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CARDIOVASCULAR HEALTH IN CHILDREN AND ADOLESCENTS WITH OBESITY: PREVALENCE, PREDICTION, AND SUPERVISED EXERCISE

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Cardiovascular health in children and adolescents with obesity: Prevalence, prediction and supervised exercise

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Till Johan, Agnes och William, jag älskar er

ABSTRACT

Background

Cardiovascular disease (CVD) is a major cause of death worldwide and is preceded by a long process in which several cardiovascular (CV) risk factors are present, sometimes already in childhood. Some of these risk factors such as non-dipping blood pressure and microvascular endothelial dysfunction and possible associated factors are little studied among children with obesity. In addition to these CV risk factors, obesity in childhood is also associated with adverse CV health in general populations adults. However, the clinical value of childhood CV risk factors for identifying children at increased risk of adverse end-organ function in young adulthood is unclear. Weight loss is effective for improving CV risk factors but is found to be hard to achieve especially among adolescents with obesity. As such, new approaches to diminish their CV risk must be evaluated.

Aims

In **Study I**, acetylcholine-induced endothelium-dependent vasodilatation was compared between children with obesity and children with normal weight. Associations between vasodilatation and potential risk factors were also studied.

In **Study II**, the prevalence of nocturnal blood pressure dipping among prepubertal and early pubertal children with obesity was studied. In addition, associations between dipping and measures of insulin-glucose metabolism or sleep-disordered breathing were evaluated.

In **Study III**, the aim was to evaluate whether well-established cardiovascular risk factors could be used clinically for the early identification of children and adolescents with obesity at risk of a rapid deterioration in cardiovascular health in young adulthood.

In **Study IV**, the aim was to evaluate whether regular supervised individualised aerobic exercise for a period of three months could increase cardiorespiratory fitness (CRF), lead to improved long-term CRF, and affect CV risk factors among adolescents in obesity treatment.

Subjects and methods

In **Study I**, endothelium-dependent vasodilatation was compared between 54 children (14.3 years old, 41% girls) with obesity and 44 (13.7 years old, 82% girls) normal-weight children. Acetylcholine was administered via transdermal iontophoresis to induce vasodilatation in the dorsal hand skin, and the subsequent change in perfusion was measured with laser Doppler flowmetry. In a subgroup of children with obesity, associations between acetylcholine-induced vasodilatation and inflammation, 24-hour ambulatory blood pressure (ABP), CRF, blood lipids, glucose/insulin metabolism, and duration of obesity were evaluated.

In **Study II**, non-dipping—measured via 24-hour ABP and defined as nocturnal blood pressure reduction lower than 10%—was studied among 76 (10.4 years old, 41% girls) prepubertal and early pubertal children with obesity. Frequently sampled intravenous

glucose-tolerance tests, fasting blood samples, and polygraph recordings were performed to study associations between dipping, insulin/glucose metabolism and sleep-disordered breathing. As a measure of end-organ damage, left ventricular mass (LVM) was measured using echocardiography and associations with the above factors were evaluated.

The longitudinal, prospective cohort of **Study III** included 49 young adults (23.4 years old, 25 females) who had been extensively examined before entering a childhood obesity treatment program at an age of 13.8 years. At inclusion, 24-hour ambulatory blood pressure, blood lipids, CRF, and metabolic syndrome score were determined. Five to fifteen years later, a comprehensive cardiovascular follow-up was performed in which left ventricular mass, carotid intima-media thickness, arterial stiffness and endothelial function were considered as main outcomes.

In **Study IV**, eight adolescents (15.1 years old) undergoing obesity treatment performed regular individualised aerobic exercise, supervised by a personal coach, for 3 months. The exercise was performed at an intensity of ≥ 150 bpm, for ≥ 30 minutes, 3 times per week. The adolescent was responsible for performing the exercise over the following 9 months. Biochemical factors (blood lipids, glucose, insulin and markers of inflammation), ambulatory blood pressure, body composition, endothelial function, arterial stiffness, CRF, metabolic syndrome score, pediatric health-related quality of life and objectively measured physical activity) were assessed before and after the 3 months of supervised exercise, and 9 months after the end of the supervised exercise.

Results

In **Study I**, endothelium-dependent vasodilatory response to acetylcholine was lower in children with obesity compared with normal-weight children ($p < 0.001$), and peak perfusion was 33% lower among children with obesity ($p = 0.001$). The lowest vasodilatation was found among children with the shortest duration of obesity ($p = 0.03$). Except for a trend in association between vasodilatation and triglycerides ($p = 0.07$) no associations were found with 24-hour ABP, CRF, inflammation, or insulin/glucose metabolism.

Study II showed a prevalence of 42% systolic non-dippers and 17% diastolic non-dippers among prepubertal and early pubertal children with obesity. Systolic and diastolic dipping were not associated with measures of insulin/glucose metabolism (adjusted for BMI SDS, sex and pubertal status) or with measures of sleep-disordered breathing. No associations were found between LVM index as a measure of end-organ damage and measures of blood pressure ($p = 0.2–0.9$), insulin/glucose metabolism ($p = 0.2–0.9$) or measures of sleep-disordered breathing ($p = 0.3–1.0$) (adjusted for BMI SDS, sex, and pubertal status).

In **Study III**, childhood total serum cholesterol, triglycerides and daytime systolic blood pressure were positively associated with carotid intima-media thickness independent of sex and change in BMI SDS at follow up ($p < 0.05$). High blood pressure or dyslipidaemia in childhood did not predict increased left ventricular mass index, arterial stiffness, or endothelial dysfunction in young adult life. The strongest tracking correlations were for

daytime diastolic blood pressure (r = 0.56, $p < 0.01$) and total cholesterol (r = 0.75, $p < 0.001$). At follow-up, severe obesity was present in 74% of subjects, although one-third had decreased their BMI SDS > 0.25 BMI SDS from childhood.

In **Study IV**, supervised exercise increased absolute and relative CRF (0.65 ± 0.41, p = 0.01 and 6.02 ± 3.69, p = 0.01, respectively), but did not improve the CV risk factor profile or long-term CRF. Arterial stiffness decreased 9 months after the supervised exercise (-16 ± 11 units, p = 0.03), whereas gynoid fat percentage (1 ± 1 %, p = 0.04) and apolipoprotein B (0.11 ± 0.08 g/L, p = 0.01) increased.

Conclusions

Children with obesity without comorbidities have impaired microvascular endothelial function compared with normal-weight children. Children with a longer duration of obesity, however, seem less affected. In addition, nocturnal non-dipping was highly present among prepubertal and early pubertal children with obesity. Compared with previous reports on children in general, non-dipping was about two times higher among children with obesity. No associations between dipping and insulin/glucose metabolism or measures of sleep-disordered breathing were found.

Few associations between childhood CV risk factors and end-organ function in young adults previously attending childhood obesity treatment were found. However, childhood serum cholesterol, triglycerides, and systolic blood pressure were associated with carotid intima-media thickness, but not with other intermediate markers of increased cardiovascular risk in young adult life. Clinicians should evaluate blood lipids and 24-hour ABP and perhaps treat these factors, if elevated, starting in childhood to reduce the future risk of CVD these subjects appear to be exposed to.

In this small but comprehensive study of the effects of supervised aerobic exercise in adolescents with obesity, it was found that short-term CRF increased, but there were no other statistically significant effects on CV risk factor profile or weight. The improved CRF was not maintained long-term when the adolescents exercised on their own, and the effect on CV risk factors was still absent, except for improved arterial stiffness. This exercise regimen cannot therefore be recommended for clinical implementation as a complement to childhood obesity treatment to reduce CV risk factors without weight reduction.

LIST OF SCIENTIFIC PAPERS

I. Obese children without comorbidities have impaired microvascular endothelial function
II. Nocturnal blood pressure non-dipping is prevalent in severely obese, prepubertal and early pubertal children
III. Weak associations between cardiovascular risk factors in childhood obesity and cardiovascular health in young adulthood
IV. Effect of supervised aerobic exercise on cardiorespiratory fitness and cardiovascular risk factors among adolescents in obesity treatment

CONTENTS

LIST OF ABBREVIATIONS

ABPM	Ambulatory blood pressure monitoring
AIR	Acute insulin responsiveness
AIx	Augmentation Index
AIx@HR75	Augmentation Index standardized for a heart rate of 75
ANCOVA	Analysis of variance with covariates
ANOVA	Analysis of variance
APO	Apolipoprotein
BMI	Body mass index
BORIS	The Swedish childhood obesity register (BarnObesitasRegistret i Sverige)
CCA	Common carotid artery
CHD	Coronary heart disease
cIMT	Carotid intima-media thickness
cpm	Counts per minute
CRF	Cardiorespiratory fitness
CV	Cardiovascular
CVD	Cardiovascular disease
FMD	Flow-mediated dilatation
FSIVGTT	Frequently sampled intravenous glucose tolerance test
DBP	Diastolic blood pressure
DXA	Dual X-ray absorptiometry
HbA1c	Glycosylated hemoglobin A1c
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment
hs-CRP	High sensitive C-reactive protein
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IOTF	International obesity task force
LDL	Low-density lipoprotein
LVH	Left ventricular hypertrophy

LVM	Left ventricular mass
METs	Metabolic equivalents
MetS	Metabolic syndrome
NCD	Non-communicable diseases
NO	Nitrogen oxide
OGTT	Oral glucose tolerance test
OSA	Obstructive sleep apnoea
PA	Physical activity
PU	Perfusion unit
RPE	Rated perceived exertion
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Standard deviation score
SPSS	Statistical package for social sciences
T2DM	Type 2 diabetes mellitus
VO_2 max	Maximal volume of oxygen consumption
WHO	World Health Organisation

1 BACKGROUND

Overweight and obesity are common not only among adults, but also among children and adolescents (1). Overweight and obesity can be caused or triggered by endocrine disorders and genetic, epigenetic, environmental and social factors. However, most of the time, the cause is excessive energy intake from too much food and food that is too energy-dense, such as fast foods, sweets, soft drinks, or snacks. Excessive energy intake is often seen in combination with low daily physical activity and low levels, or even the absence, of physical exercise. This creates an imbalance between the energy intake and energy output, leading to overweight and obesity (2).

Increased body mass index (BMI) in adolescence is related to morbidity and mortality in adulthood (3), such as cardiovascular disease (CVD). Among non-communicable diseases (NCD), CVD is the main cause of death worldwide (4) and in Sweden (5). Years of life lost for young adults aged 20 to 30 years due to obesity are 13 years for men and 8 years for women (6).

Normally, CVD is unusual among children and adolescents, but the natural development of CVD starts in childhood (7, 8) and worsens with age (9), and is modified by the presence of other cardiovascular (CV) risk factors. CVD usually occurs later in life (10). CVD (e.g. coronary heart disease (CHD) and stroke) is, however, preceded by atherosclerosis, which is the narrowing of arteries. Atherosclerosis is developed in a long process throughout life, starting in childhood, with numerous factors acting in the blood vessels contributing to its development (7, 8, 11). Low-grade inflammation, adverse levels of blood lipids and hypertension are some of the risk factors contributing to atherosclerosis. These risk factors are present at an early stage of life among those with obesity (7, 12). In autopsy studies in children and young adults, the amount of fatty streaks and plaque in the aorta and coronary arteries was associated with the number of CV risk factors (9). Circulating inflammatory markers enhance the process of accumulation of fat into fatty streaks, and these later develop into stable or unstable fibrous plaques; these are the main steps in the progress of atherosclerosis. When an unstable plaque, with its thin fibrous cap, suddenly ruptures, and clotting factors are recruited to the rupture to block the bleeding, a CVD event occurs (8, 11).

The health care costs from obesity rise along with age and the number of obesity-related diseases (13). Treating obesity as early as possible, or at least reducing the risk factors for CVD that are usually present, should be a social and health care priority.

