarded as the gold standard for evaluating arterial (i.e. aortic) stiffness. These measures might contribute to a better understanding of obesity's adverse impact on the cardiovascular system, and ultimately a better prevention and treatment of childhood obesity.

The overall aim of the present PhD thesis is to investigate arterial stiffness and 24-hour BP in obese children and adolescents, and evaluate whether these measures are influenced by weight reduction. The present PhD thesis is based on four scientific papers.

In a cross-sectional design, 104 severe obese children and adolescents with an age of 10-18 years were recruited when newly referred to the Children's Obesity Clinic, Holbæk University Hospital, and compared to 50 normal weighted age and gender matched control individuals. Ambulatory BP was measured, and cfPWV was investigated in two ways in respect to the distance measure of aorta; the previously recommended length – the so called subtracted distance, and the currently recommended length – the direct distance.

In a longitudinal design, the obese patients were re-investigated after one-year of lifestyle intervention at the Children's Obesity Clinic in purpose of reducing the degree of obesity. In the cross-sectional design, the obese group had higher measures of obesity, while matched for age, gender and height, when compared to the control group. In the longitudinal design, 74% of the 72 followed up obese patients experienced a significant weight reduction.

CfPWV was dependent on the method used to measure the length of the aorta. The subtracted distance was not consistent in its relation to height in the obese and the control group. Opposite, the direct distance was consistent in its relation to height in the two groups. Therefore, cfPWV using the direct distance (cfPWV-direct) was regarded as the appropriate measure of arterial stiffness. CfPWV-direct was reduced in the obese group after adjustment for known confounders. In the longitudinal design, weight reduction across one year did not have an impact on cfPWV-direct in the obese patients. In fact, cfPWV-direct was higher at follow up, which was explained by the increased age and partly by changes in BP and heart rate.

The obese group had a relatively higher night- than day-time BP when compared to the control group. The obesity-related elevated night-time BP was independent of arterial stiffness and insulin resistance. Although night-time systolic BP was related to arterial stiffness and tended to be related to insulin resistance, insulin resistance and arterial stiffness were not related. In the longitudinal design, changes in anthropometric obesity measures across one year were associated with changes in 24-hour, day- and night-time BP, and consistent when evaluated in standardised values that accounted for growth. No association was found between changes in anthropometric obesity measures and changes in clinic BP.

In conclusion, the results suggest that obesity in children is not 'yet' associated with structural changes in aorta when evaluated with the appropriate new method of cfPWV. In this respect, weight reduction did not have an impact on arterial stiffness. The ambulatory BP, namely the night-time BP, was elevated in the obese patients, whereas changes in anthropometric obesity measures were related to changes in ambulatory BP but not to changes in clinic BP.

In perspective, it is reassuring that weight changes are accompanied with a change in 24-hour BP as ambulatory BP is the most precise measure to evaluate the BP burden, and it emphasises the use of 24-hour ambulatory BP measurements in children and adolescents. It is important to recognise, that obese children who recover their normal weight before adulthood will have a similar cardiovascular risk as those who were never obese. Hence, early treatment and prevention of childhood obesity is important because it may prevent irreversible damage to the cardiovascular system.

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12. APPENDICES

12.1 Recruitment

Supplementary table 1:
Invited patients in the study vs. not in the study

	Included in the study N=104	Not included in the study N=38	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P value
Gender (N female/ N male)	54/50	19/19	0.84
Age (years)	12.6 (11.3-15.0)	12.2 (11.3-14.9)	0.97
Height (cm)	159.3 ± 12.1	161.3 ± 12.7	0.39
Weight (kg)	67.5 (58.4-91.0)	71.9 (59.7-84.4)	0.71
BMI (kg/m^2)	28.6 ± 5.6	28.6 ± 5.7	0.96
BMI z score	2.81 ± 0.66	2.83 ± 0.63	0.87
Social class (scale 1-5)	3 (2-4) N=87	3 (2-3.5) N=32	0.86

Data are from invited patients' first visit in The Children's Obesity Clinic. Social status was evaluated on a scale 1-5 with those having the lowest social class scoring 5 [33]. Initially, 146 patients were invited to participate in the study and 104 (71%) were examined. One control individual had a body mass index (BMI) above the 99th percentile [29] and therefore included as an obese patient. Four of the patients that were not included in the study never showed up to their appointment in the clinic.