1.1 OBESITY IN CHILDREN AND ADOLESCENTS

1.1.1 Definitions

The World Health Organisation (WHO) uses BMI to classify the degree of obesity. The range or cut-offs for overweight, obesity and severe obesity in adults are BMIs of 25.0–29.9, 30.0–34.9 and ≥ 35.0, respectively, when taking into account the risk of comorbidities (14).

Generally, it can be assumed that a BMI of 30 is equivalent to an excess of body fat, although BMI unfortunately does not discriminate between fat mass weight and muscle mass weight.

In children and adolescents, height and body composition change with age. To classify the degree of obesity in children and adolescents using BMI, age and sex have to be accounted for. The International Obesity Task Force (IOTF) has adapted the adult obesity classification to children and adolescents based on age- and sex- specific cut-offs (15, 16). The BMI standard deviation score (SDS) is suitable for following weight status over time or comparing weight status between groups. Several references are available, among them, the IOTF's international reference population (16), Roland-Cachera et al.'s French reference population (17), and Karlberg et al.'s Swedish reference population (18). It is important, however, to keep in mind that the prevalence of obesity is dependent on which reference is used.

1.1.2 Prevalences and trends

1.1.2.1 Worldwide

The trends in BMI between 1975 and 2016 among children and adolescents aged 5–19 years in 200 countries have been presented in a comprehensive worldwide population-based study of pooled data (19). Between those years, the global prevalence of obesity among children and adolescents increased from 0.7% to 5.6% in girls, and 0.9% to 7.8% in boys. The prevalence of obesity was over 30% in some countries, and about 20% in some African areas and the USA. The increase of obesity among children and adolescents has reached a plateau in many high-income countries, but at high levels. However, in parts of Asia, for example, obesity is still accelerating. On the other hand, Niger, Ethiopia, Chad and Burkina Faso are among countries with a prevalence of obesity of about 2%.

1.1.2.2 In Sweden

National data on obesity among children and adolescents in Sweden is rare. However, between the years 1999 and 2005, in selected areas of Sweden the prevalence of obesity among 10-year-old school children was about 3%–5% (20). In a later study of school children aged 7–9 in West Sweden, the prevalence of obesity was 3.2%, 3.3% and 3.1 % in 2008, 2010 and 2013, respectively (21). In a recent nationwide study of Swedish school children aged 12, 15, and 18 the prevalence of obesity was 4%, 3%, and 6%, respectively (22). However, in an additional group of adolescents aged 19 who are not attending ordinary school programmes, or are working, the prevalence of obesity was 23%; unfortunately, it was not possible to randomise this sample in order to be nationally representative (22). Overweight and obesity were more prevalent among those with a less advantaged socioeconomic status (20, 21) and lower level of education (22). There are also regional differences, with a higher prevalence of obesity in sparsely populated areas (22).

1.1.3 Tracking into adulthood

According to the results of a systematic review and meta-analysis, 55% of the children with obesity will become adolescents with obesity (23). Among the adolescents with obesity, 80%

will have obesity as adults, and 70% will still have obesity at the age of 30. However, 70% of adults with obesity were not obese during childhood or adolescence. Even though the majority of obese adults were not obese as children, the health care costs for adults with obesity will rise in the future along with the number of CV risk factors present (13). Treating obesity as early as possible, or at least improving the CV risk factors present, would be beneficial.

1.2 OBESITY-RELATED CARDIOVASCULAR RISK FACTORS

1.2.1 Risk factors versus risk markers

The expression "risk factor" is commonly used when writing about factors that increase the risk of, for example, CVD. However, some of the risk factors might by definition be risk markers rather than risk factors. Risk factors have a causal association with the outcome disease evaluated in a longitudinal study. The presence of a risk factor directly increases the risk of having a disease, but the risk will be reduced if the risk factor is absent or removed (24). There are also known risk factors that by their nature are untreatable, such as gender, age, genetics, and a family history of CVD (25). A risk marker may be associated with the disease, but not be causal (24). However, the term "risk factor" will be used for all risk factors and risk markers included in this thesis.

Another aspect of the concept of risk factors is that in the young populations studied in this thesis, manifest CVD is not present. Therefore, some of the risk factors for CVD are, when relevant, evaluated as preclinical markers of CVD, and treated as markers of end-organ function in this thesis.

1.2.2 Cardiovascular risk factors in childhood

Obesity itself is a risk factor for CVD (26), and is usually accompanied by other common risk factors for CVD (27-31). However, it must be noted that some people are considered to be metabolically-healthy individuals with obesity, meaning that obesity-related CV risk factors are not present, or at least not to the same extent as normally seen in the obese. The risk of future CVD is therefore lower for these subjects (32).

1.2.2.1 Influence of puberty on cardiovascular risk factors

Childhood obesity modifies the time of entering puberty, especially for girls (33). For boys, the data are insufficient and the association is inconsistent. In boys, the start of puberty might be delayed (34, 35) and, consequently, fewer boys than girls of the same age may have started puberty. However, metabolic health is altered during puberty in both girls and boys with obesity (36). When entering puberty—Tanner stages 2–3 (early pubertal) (37) —the risk of metabolically-unhealthy obesity is doubled. But leaving Tanner stages 2–3 and entering late puberty (Tanner stages 4–5), the chances of metabolically-healthy obesity again tripled.

Prevalences of cardiovascular risk factors such as hypertension, hypertriglyceridemia, impaired fasting glucose level, and the measure of insulin resistance became worse among children and adolescents with obesity when entering puberty. However, when entering late puberty these prevalences decreased, and the measure of insulin resistance was only weakly associated with the prevalences of these CV risk factors. This suggests that other factors besides insulin resistance contribute to metabolic changes during puberty (38).

1.2.2.2 Abnormal blood lipid levels

Abnormal levels of blood lipids are early biochemical signs of an unhealthy and excessive diet, and usually contribute to overweight and obesity. These blood lipids are involved in the formation of atherosclerotic plaque, and are therefore an example of a risk factor for atherosclerotic diseases (39). Decreased high-density lipoprotein (HDL) cholesterol and increased low-density lipoprotein (LDL) cholesterol, the ratio of LDL cholesterol and HDL cholesterol (LDL/HDL), triglycerides and total cholesterol are already present among children and adolescents with obesity (12, 31), and are traditionally used to evaluate the blood lipid profile. The apolipoproteins, apolipoprotein A1 (APO A1) and apolipoprotein B (APO B), are attached to the surface of the lipids to facilitate the transport of different kinds of hydrophobic lipid compounds in the blood. When measuring apolipoproteins, a larger fraction of blood lipids can be detected. Together with the ratio of APO A1 and APO B, it has been suggested that the apolipoproteins are a more accurate measure of the total amount of blood lipids and subsequent CV risk (40); however, according to others, they may not be superior to traditional measures (39).

1.2.2.3 Impaired insulin and glucose metabolism

Insulin resistance (41) and elevated glucose levels (42) are present in childhood obesity. In adolescents with type 1 diabetes, impaired insulin metabolism is related to the early development of CVD as a result of impairment in the nocturnal blood pressure fall (43). In otherwise healthy adolescents with obesity, a higher degree of insulin resistance was also related to lower HDL cholesterol (44). An elevated glucose level is not only a risk factor for type 2 diabetes mellitus (T2DM) (45), but glucose is also related to intima-media thickness (IMT) in adolescents with obesity (46).

1.2.2.4 Low grade inflammation

Inflammation is related to the aetiology of atherosclerosis (25, 47). There are several (45) biochemical markers that indicate an on-going inflammatory process. However, in health care and many studies, the inflammatory marker high sensitive C-reactive protein (hs-CRP) is common. Low-grade inflammation (increased hs-CRP levels) is accompanied by increased IMT not only in childhood obesity (48), but also in healthy children, although not measured as hs-CRP (49).

1.2.2.5 Hypertension

In the growing child, blood pressure naturally increases with age, and therefore references need to be based on gender, age and height (50, 51). However, blood pressure has also increased among children and adolescents over time (52), probably partly due to the parallel increase in the prevalence of overweight and obesity (53). In children and adolescents, an increased BMI is associated with an increased risk of hypertension (54). Hypertension is a strong risk factor for CVD in adults (55), and often coexists with non-dipping (56), as well as being associated with several preclinical stages of CVD in children with obesity (57).

1.2.2.6 Nocturnal blood pressure non-dipping

Normally, the blood pressure lowers by > 10% from daytime to night (referred to as dipping) in healthy children and adolescents, but if it lowers by less than 10%, it is referred to as non-dipping (58). Nocturnal blood pressure dipping is less pronounced in children with obesity (59), although the mechanisms are still unclear. Among a population of Swedish adolescents with obesity, 50% were nocturnal systolic non-dippers (60). Independent of hypertension, non-dipping in adults predicts left ventricular hypertrophy (LVH) (61, 62), end-organ function, CVD (62), and mortality (63). Non-dipping status is also related to cIMT in adults (64). However, among fairly normotensive adolescents with obesity, the association between dipping and left ventricular mass (LVM) seems to not be as pronounced (60).

In younger children with obesity, the relationship of non-dipping to CV risk factors has been little studied. Marcovecchio et al. found no associations between childhood dippers and non-dippers and obesity and measures of insulin and glucose metabolism (65). An association between non-dipping and left ventricular dysfunction (66) has been found among adolescents with type 1 diabetes. Among children and adolescents with type 1 diabetes, high nocturnal blood pressure is also associated with higher cIMT (67). In more recent studies, non-dipping has not been associated with increased LVM index in either children or adolescents with obesity and suspected hypertension (68), or among non-obese but hypertensive children (69).

1.2.2.7 Endothelial dysfunction

Endothelial dysfunction is one of the first detectible steps in the development of atherosclerotic diseases (70). Endothelial dysfunction contributes to conditions such as increased blood pressure and atherosclerosis through mechanisms involving the production of inflammatory and vasoconstriction factors, the uptake of lipids to the vascular structure, and the reduced production of vasodilating factors such as the highly important nitrogen oxide (NO) produced by the endothelial cells (71). Endothelial function can be evaluated both in the macrovasculature (brachial and femoral arteries) and microvasculature (arterioles and capillaries) (72). Microvascular endothelial function among children and adolescents with obesity has been poorly studied compared to macrovascular endothelial function. In high cardiovascular risk adults, macrovascular and microvascular dysfunction are correlated, but this relationship is unclear in children with obesity (72).

1.2.2.8 Arterial stiffness

Arterial stiffness is an early subclinical marker of CVD (73) that can be detected before CVD appears. It is possible to measure arterial stiffness at different sites in the vascular tree, depending on the method used (74). Arterial stiffness is already present in children with obesity (75-77).

1.2.2.9 Increased intima-media thickness

Increased cIMT is a marker of the early atherosclerotic process involving plaque formation in the intima layer of the artery (47), and predicts the risk of CVD in adults (78). Increased cIMT is found in children and adolescents with obesity (28, 79), though it may not be dependent on the adiposity itself (80), but on the presence of other risk factors, at least in children (81).

1.2.2.10 Left ventricular hypertrophy

LVH is a marker of increased risk for CVD (82). LVH is present in children and adolescents with obesity (83, 84) irrespective of blood pressure status (85), though it is thought to be affected to a greater extent when both obesity and hypertension are present (86).

1.2.2.11 Metabolic syndrome

In children and adolescents, the prevalence of metabolic syndrome (MetS) increases with the degree of obesity. In severely obese youths, the prevalence is about 50%, and is related to insulin resistance (87). Among adolescents, most of them obese, MetS is also associated with a higher prevalence of LVH (88).

1.2.2.12 Influence of obstructive sleep apnoea on cardiovascular risk factors

Obstructive sleep apnoea (OSA) is present in children with obesity (31). In children, OSA is associated with insulin resistance, hypertension, and metabolic consequences (89). In adults, OSA also increases the risk of CVD and overall mortality (90).

1.2.2.13 Influence of low physical activity and cardiorespiratory fitness on cardiovascular risk factors

Physical activity involves any bodily movement produced by skeletal muscles that results in energy expenditure higher than basal levels, and is related to physical fitness. The effect of physical activity depends on the intensity, frequency and duration. The intensity of physical activity can be expressed as the metabolic equivalent (MET), which is related to the oxygen consumption per kilogram of body weight and minutes of physical activity performed (91).

Exercise is one part of physical activity, and is planned, structured and repetitive bodily movement related to physical fitness. Exercise is intended to maintain or improve components of physical fitness. Cardiorespiratory fitness (CRF) is one component of physical fitness, and refers to the ability of the circulatory and respiratory systems to supply the skeletal muscles with oxygen during work (91). CRF is measured as the maximum oxygen

uptake (VO_2 max), which is limited by the capacity of heart, lungs and blood (the cardiorespiratory system) (92).

Low physical activity levels have been associated with an increased degree of obesity among children and adolescents (93). In adults, low physical activity levels and CRF are risk factors for CVD, but also interact with other CV risk factors, such as increased blood glucose, blood pressure, and blood lipids (94), and increased cIMT (95). Although it has not been studied to the same extent as in adults, the relationship of low physical activity and CRF with CV risk factors is also present among children and adolescents both with (96) and without obesity (97). CRF has been found to be more strongly correlated to risk factors than physical activity (98). In children and adolescents, greater CRF was associated with better arterial stiffness as measured by augmentation index, but not by pulse wave velocity (99). In another population-based study of adolescents, CRF was associated with arterial stiffness, but not cIMT (100). Central and total adiposity are higher in parallel with low CRF among children with obesity (101).

1.2.2.14 Influence of smoking and parental smoking on cardiovascular risk factors

Both smoking and passive smoking (102) exert a direct adverse effect on health in children and adults. In addition, endothelial function (103) and increased cIMT (104) in adulthood have been associated with exposure to environmental or parental smoking during childhood, even when adjusted for adult smoking status.

1.2.3 Tracking of childhood cardiovascular risk factors into adulthood

About 55% of children with obesity will still have obesity in adolescence. About 80% of adolescents with obesity will have obesity in adulthood, and 70% will have obesity after the age of 30. However, 70% of the adults with obesity did not have obesity in childhood (23). As described above, CV risk factors are present along with obesity, and several of these CV risk factors are also seen tracking into adulthood.

Blood pressure (both in-office and 24-hour ambulatory blood pressure monitoring (ABPM) (105, 106)), blood lipids, and BMI measured in childhood are strongly related to levels seen in adulthood (107), although measurements of glucose are not (108). CRF also tracks into adulthood, although not very strongly (109). There is low tracking for the dichotomised MetS variable from adolescence to young adulthood, but the tracking of the cluster score of the MetS-components seems more relevant (110). Those categorized as metabolically-healthy obese in childhood were more likely to remain so into adulthood (32).Tracking of vascular structure and function from childhood to adulthood is less studied, however arterial stiffness seems to track over a 5-year period from early to late adolescence (75).

Even though childhood BMI tracks into adulthood (23, 107), changes in the degree of obesity from childhood to adulthood influence the tracking of CV risk factors, and hence the risk of CVD (111).

1.2.4 Associations between childhood cardiovascular risk factors and end-organ function in adulthood

Associations between childhood CV risk factors and adverse end-organ function in adulthood have been examined previously in large prospective studies of general populations, such as the Muscatine study (112), the Bogalusa Heart Study (9, 113-116), the Cardiovascular Risk in Finns Study (117-121), the Childhood Determinants of Adult Health Study (122) and the European Youth Heart Study (123). Some of these studies have also been evaluated together in reviews and/or meta-analyses (122, 124-126). However, other longitudinal studies also exist (127-131). Longitudinal studies following only those with obesity from childhood to adulthood are few. Some of the above studies evaluate adverse end-organ function in adulthood (near or in middle age), when ageing itself has started to have a crucial role for the CV outcome studied, and must be adjusted for. In addition, in most of these longitudinal studies, childhood measurements were performed before or at the beginning of the rise of the obesity epidemic (128, 130-132), and therefore may not reflect the future risks for today's children with a higher prevalence of overweight and obesity, and exposed to a different environment, with for example more fast food which also is more available in different shops and supermarkets, and different transportation facilities decreasing the natural level of physical activity.

1.2.4.1 Measures of obesity in childhood

Childhood BMI increases the risk of risk factors for CVD in young adulthood (133), increases cIMT (114, 134), and increases the risk of premature death up to the age of 55 (131). Childhood obesity is associated with an increased risk of both nonfatal and fatal coronary heart disease (CHD) from the age of 25 to the age of 60 (130). Childhood adiposity predicts cardiac mass in adulthood (135). In addition, having obesity since childhood is associated with LVH in adults (116). Childhood BMI and increased BMI from childhood to adulthood predict higher LVM in adulthood, even after adjusting for adult systolic blood pressure (SBP) and fasting glucose levels (136).

1.2.4.2 Blood pressure in childhood

Childhood SBP predicts arterial stiffness in young adulthood (115). A high SBP in adolescence (males only) but not in childhood (males and females) predicted adult brachial endothelial dysfunction (119). Childhood hypertension predicts higher cIMT in adulthood if the hypertension persists into adulthood (137). The change in adiposity and SBP from childhood to adulthood predicted the LVM index in young adulthood (135).

Blood pressure in childhood is often assessed via in-office blood pressure measurement. Longitudinal studies evaluating the association between childhood 24-hour ABPM and adult end-organ function seem to be lacking. In hypertensive adults, a non-dipping pattern is related to arterial stiffness (138).

1.2.4.3 Blood lipids in childhood

Adult cIMT is predicted by childhood LDL cholesterol (114), APO B and APO A1 (40), and total cholesterol (112). Compared with normal levels, high levels of childhood LDL cholesterol and triglycerides were associated with increased cIMT in adulthood. This was worsened by the presence of increased numbers of non-lipid CV risk factors or MetS (139). Childhood APO B and APO A1 also predict brachial endothelial function (40).

1.2.4.4 Physical activity and cardiorespiratory fitness in childhood

CRF in adolescence did not predict CVD risk factors in adulthood more accurately than childhood measures of body fat (140). Adolescents with a greater decrease in moderate- to vigorous-intensity physical activity from adolescence to young adulthood had more pronounced arterial stiffness as young adults than those with preserved levels of moderate- to vigorous-intensity physical activity (123). Childhood CRF measured at the age of 13 was associated with blood pressure in young adulthood, but was not associated at ages 25 and 40. The relationship to blood lipids was absent short after the age of 13 at baseline (141).

1.2.4.5 Metabolic syndrome in childhood

Childhood and adolescent MetS predicts MetS, high cIMT and T2DM in young adulthood (125). MetS in childhood may also predict a higher risk of developing MetS, myocardial infarction and stroke in middle age, although longitudinal changes in BMI also affect the associations (128). In another study, childhood MetS cluster scores, but not the dichotomous MetS, predicted MetS in young adulthood (110). A lower CV risk in adulthood was found among those who had lower levels of the variables included in MetS at childhood (142).

Those categorized as metabolically-healthy obese in childhood were more likely to remain so into adulthood, and were hence at a lower risk of CVD (32).

1.2.4.6 The influence of change in post-childhood BMI

Some previous studies on the influence of a change in BMI or obesity status on associations between childhood CV risk factors and CV outcomes in adulthood indicate that the change alters the association if taken into account in the analyses. After adjusting for adult BMI, the associations between childhood BMI and both adult blood pressure and cIMT were weakened (111). Those at risk of the highest blood pressure in adulthood were those with a lower BMI in childhood but overweight in adulthood, suggesting that the change in BMI status might be of higher importance than childhood BMI for predicting future CV health (111). Another study showed that a higher BMI from childhood to adulthood compared with a normal BMI in childhood and non-obese state in adulthood increased the risk of hypertension, T2DM, dyslipidaemia, and increased cIMT (124). Associations found between childhood BMI and the risk of T2DM, hypertension and CHD are dependent on adult BMI since associations were attenuated when adjusting for adult BMI. Longitudinal studies would therefore adjust for adult BMI but this is seldom done or studies fail to show associations when adjusting for adult BMI (143). Overweight is associated with adverse CV outcomes in adolescence and

adulthood if not resolved by this point in time (144). Associations between measures of obesity and vascular structure and function were influenced by the tracking of obesity (121). Childhood overweight was associated with increased cIMT when participants became obese in adulthood, but not if they were overweight (134).

1.2.4.7 Influence of age at the time of measurement of risk factors

The age at the time of measurement of CV risk factors in childhood might influence the associations with the outcome measures in adulthood. CV risk factors measured before the age of 9 were not predictive of adult cIMT as CV risk factors measured after the age of 9 (145).

Although there are longitudinal studies of general populations in which the impact of a high BMI or the state of obesity are evaluated, long-term follow-up studies of the relationship between childhood CV risk factors and end-organ function in young adults who have been attending childhood obesity treatment is lacking. To optimise resources within childhood obesity treatment, the evaluation of childhood risk factors of importance for future CV risk in this specific group is important. A previous study of young Japanese adults previously treated for childhood obesity showed a high prevalence of persistent obesity in adulthood and a higher prevalence of chronic diseases such as hypertension, dyslipidaemia, T2DM, atherosclerosis and stroke among those still having obesity in adulthood. However, the follow-up was performed via questionnaire, which is a weakness (146).

1.2.5 Weight loss and cardiovascular risk factors

After attending a 1-year intervention program, a weight loss of ≥ 0.5 BMI SDS in children with obesity decreased SBP, DBP, LDL cholesterol, triglycerides, and measures of insulin, and increased HDL cholesterol (147, 148). One-year lifestyle modifications in adolescents with obesity resulted in a decrease of ≥ 0.5 in BMI SDS, and further improvements in triglycerides, LDL cholesterol and CRP (149). An effect was also found with a reduction of ≥ 0.25 in BMI SDS, although it was less pronounced (149).

Improvement in LVH can be achieved via weight reduction in children and adolescents with obesity, but is strengthened by a simultaneous normalization of elevated blood pressure, if present (86). A 1-year weight loss intervention in children and adolescents with obesity found effects from weight loss on 24-hour ABPM, but no convincing effects on arterial stiffness (150).

In a 1-year outpatient intervention in children with obesity, cIMT was improved after a weight loss of 0.5 BMI SDS in parallel with improved CV risk factors such as blood pressure, blood lipids, and insulin (151). Among those with a lower decrease or no decrease in BMI SDS, no significant improvements in CV risk factors or cIMT were detected (151).

Weight reduction among children with obesity also improves OSA (152).

Gaining weight (111) or having obesity (124) from childhood to adulthood are associated with adverse CV risk factors in adulthood. Even though there is convincing evidence that weight loss is effective in improving CV risk factors, and that children with obesity who become normal weight at adulthood do not have an increased risk of CVD compared to those who never had obesity, obesity in childhood and adolescence persist into adulthood at a high rate (23). In addition, weight loss has been found to be hard to achieve, especially among adolescents with obesity, despite their participation in childhood obesity treatment (153). Therefore, approaches other than achieving an adequate weight reduction to acutely improve CV risk factors in children and, especially, adolescents with obesity, must be studied.

1.2.6 Effect of aerobic exercise in childhood on cardiovascular risk factors

The obesity paradox is considered to be modified by CRF. Subjects with obesity improve their CV risk factors or CVD outcomes when they improve their CRF, at least when starting from an unfavourable CRF. But when high CRF already exists, increasing the CRF further may not modify CV risk factors (154). In addition, exercise did not affect flow-mediated dilatation (FMD) among children with already normal vascular function (155).