12.2 Supplemental material for paper II

Supplementary table 1 (paper II):
Clinic and ambulatory blood pressure z scores

	Obese Group N=92	Control Group N=49	
	Mean ± SD	Mean ± SD	P value
Clinic brachial systolic BP z score	1.57 ± 1.10	1.29 ± 0.85	0.13
Clinic brachial diastolic BP z score	0.42 ± 0.61	0.22 ± 0.51	0.047
24-hour systolic BP z score	1.13 ± 1.03	0.33 ± 1.27	<0.0001
24-hour diastolic BP z score	0.53 ± 0.89	0.22 ± 1.08	0.072
24-hour MAP z score	0.95 ± 1.02	0.45 ± 1.14	0.009
24-hour HR z score	-0.21 ± 0.97	-0.73 ± 1.08	0.004
Day-time systolic BP z score	0.91 ± 1.06	0.30 ± 1.36	0.008
Day-time diastolic BP z score	0.11 ± 1.08	-0.01 ± 1.26	0.54
Day-time MAP z score	0.67 ± 1.20	0.37 ± 1.26	0.18
Day-time HR z score	-0.79 ± 0.95	-1.24 ± 1.09	0.01
Night-time systolic BP z score	0.75 ± 1.22	-0.16 ± 1.00	<0.0001
Night-time diastolic BP z score	0.70 ± 1.02	0.18 ± 0.82	0.002
Night-time MAP z score	0.79 ± 1.17	0.03 ± 0.90	0.0001
Night-time HR z score	0.14 ± 0.95	-0.51 ± 0.99	0.0002

Mean clinic and ambulatory BP in mmHg are calculated into BP z scores in respect to recommended normative populations; clinic BP z scores in respect to gender, age and height [82], whereas ambulatory BP z scores in respect to gender and height [84]. The 95th percentile equals a z score of 1.645, and is used as the cut-off level in the BP classification. HR: heart rate. MAP: mean arterial pressure.

12.3 Supplemental material for paper III

Supplementary table 1 (paper III):
Potential gender differences in the followed up patients at baseline

	Girls N=37 (51%)	Boys N=35 (49%)	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P value
Age (years)	13.3 ± 2.5	12.8 ± 2.0	0.30
Height (cm)	160.3 ± 10.5	158.6 ± 12.7	0.54
Weight (kg)	77.7 (58.3-90.9)	62.5 (53.1-78.3)	0.12
BMI (kg/m2)	28.0 (25.7-32.8)	25.7 (22.5-29.1)	0.071
BMI z score	2.75 (2.39-3.10)	2.53 (2.11-3.50)	0.91
Waist circumference (cm)	98.9 ± 14.3	94.6 ± 15.7	0.23
Waist-height ratio	0.61 ± 0.07	0.59 ± 0.07	0.20
DXA total body fat (%)	45.4 (43.0-49.0)	42.6 (38.2-47.0)	0.018
Clinic brachial systolic BP (mmHg)	110.8 ± 8.8	110.5 ± 9.1	0.88
Clinic brachial diastolic BP (mmHg)	63.6 ± 5.7	60.5 ± 5.3	0.02
Clinic brachial PP (mmHg)	47.2 ± 7.3	50.1 ± 8.3	0.13
Clinic brachial systolic BP z score	1.53 ± 1.10	1.48 ± 1.19	0.84
Clinic brachial diastolic BP z score	0.67 ± 0.61	0.20 ± 0.52	0.0007
Heart rate (bpm)	68.5 ± 10.9	65.6 ± 8.1	0.20
cfPWV (m/s)	4.94 ± 0.58	4.73 ± 0.55	0.12
Central systolic BP (mmHg)	93.3 ± 7.6	93.0 ± 7.8	0.86
Central diastolic BP (mmHg)	64.6 ± 6.2	61.6 ± 5.3	0.03
Central MAP (mmHg)	77.9 ± 6.7	76.2 ± 6.1	0.26
Central PP (mmHg)	28.7 ± 5.4	31.4 ± 5.6	0.04
AIx@HR75	-1.67 ± 10.64	-0.35 ± 10.23	0.59

Seventy-two patients were included in the follow up study. Only 60 (83%) patients (girls 34 (92%) and boys 27 (77%)) had a dual energy x-ray absorptiometry (DXA) scan at baseline. BMI: body mass index. CfPWV: carotid-femoral pulse wave velocity. MAP: mean arterial pressure. PP: pulse pressure.

Repeatability sub study

Twenty-five patients (35% of the 72 followed up patients) participated in the repeatability sub study. The two patients who were excluded from the analyses (due to a kidney disease or influenza symptoms at follow up) were not included in the repeatability sub study.