In a recent review, exercise interventions among children and adolescents with obesity had an effect on weight loss, but there was limited evidence of an effect on CV risk factors (156). However, CRF in a general population of children is associated with metabolic risk factors (97), and effects have also been demonstrated from physical activity interventions on physical fitness and CV risk factors among adolescents with overweight and obesity (157).

In addition, aerobic exercise without weight loss has improved several CV risk factors in children and adolescents with obesity: CRF, HDL cholesterol and FMD (158); abdominal and truncal fat (159); inflammatory markers (160); lipoprotein particle size and cholesterol distribution (161); macrovascular and microvascular function, without improvements in CRF or whole body insulin sensitivity (162); macrovascular endothelial function (163); and insulin resistance (164). Physical activity also has positive effects on components of metabolic syndrome in childhood (165).

One consequence of increased structured exercise in children and adolescents is that total physical activity may not increase (166), as per the "ActivityStat hypothesis" (167). The "ActivityStat hypothesis" says that overall physical activity is hard to change in individuals, despite the introduction of structured exercise sessions. Total physical activity might even decrease, as has been seen among adolescents with obesity (168).

Adolescent CRF seems to not be associated with CV risk factors in adulthood, but indicates a relationship with measures of body fat in adulthood (140). However, improved CRF from childhood to young adulthood has been found to improve arterial stiffness, but not cIMT (127).

1.2.6.1 Intensity and frequency of exercise

Exercise may need to be performed at high intensities in adolescents in order to improve vascular function such as FMD (159); low intensities did not improve FMD (155). In sedentary adults with obesity, high-intensity exercise was required to improve CRF (169). Intensities of around 150 bpm during at least 30 minutes, 3 times a week seem to be sufficient to improve insulin sensitivity (164) and FMD (159) in children with obesity. Higher amounts of moderate- to vigorous-intensity physical activity among children and adolescents is associated with preferable cardiometabolic risk factors, regardless of time spent sedentary (170). However, another study in children and adolescents concluded that only vigorous-intensity physical activity was advantageous (171).

1.2.6.2 Factors affecting participation and compliance with exercise

Attitudes towards physical activity appear to have an important effect on participation in physical activity. Attitudes towards physical activity are less positive, and rates of participation in sports are lower among adolescents with overweight and obesity compared to normal-weight adolescents (172). Lack of interest and time have been shown to be a hindrance to performing exercise among adolescents with overweight and obesity (173). In a study examining what adolescents want in order to become more active, it was shown that adolescents with overweight and obesity were less likely to be physically active with friends compared with normal-weight adolescents (174). Social support from parents and friends and self-efficacy have an impact on physical activity among adolescents (175). The intensity of the exercise might also affect compliance through modifying the pleasure of exercise. In adult woman with overweight, a small increase in intensity above a self-selected intensity of exercise decreased the pleasure of exercise (176).

1.3 GAPS IN THE KNOWLEDGE WITHIN THE FIELD OF CARDIOVASCULAR RISK FACTORS IN CHILDREN AND ADOLESCENTS WITH OBESITY

Children with various CV risk factors have been found to have endothelial dysfunction. Microvascular endothelial dysfunction seems to be a first sign of atherosclerosis. However, studies in children, particular those measuring endothelial function in the microvasculature, are limited. Studies of microvascular acetylcholine-induced endothelial-dependent vasodilatation in children with obesity, without comorbidities, and associations with other potential risk factors, are lacking.

Blunted nocturnal blood pressure dipping, associated with a risk of CVD in adults, is also present in adolescents to a surprisingly high degree, but whether this is true for younger children as well, and whether there are associations with measures of insulin-glucose metabolism or sleep-disordered breathing is unknown.

In longitudinal studies of the general population, the presence of CV risk factors in childhood is associated with adverse CV outcomes in adulthood, but these are probably more affected by changes in weight from childhood to adulthood. The long-term effects of childhood CV risk factors on CV health in young adults treated for childhood obesity are unknown.

Weight reduction improves several obesity-related CV risk factors, but weight loss in children and adolescents with obesity is hard to achieve, even when they participate in obesity treatment programmes. Aerobic exercise with improved CRF also improves CV risk factors in different exercise programme designs. However, children and adolescents with obesity are less prone to participate in exercise if it is performed in a group and/or the exercise is too inaccessible. Studies to evaluate the performance of individualised aerobic exercise with a personal coach close to the school or home of adolescents with obesity in order to increase accessibility, as well as the maintenance of exercise, with the goal of increasing CRF and affecting CV risk factors, are lacking.

2 AIMS

2.1 OVERALL AIMS

The main aims of this study are to investigate the following:

- The prevalence of cardiovascular risk factors in children and adolescents who are in childhood obesity treatment.
- The predictive value of childhood cardiovascular risk factors for early signs of cardiovascular end-organ function in young adulthood.
- The effect of regular supervised individualized aerobic exercise on short- and long-term cardiorespiratory fitness and associated cardiovascular risk factors.

2.1.1 Specific aims

The specific aims of this paper are as follows:

- ✓ To test acetylcholine-induced endothelium-dependent vasodilatation in children with obesity but without comorbidities, compared with normal-weight children controls (Study I).
- ✓ To analyse associations between vasodilatation and other potential risk factors in children with obesity but without comorbidities (Study I).
- ✓ To analyse the prevalence of nocturnal blood pressure dipping among prepubertal and early pubertal children with obesity (Study II).
- ✓ To analyse the relationship between dipping and measures of insulin-glucose metabolism or sleep-disordered breathing among prepubertal and early pubertal children with obesity (Study II).
- ✓ To evaluate if well-established cardiovascular risk factors can be used clinically for the early identification of children and adolescents with obesity who are at risk of a rapid deterioration in cardiovascular health in young adulthood (Study III).
- ✓ To evaluate if 3 months of regular supervised individualised aerobic exercise can increase cardiorespiratory fitness and implement improved long-term cardiorespiratory fitness among adolescents in obesity treatment (Study IV).
- ✓ To evaluate the effect of 3 months of regular supervised individualised aerobic exercise on cardiovascular risk factors among adolescents in obesity treatment (Study IV).

3 METHODS

3.1 ORIGIN OF STUDY POPULATIONS

The populations included in the studies in this thesis were mainly recruited from the National Childhood Obesity Centre in Stockholm, Sweden and the Swedish Childhood Obesity Register (BORIS). A summary of the included studies is presented in Table 1.

3.1.1 The National Childhood Obesity Centre

It is possible to carry out obesity treatment in various health care settings all over Sweden. However, when morbid obesity or complicated obesity with comorbidities are present, children and adolescents can be referred to the National Childhood Obesity Centre in Stockholm, which was founded in 1997. The main goals of treatment are to involve the family, highlight the severity of the disease and focus on lifelong treatment. The treatment consists mainly of behavioural treatment regarding diet and physical activity, but may also be accompanied by low- and very-low-calorie diets and/or pharmacological treatment when necessary. Comprehensive obesity-related physical examinations are performed routinely at admission for treatment. During the course of treatment, examinations of importance for the treatment of a specific individual are repeated when required.

3.1.2 The Swedish Childhood Obesity Register

To be able to follow the treatment of children and adolescents with obesity and evaluate their treatment, the nationwide web-based register BarnObesitasRegistret i Sverige (BORIS) was started in 2005 (www.e-boris.se). Children attending childhood obesity treatment are supposed to be registered in the BORIS register, which can be used for evaluation of childhood obesity treatment both regionally and nationally, but also used for clinical research.

Table 1. Summary of the studies included in the thesis.

	Study I	Study II	Study III	Study IV
Aim	To test acetylcholine-induced endothelium-dependent vasodilatation in children with obesity without comorbidities, compared with normal-weight controls, and to analyse associations between vasodilatation and other potential risk factors.	To investigate the prevalence of nocturnal blood pressure dipping among obese prepubertal and early pubertal children and to analyse the relationship between dipping and measures of insulin-glucose metabolism or sleep-disordered breathing.	To evaluate if well-established cardiovascular risk factors can be used clinically to early identify children and adolescents with obesity at risk of a rapid deterioration of cardiovascular health in young adulthood.	To study the effect of three months regular supervised individualised aerobic exercise on short- and long-term cardiorespiratory fitness and cardiovascular risk factors among adolescents in obesity treatment.
Design	Retrospective Cross sectional Observational A normal-weight group for comparison.	Retrospective Cross sectional Observational	Prospective Longitudinal Observational	Prospective Longitudinal Observational Intervention
Population	Children with obesity, admitted to the National Childhood Obesity Centre in Stockholm, Sweden between 1996 and 2007. Normal-weight children for comparison.	Children with obesity, enrolled to the National Childhood Obesity Centre in Stockholm, Sweden and registered in the BORIS register.	Young adults who have attended a treatment program for severe childhood obesity at the National Childhood Obesity Centre in Stockholm, Sweden and registered in the BORIS register.	Adolescents with obesity attending a treatment program at the National Childhood Obesity Centre in Stockholm, Sweden, the pediatric outpatient clinic in Huddinge or the pediatric outpatient clinic at Södertälje Hospital, Sweden.

3.2 STUDY I

3.2.1 Design and population

The population in this retrospective cross-sectional study consisted of children with obesity admitted to the National Childhood Obesity Centre in Stockholm, Sweden between 1996 and 2007. Data from 49 healthy Swedish children with normal weight, previously investigated with the same apparatus as the children with obesity, were available for comparison (177, 178).

3.2.1.1 Inclusion and exclusion criteria

Sixty-eight children with obesity who had undergone an endothelial functioning test at admission to the obesity treatment were included. Exclusion criteria were invalid endothelial functioning test, type 2 diabetes (179), hypertension (50) or hyperlipidemia (according to accredited laboratory cut-offs). In order to study associations between microvascular acetylcholine-induced vasodilatation and obesity-related risk factors and confounders, a subgroup of the children with obesity was studied. There was information from biochemical analyses of these children, and there was also information about additional obesity-related risk factors and confounders within the same time period as the endothelial functioning test for most of them.

3.2.2 Data collection

Data on microvascular endothelial function (acetylcholine-induced endothelium-dependent vasodilatation), anthropometric assessments—weight and height, dual X-ray absorptiometry (DXA), 24-hour ABPM, frequently sampled intravenous glucose tolerance test (FSIVGTT), submaximal ergometer cycle test, and duration of obesity for the children with obesity were extracted from medical records or the BORIS register. Data on microvascular acetylcholine-induced vasodilatation and descriptive and anthropometric data for the normal-weight children (177, 178) were obtained from the authors. Other data included for all children were classification of normal weight and obesity (15), and calculated BMI SDS (18).

3.3 STUDY II

3.3.1 Design and population

The population in this retrospective cross-sectional study consisted of children with obesity enrolled at the National Childhood Obesity Centre in Stockholm, Sweden, and registered in the BORIS register.

3.3.1.1 Inclusion and exclusion

This study included 115 prepubertal and early pubertal children for whom information on BMI was available, and who had performed a complete 24-hour ABPM at admission to obesity treatment. Excluded were subjects lacking data from 24-hour ABPM due to technical problems, or poor compliance with the measurement.

3.3.2 Data collection

Data from 24-hour ABPM, echocardiography, polygraph recordings, biochemical variables and anthropometric measurements including DXA were obtained from the BORIS register.

3.4 STUDY III

3.4.1 Design and population

The population examined in this longitudinal prospective study was young adults who had attended a treatment program for severe childhood obesity at the National Childhood Obesity Centre in Stockholm, Sweden and registered in the BORIS register.

3.4.1.1 Inclusion and exclusion

In order to be included, subjects had to have information from having performed an intravenous frequency sampling glucose tolerance test (not included here) and a 24-hour ABPM performed within a reasonable timeframe from each other, a minimum of 5 years; these criteria were met by 151 subjects. Exclusion criteria were: bariatric surgery, pharmaceutical treatment or diagnoses affecting the ability to participate, or variables measured at follow-up. The inclusion process is presented in Figure 1.

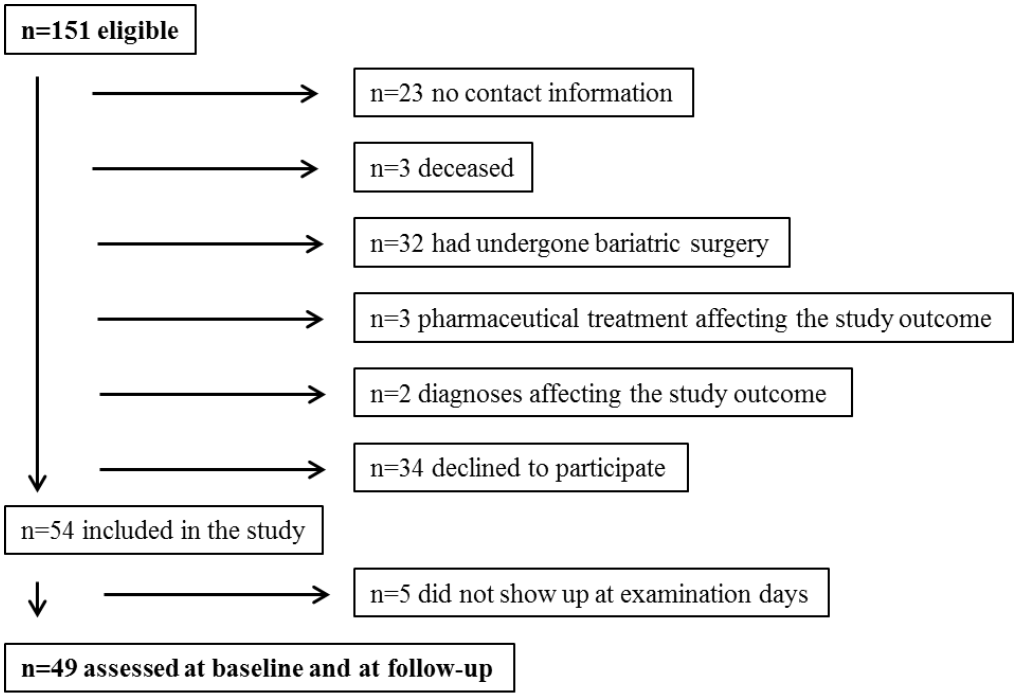

Figure 1. Flow chart of the inclusion process in Study III.

3.4.2 Data collection

Subjects, now in young adulthood, who met the inclusion criteria were identified using the BORIS register. They were informed about the study via mail and telephone. Those who fulfilled the exclusion criteria were invited to participate in the study. In young adulthood, the examinations were performed over 2 days at the Karolinska University Hospital in Huddinge after an overnight fast each day. Measurements performed both in childhood and in young adulthood were anthropometry, 24-hour ABPM, echocardiography, biochemical analyses, and submaximal bicycle test. In addition, the presence of MetS was determined. In addition, in young adulthood, microvascular acetylcholine-induced endothelium-dependent vasodilatation, pulse wave analysis, and ultrasonography of the carotid arteries were measured. In childhood, information on pubertal status also was collected. Data on measurements performed in childhood were collected from medical records and/or the BORIS register.

3.5 STUDY IV

The original aim of this randomised controlled clinical intervention was to study the impact of aerobic exercise or metformin therapy on nocturnal blood pressure and related risk factors for CVD in adolescents with obesity.

The design had three groups: the first was treated with a daily dose of 2000 mg of Metformin over 12 months; the second group performed regular individualised aerobic exercise 3 times a week over 12 months, led by a personal coach during the first 3 months; the third was a control group.

Unfortunately, the recruitment process turned out to be more difficult than expected. In addition, the dropout rate, mainly among the controls, was large soon after randomisation. It was decided to terminate the study, and primarily evaluate those who performed the aerobic exercise, which is Study IV in this thesis.

3.5.1 Design and population

This longitudinal, prospective intervention study was performed among adolescents with obesity attending a treatment program at the National Childhood Obesity Centre in Stockholm, Sweden, the outpatient clinic in Huddinge, or the outpatient clinic at Södertälje Hospital, both in Sweden.

3.5.1.1 Inclusion and exclusion

Inclusion criteria were adolescents with obesity (15) aged 13–19 who had recently completed a 24-hour ABPM. Excluded were those with diagnoses or treatments affecting the studied variables; medical treatment contraindicated for the original study aim; and mental or physical conditions preventing sufficient compliance with the original study protocol.

3.5.2 Data collection

Physiological examinations were performed at baseline, after the 3-month exercise period with the personal coach, and 9 months after the coaching period. The physiological examinations were performed over 2 days at the Karolinska University Hospital in Huddinge after an overnight fast each day. The examinations consisted of anthropometry including DXA, a submaximal bicycle test, biochemical analyses, an oral glucose tolerance test, 24-hour ABPM, pulse wave analyses, microvascular acetylcholine-induced endothelium-dependent vasodilatation, accelerometry, a questionnaire on health-related quality of life and pubertal status. In addition, the presence of MetS was determined.

Subjects followed their ordinary plans for obesity treatment during the study.

3.5.3 Aerobic exercise intervention

Appropriate, individualised, moderate- to high-intensity aerobic exercise was chosen by the subject and the personal coach together in Study IV. Both type of exercise and location were carefully chosen to increase the chances of getting the adolescent to attend, perform and also continue with the exercise after the supervised period. To make the exercise as accessible as possible for the adolescent, the exercise was made available in a location near the subject's home or school. The exercise was performed for 45 minutes, 3 times per week (159) under the supervision of the personal coach during the first 3 months. During this time, the subject wore a heart rate monitor (Polar RS400, Polar Electro Sverige AB, Bromma, Sweden) to ensure sufficient intensity at a mean heart rate of 150 beats per minute (bpm) for at least 30 minutes (164). During the following 3 months, the subject performed aerobic exercise at the same intensity and frequency, but without the coach; instead, the personal coach or someone from the research team made weekly contact with the subject for support. During the final 6 months, the subject performed aerobic exercise without any support. The subject was instructed to fill out an exercise diary after each exercise session during the entire year, and mail it monthly to the research team to monitor exercise compliance. Those with a compliance of < 75% were excluded from statistical analyses.

3.6 MEASUREMENTS

A summary of measurements and variables included in the studies is presented in Table 2. All examinations were performed by trained or specialised staff either in the research group, at the National Childhood Obesity Centre in Stockholm, or at specialised clinics at the Karolinska University Hospital in Huddinge, depending on which examination was performed.

Table 2. Overview of measurements and related variables included in each study of this thesis.

Variable	Study I	Study II	Study III	Study IV
Age	x	x	x	x
Sex	x	x	x	x
Pubertal status		x	x	x
Weight	x	x	x	x
Height	x	x	x	x
Waist circumference				x
BMI			x	x
BMI SDS, Karlberg et al. (18)	x			
BMI SDS, Roland-Cachera et al. (17)		x		
BMI SDS, Cole et al. (16)			x	x
delta BMI SDS, Cole et al. (16)			x	x
Classification of BMI (15)			x	
Duration of obesity	x			
Time to follow-up			x	
Time in treatment			x	
24-hour Ambulatory Blood Pressure Monitoring	x	x	x	x
24-h SBP	x	x		
24-h DBP	x	x		
Daytime SBP	x	x	x	x
Daytime DBP	x	x	x	x
Night-time SBP	x	x	x	x
Night-time DBP	x	x	x	x
SBP dipping	x	x	x	x
DBP dipping	x	x	x	x
Acetylcholine-induced endothelium-dependent vasodilatation	x		x	x
Basal Perfusion	x		x	
Peak perfusion	x		x	x
Echocardiography		x	x	
Left Ventricular Mass			x	
Left Ventricular Mass Index		x	x	
Ultrasonography			x	
Carotid Intima-Media Thickness			x	
Pulse Wave Analysis			x	x
Arterial stiffness (AIx@HR75)			x	x
Intravenous frequent sampling glucose tolerance test	x	x		
Acute insulin responsiveness to glucose	x	x		
Insulin sensitivity index	x	x		
Glucose effectiveness	x	x		
Disposition index	x	x		
The Homeostasis model assessment index	x	x		
Oral glucose tolerance test				x
2-hour glucose				x
2-hour insulin				x

Table 2. Overview of measurements and related variables included in each study of this thesis. Continued.

Variable	Study I	Study II	Study III	Study IV
Dual X-ray Absorptiometry		x		x
Body fat per cent		x		x
Lean body mass per cent				x
Android fat per cent				x
Gynoid fat per cent				x
Abdominal fat per cent		x		
Submaximal bicycle test	x		x	x
Absolute maximal oxygen consumption			x	x
Relative maximal oxygen consumption	x		x	x
Polygraph recording		x		
Apnoea-hypopnoea index		x		
Oxygen desaturation index		x		
Lowest oxygen saturation		x		
Biochemical variables	x	x	x	x
Glycosylated hemoglobin A1c	x	x		x
Glucose	x	x		x
Insulin	x	x		x
HDL cholesterol	x		x	
LDL cholesterol	x		x	
LDL/HDL ratio			x	x
Total cholesterol	x		x	x
Triglycerides	x		x	x
hs-CRP	x			x
Apo B				x
Apo A1				x
Apo B/Apo A1 ratio				x
Metabolic syndrome score			x	x
Smoking prevalence			x	
Quality of life questionnaire				x
Physical Health score				x
Psychosocial Health score				x
Total score				x
Accelerometry				x
Mean counts per minute/day				x
Minutes/day < 1.5 METs				x
Minutes/day > 3 METs				x
Minutes/day > 6 METs				x

3.6.1 Anthropometry

Weight and height were measured with a calibrated wall-mounted scale to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated, and was classified in children and adolescents by age and sex specific cut-offs (15) corresponding to the adult cut-offs for normal weight, overweight, obesity and severe obesity, defined as 18.5–24.9 kg/m^2, 25.0–29.9 kg/m^2, 30.0–34.9 kg/m^2 and ≥ 35.0 kg/m^2, respectively.

In order to compare BMI between groups, ages or sexes, and to follow BMI over time, BMI SDS was calculated according to Karlberg et al. in Study I (18), Roland-Cachera et al. in Study II, and the International Obesity Task Force (IOTF) in Studies III and IV (16). In order to follow BMI from childhood to young adulthood in Study III, BMI SDS was calculated for the young adults also, and it was estimated that all were 18 years old at follow-up.

Body composition and fat distribution were studied via DXA in Study II (Lunar Prodigy, software version 8) and Study IV (iDXA). Waist circumference was measured with a tape measure to the nearest 0.5 cm (Study IV).

3.6.2 Biochemical analyses

Fasting venous blood samples was collected in order to measure blood lipids (Studies I, III, and IV), hs-CRP (Studies I and IV), glycosylated hemoglobin A1c (HbA1c) (Studies I and II), insulin and glucose (Studies I, II and IV) were drawn in the morning after an overnight fast starting at midnight. Homeostasis model assessment (HOMA) was calculated based on fasting insulin and glucose (180) in Studies I and II. Biochemical analyses were performed in the laboratory at Karolinska University Hospital in Huddinge, according to accredited standard procedures present at the time the blood samples were drawn and analysed in each study.

Reference data for abnormal levels differs between studies. The reference from the expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents was used for children and adolescents (39) in Studies III and IV, and the Third Report of the National Cholesterol Education Program was used for adults in Study III (181). References for variables (Studies III and IV) not included in these reports came from the chemical laboratory at Karolinska University Hospital.