Patients included in the repeatability sub study had three examinations:
Day 1 = Baseline
Day 2 = First day at follow up
Day 3 = Second day at follow up (the repeatability examination)

Supplementary table 2 (paper III):
Basic characteristics in followed up patients

	In repeat sub study N=25 (35%)	Not in repeat sub study N=47 (65%)	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P value
Gender (N girls/ N boys)	14/11	23/24	0.57
Age (years)	12.6 (11.2-15.44)	12.5 (11.4-14.4)	0.88
Height (cm)	158.8 ± 11.8	159.8 ± 11.6	0.73
Weight (kg)	63.7 (58.3-86.6)	67.4 (55.8-90.2)	0.81
BMI (kg/m2)	27.1 (24.1-29.5)	26.7 (23.8-31.9)	0.94
BMI z score	2.76 ± 0.64	2.74 ± 0.62	0.92

All variables are baseline (day 1) measurements. BMI: body mass index.

12.4 Supplemental material for paper IV

Supplementary table 1 (paper IV):
Potential gender differences in ambulatory blood pressures at baseline

	Female N=33 (54%)	Male N=28 (46%)	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P value
24-hour systolic BP (mmHg)	121.1 ± 7.2	121.2 ± 7.4	0.93
24-hour diastolic BP (mmHg)	70.8 ± 5.5	70.6 ± 4.0	0.88
24-hour MAP (mmHg)	87.6 ± 5.7	87.5 ± 4.7	0.95
24-hour HR (bpm)	80.2 ± 8.0	78.8 ± 7.3	0.47
Day-time systolic BP (mmHg)	125.0 ± 7.8	124.7 ± 8.0	0.91
Day-time diastolic BP (mmHg)	74.0 ± 6.6	73.2 ± 4.9	0.59
Day-time MAP (mmHg)	91.0 ± 6.7	90.4 ± 5.4	0.68
Day-time HR (bpm)	82.4 ± 7.9	81.8 ± 8.2	0.76
Night-time systolic BP (mmHg)	107.0 ± 9.5	109.1 ± 9.4	0.39
Night-time diastolic BP (mmHg)	58.7 ± 6.1	61.5 ± 6.8	0.09
Night-time MAP (mmHg)	74.8 ± 6.7	77.4 ± 7.3	0.15
Night-time HR (bpm)	71.8 (65.2-76.4)	68.8 (63.7-73-7)	0.11

Sixty-one of the followed up patients had a valid ambulatory BP monitoring at baseline and follow up. HR: heart rate. MAP: mean arterial pressure.

Supplementary table 2 (paper IV):
Potential gender differences in ambulatory blood pressures at baseline

	Female N=33 (54%)	Male N=28 (46%)	
	Mean ± SD or Median (IQR)	Mean ± SD or Median (IQR)	P value
24-hour systolic BP z score	1.31 (0.56-1.81)	0.93 (0.29-1.43)	0.12
24-hour diastolic BP z score	0.62 (0.14-1.19)	0.66 (0.17-0.89)	0.90
24-hour MAP z score	1.14 (0.41-1.57)	0.94 (0.39-1.16)	0.22
24-hour HR z score	-0.20 ± 0.96	-0.23 ± 0.96	0.89
Day-time systolic BP z score	1.16 (0.33-1.81)	0.62 (0.31-1.28)	0.24
Day-time diastolic BP z score	0.09 (-0.21-0.68)	0.22 (-0.32-0.73)	0.78
Day-time MAP z score	0.73 (0.16-1.20)	0.69 (0.11-1.17)	0.57
Day-time HR z score	-0.78 ± 1.01	-0.79 ± 0.93	0.99
Night-time systolic BP z score	0.51 (-0.03-1.53)	0.65 (0.19-1.10)	0.85
Night-time diastolic BP z score	0.56 ± 0.90	0.87 ± 1.12	0.23
Night-time MAP z score	0.65 ± 1.09	0.93 ± 1.24	0.36
Night-time HR z score	0.26 (-0.40-0.98)	0.24 (-0.57-0.53)	0.22

Sixty-one of the followed up patients had a valid ambulatory BP monitoring at baseline and follow up. Mean ambulatory BP in mmHg were calculated into BP z scores in respect to gender and height [84]. HR: heart rate. MAP: mean arterial pressure.

12.5 Corrections since final publication of papers

Correction to section 5.2.3 (paper III): In multiple regression analysis, changes in AIx@HR75 were related to changes in DXA total body fat percent (β = 0.70, 95% CI 0.11–1.29, P = 0.02, model: R^2 = 0.415, P<0.0001) when adjusted for gender and relevant baseline confounders.