3.6.3 Metabolic syndrome score

The MetS score was calculated according to Zimmet et al. in Study IV and at baseline in Study III (182). At follow-up in Study III, Alberti et al. was used since the criteria are different for adults (183). The five possible risk factors included in the concept of MetS are obesity or elevated waist circumference, lowered HDL cholesterol, elevated fasting glucose, casual blood pressure, and triglycerides.

3.6.4 24-hour ambulatory blood pressure monitoring

The 24-hour ABPM was measured in all studies using the Space Labs 90 207/90 217 apparatus (Space Labs, Workingham, UK) to evaluate circadian variations in blood pressure. Overall, the consensus guidelines for 24-hour ABPM were followed (184). A cuff of appropriate size was placed on the upper non-dominant arm and connected to a small apparatus attached to a belt around the waist. Subjects were instructed to live normally as much as possible, but not to move the arm during readings. The participants were told to fill in a diary with wake and sleep times and other events possibly affecting the 24-hour measurement. If required, the standard daytime (8 a.m. to 8 p.m.) and night-time (12 p.m. to 6 a.m.) were adjusted to conform to what was reported in the diary. If possible, the night-time was offset to still include 6 hours of sleep. A minimum of 1 hour after bedtime and awakening was excluded from sleep time and waking hours, respectively, if within or very close to the standard night-time and daytime, to minimize incorrect night-time and daytime mean values. The aim was to have a minimum of 70% of possible readings during daytime and night-time respectively for a measure to be valid (184). However at baseline in Study III, several of the measurements were missing blood pressure readings, and when necessary, the cut-off for the percentage of valid readings was lowered to 50%. At follow-up in Study III, the cut-off was also lowered to 50% in some measurements.

Dipping is a measure of the per cent difference between daytime blood pressure and night-time blood pressure; if the dip is less than 10%—a commonly-used and widely-accepted cut-off for an absent decrease of nocturnal blood pressure—this is referred to as non-dipping.

For determining hypertension from 24-hour ABPM in children, the reference from Wühl et al. (50) was used in Studies I and II, and the reference from Lurbe et al. (51) in Study III.

3.6.5 Submaximal bicycle test

Submaximal tests were performed according to Åstrand (185) to estimate CRF as absolute and relative maximal volumes of oxygen consumption (VO_2 max). The subject is supposed to cycle for 6 minutes at an appropriate workload in order to reach a steady state in heart rate between minutes 5 and 6. At the end of every minute, the subject estimated the exertion on the Borg Rated Perceived Exertion (RPE) scale (186) to ensure and confirm the right workload had been chosen or adjust it at the beginning of the test and restart. Heart rate and level on the Borg RPE scale were noted every minute. The tests were performed on two different bicycle models in the included studies. A mechanically-braked bicycle (Monark, 864, Varberg, Sweden) was used in Study I, at baseline in Study III, and in some of the tests in young adulthood in Study III, where the method has been described previously (187). In most of the tests in young adulthood in Study III and all tests in Study IV, an electronically-braked bicycle (Siemens Elma, Rodby elektronik AB) was used. Compared to the mechanically-braked bicycle, where the frequency of pedalling is important for the workload, the frequency of pedalling is unimportant when using the electronically-braked bicycle, since the bicycle adjusts its own resistance in order to provide the selected workload. In order to

make the results of the tests performed with the different bicycles comparable, maximum VO_2 estimates were made. In Study III, the degree of CRF was classified according to Anderson et al. (188).

3.6.6 Accelerometry

The Actiwatch® accelerometer (AW, Model 4; Cambridge Neurotechnology Ltd, Cambridge, United Kingdom) was used to measure physical activity (PA) in Study IV, and worn as a watch on the non-dominant arm for 1 week. It was only removed for bathing. A minimum of 3 valid days (189), including 1 weekend day, were required to produce a valid measurement. PA is presented as mean counts per minute (cpm) of total days recorded, time spent in sedentary PA (< 1.5 METs = < 320 cpm), time spent in moderate to vigorous intensity PA (> 3 METs = > 1048 cpm), and time spent in vigorous intensity PA (> 6 METs = > 1624 cpm) (190).

3.6.7 Frequently sampled intravenous glucose tolerance test

With FSIVGTT (191) and minimal model analysis (192) it is possible to evaluate several aspects of insulin and glucose metabolism: insulin sensitivity (how well insulin enhances glucose disposal and inhibits endogenous glucose production); glucose effectiveness (glucose-mediated glucose disposal); acute insulin responsiveness (AIR) to glucose (endogenous insulin secretion); and disposition index (AIR x insulin sensitivity). Details of the protocol used in Studies I and II are described elsewhere (60). After an overnight fast starting at midnight, a peripheral intravenous catheter was inserted in each arm. Fasting insulin and glucose were drawn before the glucose was injected over the course of 1 minute. The intravenous dose of insulin was given 20 minutes after the glucose injection. Glucose and insulin blood samples were drawn at specific time points after the glucose was injected and until the examination finished 3 hours later.

3.6.8 Oral glucose tolerance test

An extended oral glucose tolerance test (OGTT) was used to determine glucose tolerance after oral intake of 75 g of glucose per kilogram of body weight in Study IV (193). After an overnight fast, venous blood samples of glucose and insulin were drawn 5 minutes before and again just before the oral glucose load was given. Blood sampling continued every 30 minutes until 120 minutes after the glucose load was given. The last glucose sample was taken to determine glucose tolerance (179).

3.6.9 Acetylcholine-induced endothelium-dependent vasodilatation

In Studies I, III and IV, acetylcholine chloride was transferred with a weak anodal current through the left dorsal hand skin using a micropharmacology system, iontophoresis, to induce endothelium-dependent vasodilatation. This was repeated 6 times at 1 minute 20 second intervals. Laser Doppler flowmetry was used to measure endothelial function as perfusion in the microvasculature after the administration of acetylcholine using iontophoresis. The laser Doppler signal, expressed as perfusion units (PU), is proportional to the number and velocity

of moving blood cells in the skin. If movement artefacts were within the perfusion change area for one dose, that value was excluded from the measurement; however, a maximum of one excluded value at each valid measurement was accepted. Basal perfusion ≥ 15 PU was a criterion for exclusion according to the manufacturer's instructions. The perfusion after the sixth dose was used as the peak (maximum) perfusion. Details of the methodology and the performance have been described elsewhere (177).

3.6.10 Pulse wave analysis

Pulse wave analysis (194) measures systemic arterial stiffness; it was performed with the SphygmoCor applanation tonometer device (AtCor Medical, Sydney, Australia) placed at the radial artery of the non-dominant arm (almost exclusively the left arm) to gently compress the artery against the radius bone. The participants had fasted and refrained from exercise and smoking overnight, and rested in a supine position in a quiet and temperature-controlled room for at least 20 minutes prior to the examination. Three sequential systolic and diastolic blood pressures were measured with an Omron M6 Comfort device (Omron Healthcare Europe B.V. Hoofddorp, The Netherlands) on the same arm as the pulse wave analysis and entered into the software to be included in the software calibration of the radial waveform. Blood pressure was measured every 5 minutes until the required number of measurements with good quality control (according to the manufacturer's guidelines) had been captured.

A general transfer function in the software converted the radial pressure waveform into an aortic pressure waveform (195). The Augmentation Index (AIx) standardized for a heart rate of 75 (AIx@HR75) (units in per cent) is a measure of arterial stiffness why written as arterial stiffness in this thesis. Only the two closest measurements of the three obtained were used, provided they passed the quality control and the AIx@HR75 did not differ more than 4% between the measurements (196).

3.6.11 Echocardiography

Two- dimensional echocardiography was performed at an accredited laboratory in Studies II and III according to established standards (197, 198). LVM was calculated using the Devereux equation (199). To account for differences in body size, the LVM index was calculated by dividing LVM in grams by the 2.7^{th} power of height ($g/m^{-2.7}$), and was used as a measure of cardiac structure (200). In Study III, different references for adverse levels were used in childhood (201) and adulthood (197).

3.6.12 Ultrasonography of the carotid arteries

The ultrasonography in Study III was performed in a supine position on the left and right common carotid arteries (CCA) to measure cIMT (78). Three ultrasound images were taken in systole on the right and left CCAs; however, only the image in which the intima-media complex of the far wall was clearest was used for analysis. A mean value of the right and left cIMT was calculated. The reference from Engelen et al. was used for elevated cIMT levels, in which the 75^{th} percentile was used for indication of increased risk of CVD (202).

3.6.13 Polygraph recordings

Sleep-disordered breathing (apnoea-hypopnoea index ≥ 1.5), oxygen desaturation index (the average number of desaturation dips per hour of sleep) and the lowest oxygen saturation were determined after in-hospital overnight sleep polygraph recordings sampled by the Micro Digitrapper SAS (Synectics Medical AB, Stockholm, Sweden) according to the hospitals standard protocol in Study II.

3.6.14 Pubertal status

Pubertal stage (genital and pubic hair development) (37) was examined in Studies II and IV. Pubertal stage one is considered prepubertal, stages two and three early pubertal, and four and five late pubertal.

3.6.15 Health-related quality of life

The Pediatric Quality of Life Inventory version 4.0 for teens, ages 13–18, was used to assess health-related quality of life (203) in Study IV. The questionnaire was composed of 23 items comprising four dimensions: physical functioning, emotional functioning, social functioning and school functioning. The final score was presented as a psychosocial health summary score (including emotional, social, and school functioning scales), a physical health summary score (including the physical functioning scale), and a total score.

3.7 STATISTICAL METHODS

The STATISTICA 10 data analysis software system, StatSoft, Inc. (www.statsoft.com), was used in Studies I and II for statistical analyses. In Studies III and IV, statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 22. A summary of statistical methods used in the studies is presented in Table 3.

Table 3. Summary of statistical methods.

	Study I	Study II	Study III	Study IV
Descriptive statistics	x	x	x	x
Student´s *t*-test	x	x		x
Mann-Whitney U-test		x		
Paired sample t-test				x
Non-parametric related sample test				x
Linear regression			x	
ANOVA		x		
ANCOVA	x	x	x	
Chi-square test	x	x	x	
McNemar's test			x	

3.8 ETHICAL APPOVAL

All ethical approvals were granted by the Stockholm Regional Ethical Review Board.

- Study I dnr; 2010//805-31/2.
- Study II dnr; 2005/1213-31/2 for the BORIS register, from which data was obtained.
- Study III dnr; 2010/1089-31/1 and 2011/2074-32.
- Study IV dnr; 2007/1031-31/2 ; 2009/73-32 and 2010/565-32.

4 RESULTS

Characteristics of subjects included in all studies are presented in Table 4.

Table 4. Characteristics of included subjects.

	Study I		Study II	Study III		Study IV	
						Exercise group	Non-exercise group
	Normal-weight children	Extensively investigated children with obesity	Prepubertal and early pubertal children with obesity	Childhood	Young adulthood	Before supervised exercise	
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
Women/men (*n*)	36/8	12/25	33/76	25/24	---	5/3	5/4
Age (years)	13.7 (3.7)	14.0 (2.3)	10.4 (1.7)	13.8 (2.3)	23.4 (3.0)	15.1 (1.2)	14.7 (1.4)
Weight (kg)	45.7 (14.0)	102. 3 (27.1)	73.1 (15.9)	95.0 (22.6)	119.0 (29.6)	99.9 (15.0)	99.1 (21.1)
Height (m)	1.55 (0.15)	1.67 (0.12)	1.51 (0.11)	1.65 (0.11)	1.74 (0.10)	1.68 (0.07)	1.67 (0.09)
BMI SDS	0.9 (1.4)	3.8 (0.6)	6.2 (1.6)	3.0 (0.4)	3.0 (0.9)	2.9 (0.5)	3.0 (0.5)

4.1 CARDIOVASCULAR RISK FACTORS IN CHILDHOOD AND CHANGE TO YOUNG ADULTHOOD

An overview of prevalences of CV risk factors in childhood and young adulthood are shown in Figure 2.

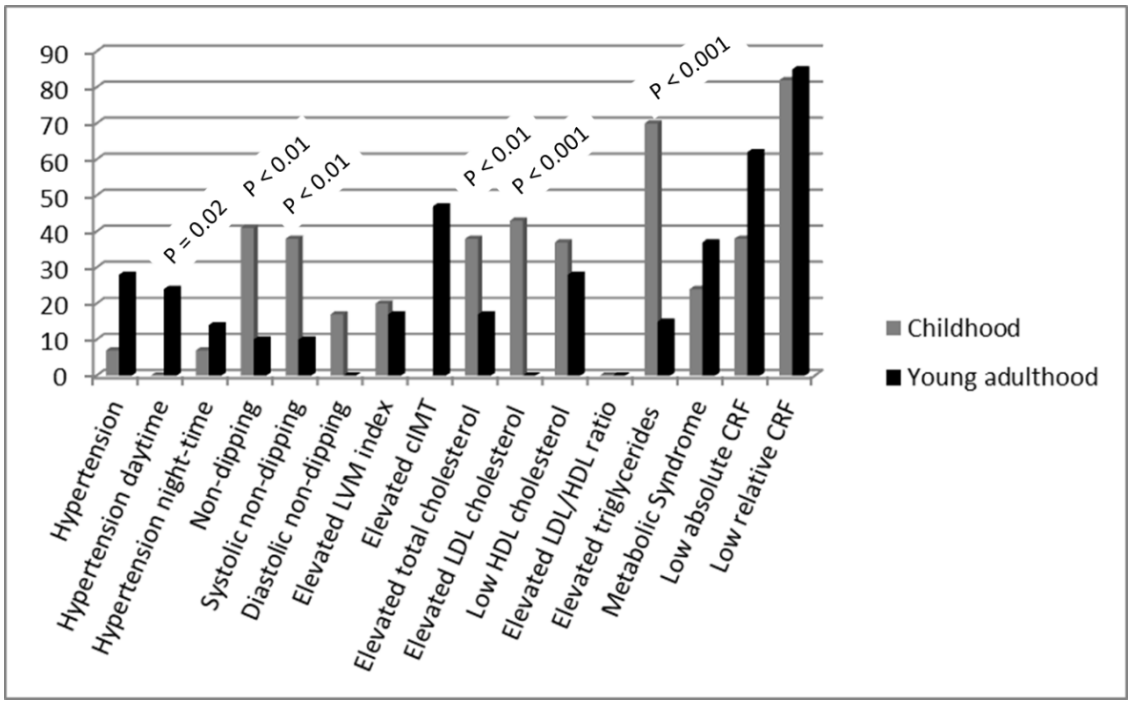

Figure 2. Prevalences of cardiovascular risk factors in childhood and young adulthood. P-values indicate statistically significant changes in prevalence from childhood to young adulthood among those with data at both time points (n = 20–48).

4.1.1 Dyslipidemia (Study III)

High total cholesterol and LDL cholesterol were both present in about 40% of subjects in childhood, and high triglycerides were present in as many as 70% in childhood; all prevalences, however, were significantly lower in young adulthood ($p = 0.00–0.03$). The prevalence of high triglycerides was resolved in about 80% of subjects, and high LDL cholesterol was totally resolved in young adulthood. For those with low HDL cholesterol in childhood, about 50% saw a resolution in young adulthood, however a 15% onset of low HDL cholesterol levels was found in young adulthood resulting in a non-significant change ($p = 0.39$). HDL cholesterol and LDL cholesterol tracked into young adulthood, although low to moderate ($r = 0.35$ and $r = 0.44$, respectively, with $p = 0.02$ for both). Total cholesterol carried into young adulthood at high rates ($r = 0.75$ and $p < 0.01$). Triglycerides and the LDL/HDL ratio did not track into young adulthood ($p = 0.05$ for both).

4.1.2 Hypertension (Study III)

The low prevalence of night-time hypertension present in childhood did not change statistically in young adulthood ($p = 0.63$). The prevalence of daytime hypertension increased from zero in childhood to 24 % in young adulthood ($p = 0.02$). Daytime SBP tracked into young adulthood ($r = 0.56$, $p < 0.01$), but this was not the case for other measures of daytime or night-time BP ($p = 0.17–0.46$).

4.1.3 Non-dipping (Studies II and III)

SBP and DBP non-dipping were present at about 40% and 17% in childhood, respectively. Prevalences did not differ between pre- and early pubertal children ($p = 0.91$ and $p = 0.25$, respectively; Studies II and III). Non-dipping resolved to 75% in young adulthood ($p < 0.01$; Study III). SBP dipping tracked into young adulthood ($r = 0.43$, $p = 0.03$).

4.1.3.1 Associations with non-dipping in childhood obesity (Study II)

BMI SDS, sex, and pubertal status were not associated with SBP or DBP dipping ($p = 0.1–0.8$); nor were measures of insulin and glucose metabolism (adjusted for BMI SDS, sex, and pubertal status; $p = 0.3–1.0$) or measures of obstructive sleep-disordered breathing ($p = 0.4–0.7$).

4.1.4 Microvascular endothelial dysfunction (Study I)

Compared with normal-weight children, microvascular endothelial function was 33% lower among children with obesity ($p < 0.01$). In addition, the change over time in vasodilatation from doses 1 to 6 was lower and slower for the children with obesity (adjusted for basal perfusion) (Figure 3).

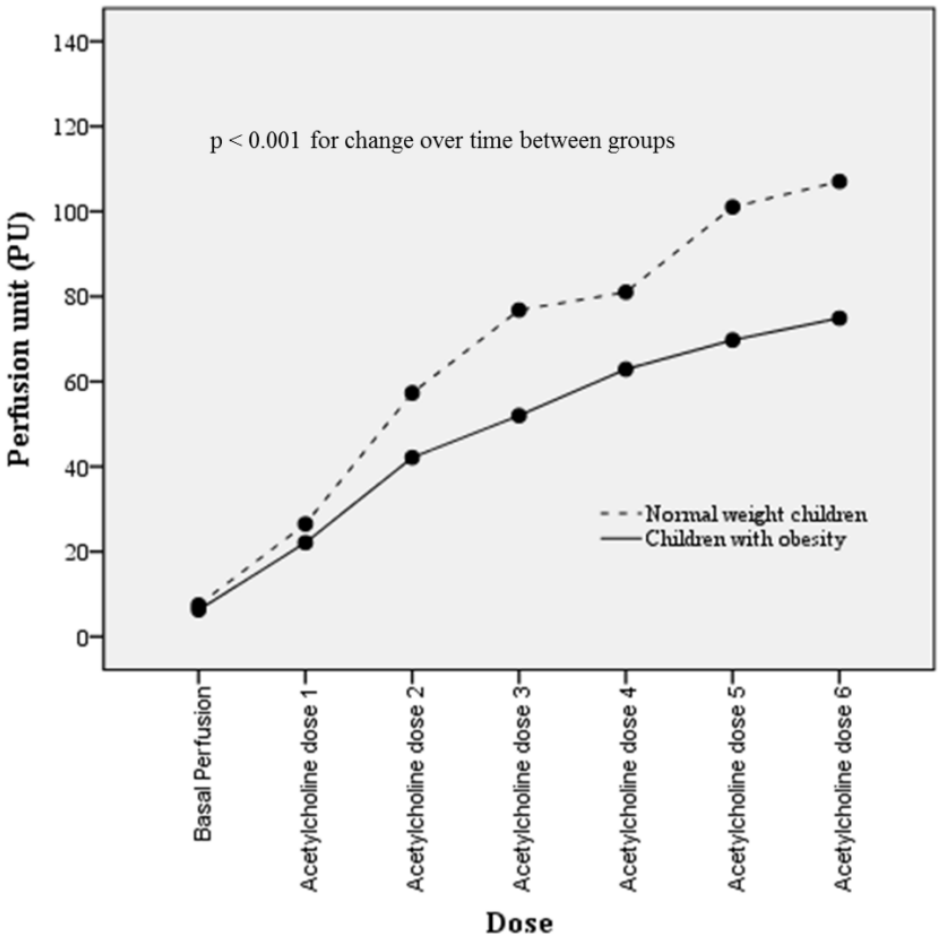

Figure 3. Dose-response curve after acetylcholine-induced endothelium-dependent vasodilatation in normal-weight children and children with obesity.

4.1.4.1 Associations with microvascular endothelial function in childhood obesity

Duration of obesity was positively associated with microvascular endothelial function (p = 0.03). Sex, age, BMI SDS, measures of insulin and glucose metabolism, hr-CRP, blood lipids, measures of blood pressure (including dipping) and CRF were not associated with microvascular endothelial function (p = 0.10–0.86).

4.1.5 Left ventricular mass hypertrophy (Studies II and III)

The prevalence of increased LVM index was stable at about 20% from childhood to young adulthood (p = 1.00; Study III). There was no tracking of LVM index into young adulthood (p = 0.16).

4.1.5.1 Associations with left ventricular mass index in childhood obesity (Study II)

BMI SDS was positively associated with LVM index (adjusted for sex, pubertal status and measures of SBP and DBP; p < 0.001). Measures of blood pressure (including dipping),

insulin and glucose metabolism and sleep-disordered breathing (adjusted for BMI SDS, sex and pubertal status) were not associated with LVM index ($p = 0.2–1.0$).

4.1.6 Low cardiorespiratory fitness (Study III)

The prevalences of low absolute and relative CRF were stable at levels of about 40%–60% and 80% (respectively) into young adulthood ($p = 0.12$ and $p = 0.22$, respectively). There was both high remission and onset of low absolute CRF in young adulthood. Relative CRF tracked into young adulthood ($r = 0.47$, $p = 0.01$), but absolute CRF did not ($p = 0.07$).

4.1.7 Metabolic syndrome (Study III)

The presence of MetS was stable at about 30% from childhood to young adulthood ($p = 0.96$), although both remissions and onset of MetS were observed; however, no tracking was found ($p = 0.88$).

4.2 PREDICTION OF MARKERS OF END-ORGAN FUNCTION IN YOUNG ADULTHOOD FROM CHILDHOOD CARDIOVASCULAR RISK FACTORS (STUDY III)

In young adults who had been in childhood obesity treatment, higher childhood triglycerides ($p = 0.02$), total cholesterol ($p < 0.01$) and daytime SBP ($p = 0.04$) predicted a higher cIMT adjusted for sex and change in BMI SDS. No other adverse effects on cIMT, LVM index, microvascular endothelial function, or arterial stiffness were found in young adulthood based on relevant childhood CV risk factors (all $p \geq 0.05$).

4.3 THE EFFECT OF 3 MONTHS OF REGULAR SUPERVISED AEROBIC EXERCISE ON ADOLESCENTS IN OBESITY TREATMENT (STUDY IV)

4.3.1 Short- and long-term cardiorespiratory fitness

Mean values of absolute and relative CRF at each time of measurement in the exercise and non-exercise group during the study are shown in Table 5. Absolute and relative CRF improved during 3 months (short-term CRF) of supervised aerobic exercise ($p = 0.01$ for both), and also improved compared to the non-exercise group ($p = 0.01$ for both the difference in change in absolute CRF and relative CRF between groups). Absolute and relative CRF ($p = 0.75$ and $p = 0.63$, respectively) did not change in the non-exercise group.

Absolute and relative CRF were not further improved 9 months after the end of the 3 months of supervised aerobic exercise (long-term CRF), but returned to about the levels present prior to the supervised exercise ($p = 0.04$ and $p = 0.03$, respectively). The impairment of absolute CRF ($p = 0.04$) was different compared to the non-exercise group, but not relative CRF ($p = 0.07$). Absolute and relative CRF in the non-exercise group did not change during these 9 months ($p = 0.50$ and $p = 0.94$, respectively).

Table 5. Cardiorespiratory fitness in those who performed supervised aerobic exercise compared with a non-exercise group.

	Exercise group	Non-exercise group	Exercise group	Non-exercise group	Exercise group	Non-exercise group
	Before supervised exercise		After 3 months of supervised exercise		Nine months after the supervised exercise	
	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)
Absolute CRF (L/Minute)	3.3 (0.7)	2.8 (0.6)	4.0 (0.8)	2.7 (0.5)	3.4 (0.9)	2.8 (0.6)
Relative CRF (ml/kg*minute)	34.5 (12.1)	29.2 (8.1)	40.5 (11.9)	28.3 (7.4)	31.3 (10.1)	28.4 (8.4)

4.3.2 Cardiovascular risk factors

No statistically significant effect on CV risk factors was found after the 3 months of supervised aerobic exercise (all $p \geq 0.05$). However, after the 3 months of supervised aerobic exercise, a total remission of MetS was observed in the 2 adolescents with MetS at baseline.

Nine months after the supervised period ended, no statistically significant favourable CV effect was found (all $p \geq 0.05$) with the exception of improvements in arterial stiffness. Arterial stiffness improved and elevated by 16 units during the 9 months after the supervised period ended ($p = 0.03$).

5 DISCUSSION

5.1 MAIN FINDINGS

This thesis highlights the presence of CV risk factors that accompany childhood obesity, and evaluates important CV risk factors in childhood for the prediction of early signs of end-organ dysfunction in young adulthood in order to provide clinicians with factors to focus on during childhood obesity treatment to improve future CV health. Further, this thesis addresses supervised individualised aerobic exercise as a complement to childhood obesity treatment for improving CRF and subsequent CV health in adolescents with obesity.

Study I showed that microvascular endothelium-dependent vasodilatory response to acetylcholine was 33% lower among children with obesity compared to normal-weight children. Microvascular endothelial function seemed less affected among those who had been obese for a longer time. No associations were found with CRF, 24-hour ABPM, inflammation, or insulin/glucose metabolism.

In Study II, a 42% prevalence of systolic non-dipping was found among prepubertal and early pubertal children with severe obesity. Dipping was not associated with measures of insulin-glucose metabolism after adjustments for BMI-SDS, sex, and pubertal status or between dipping and measures of sleep-disordered breathing.

In Study III, certain childhood CV risk factors—high total cholesterol, high triglycerides and high daytime SBP—predicted high cIMT, independent of sex, change in BMI SDS at adulthood, and smoking habits at adulthood in young adults who had previously attended childhood obesity treatment. The strongest tracking of CV risk factors from childhood to adulthood was found in diastolic BP and total cholesterol. Several of the prevalences of CV risk factors at childhood were lower in adulthood, despite a high persistence of severe obesity. Increased cIMT and low CRF were highly prevalent in young adulthood, however.

Study IV showed that regular supervised individualised aerobic exercise for 3 months was sufficient to improve short-term CRF among adolescents in obesity treatment, but not to implement sustained aerobic exercise habits in order to maintain long-term CRF. The supervised exercise did not improve CV risk factors during the 3-month coaching period or during the following 9 months without the coach, except for improvements in arterial stiffness during the last 9 months.

5.2 ACETYLCHOLINE-INDUCED ENDOTHELIUM-DEPENDENT VASODILATATION IN CHILDREN WITH OBESITY

In children and adolescents with obesity, microvascular endothelial function is much less studied than macrovascular endothelial function (72). Endothelial function can be determined by assessing vasodilatation by measuring the response to increased blood flow after occlusion of the vessel, or by stimulating the endogenous release of nitric oxide from the endothelium

using local administration of drugs such as acetylcholine. The latter method is the only non-invasive method of selectively stimulating endothelium-dependent vasodilatation. Non-invasive flow-mediated dilatation (FMD) is considered to be the gold standard method for measuring endothelial function, but is commonly used at the macrovascular brachial artery site. Microvascular endothelial function in children can be measured non-invasively in the peripheral cutaneous microvasculature. In any case, these methods are correlated to each other (204) and hence the acetylcholine-induced endothelium-dependent vasodilatation can be used as a measure of endothelial function in the microvasculature. More discomfort might be experienced by children when occlusion is used, whereas acetylcholine-induced vasodilation does not hurt; because of this, use of the latter method in children seemed more suitable (72). In addition, this method is reproducible (205), easy to use, quick, and operator independent.

Few previously published studies of microvascular endothelial function in children with obesity used acetylcholine-induced vasodilatation for assessment (206, 207), though some used post-occlusive reactive hyperemia (208-212). Some of the previous studies were performed in hypertensive children with obesity (207) or normal-weight children with abnormal glucose tolerance or T2DM (206). Impaired microvascular endothelial function among children with obesity but without comorbidities compared to normal-weight children in Study I was in line with other studies that mostly used different vascular provocation methods (207-210, 212). Worsened acetylcholine-induced vasodilatation has also been seen among normal-weight children with a higher body fat percentage or a higher 2-hour post-feeding glucose level using the same method as in Study I (206).

The duration of obesity at the time of measurement, although not often mentioned in studies of obesity, seems to play a role in the presence of comorbidities such as the microvascular endothelial dysfunction found in Study I. This relationship has also been noted in a previous study of endothelial function among normal-weight children (208). Lower levels of endothelial progenitor cells have been detected in adult obesity (213) and the insulin resistance state (214), and have been associated with lower brachial endothelial function (215). Circulating endothelial progenitor cells in the bloodstream mature into active endothelial progenitor cells on demand, and hence repair the damaged endothelium.

One hypothesis might be that the endothelial progenitor cells have not yet been fully activated for repairing the endothelium in subjects having obesity for only a short time. Later, the repair process may be stabilized and maintained, and therefore less impaired endothelial function is present among those with a longer duration of obesity. But in addition, depending on the age at onset of obesity, this adaptation/up-regulation of repair might not be as pronounced as in childhood. After the age of 20 years, adults have lower levels of circulating progenitor cells than children (216), and if they become obese, their bodies might not be able to maintain the same level of repair. This is probably only one of several explanations of the relationship between endothelial function and the duration of obesity.

Further, children with obesity who were included in the analyses of associations between acetylcholine-induced vasodilatation and other potential risk factors may have been healthier than those excluded because of missing data in the extended clinical investigation. Those included had lower BMI SDS, which may have contributed to the absence of associations between acetylcholine-induced vasodilatation and measures of insulin and glucose metabolism, 24-hour ABPM, CRF, inflammation, and blood lipids.

Reference values for impaired acetylcholine-induced vasodilatation related to the future risk of CVD are not available, and hence comparison with normal-weight subjects was the natural choice to evaluate whether impairment was present.

5.3 NOCTURNAL BLOOD PRESSURE DIPPING AMONG PREPUBERTAL AND EARLY PUBERTAL CHILDREN WITH OBESITY

24-hour ABPM is preferable to in-office blood pressure measurement when measuring blood pressure in children and adolescents, since they are mobile and their blood pressure fluctuates with their movements not only during waking hours, but also during sleep. With ABPM, short-term and 24-hour blood pressure are recorded and nocturnal blood pressure dipping can be studied (184). Reduced nocturnal blood pressure dipping in adolescents with obesity has been recorded using 24-hour ABPM (59, 60). In Study II, 42% of prepubertal and early pubertal children with obesity were systolic non-dippers, a figure almost as high as that for adolescents with obesity (60). The effect of non-dipping in children and adolescents has been less studied than in adults, and the associations found with CV risk factors inconclusive. In adults, non-dipping is associated with LVH (61), CVD (62) and increased mortality (63), which would make this high prevalence even more worrisome if the non-dipping persisted into adulthood. However, in Study III, tracking only of SBP dipping was evident, and the non-dipping present in childhood was resolved to a large extent, and in addition did not predict end-organ damage in young adulthood. This might indicate that the high prevalence of non-dipping seen in childhood is less important for the future CV health of these individuals. On the other hand, the prevalence of hypertension, which influences the dipping, seems to fluctuate throughout adulthood (217), and may affect dipping and other factors measured more or less depending on at what age it is studied. Despite the absence of prediction of end-organ damage in Study III at the specific ages evaluated, childhood non-dipping may play a role in future CV health if followed up later.

The mechanisms behind dipping are not fully understood, but the duration of obesity might also have an impact here (218). A sympathovagal imbalance is present in children with obesity compared to those without obesity (219), but is especially evident among those with a shorter duration of obesity (218). Among these non-diabetic prepubertal and early pubertal children (Study II), measures of insulin metabolism were not associated with dipping as have been found among adolescents (60) and adults (220), high insulin levels developing later, probably will enhance the imbalance further. Despite the fact that OSA was present in some children, it was not related to dipping or the LVM index in this group. Measures of insulin and glucose metabolism or blood pressure, including dipping, were not associated with the

LVM index, even though the prevalence of non-dipping was high, and some subjects had hypertension. In more recent studies, non-dipping was again not associated with an increased LVM index among children and adolescents with obesity and suspected hypertension (68), or among non-obese but hypertensive children (69). The non-diabetic state and fairly healthy levels of most factors may have contributed to the absence of associations in Study II.

5.4 CLINICAL SIGNIFICANCE OF MEASURING CARDIOVASCULAR RISK FACTORS IN CHILDHOOD

When children with a high degree of obesity are admitted to treatment at our highly specialized clinic, the National Childhood Obesity Centre, an extended clinical investigation is performed. The goal of treatment is weight loss, which is hard to achieve, especially among adolescents (153). Effective treatment requires very intensive behavioural intervention (221), which is difficult to offer to all adolescents with obesity.

In a very recent study in children with obesity, association between high fat mass through adolescence and arterial stiffness was found (222). In studies of general populations, associations between CV risk factors in childhood and measures of end-organ function in adulthood have been found (122, 124-126). Although it is important to study large cohorts, it is unclear whether monitoring different CV risk factors are of value for clinical decision-making and the identification of children and adolescents with obesity with the highest risk of early development of CVD. The well-established CV risk factors measured during treatment might therefore contribute to identifying those at highest risk of developing obesity-related comorbidities in young adulthood, and enable offering them optimal treatment.

In the longitudinal study of young adults who had previously been in childhood obesity treatment (Study III), associations were found between elevated total cholesterol, triglycerides, and daytime SBP in childhood and increased cIMT in young adulthood. The associations with cIMT were adjusted for sex, smoking in young adulthood, and changes in BMI SDS in young adulthood in order to adjust for known differences between the sexes in the studied variables, diminish the known effects of smoking on cIMT, and reduce the known effects of weight change. Adjustments for passive smoking during childhood were not performed, but may have affected the associations found, since passive smoking has also been found to be related to cIMT (104) and endothelial function (103).

The hypothesis that children and adolescents with obesity and present CV risk factors are predisposed to develop early signs of adverse CV end-organ function in young adulthood, and that these childhood risk factors might be used clinically must be revised: most of the childhood risk factors do not seem to predict adverse end-organ function in these young adults as in adults older than the cohort in Study III. Juonala and colleagues (145) showed that CV risk factors measured from the age of 9 were predictive of end-organ function in adulthood. In Study III, all subjects were at minimum 9 years old with a mean age of 14 years, but even so, few associations were found. Obesity during childhood and adolescence may involve mechanisms increasing a physiological plasticity not possible if obesity occurs

in adulthood why the predictive value of childhood CV risk factors in children and adolescents with obesity is inconsistent and reduced. Or, the individuals included in the study may have been healthier than non-participants, and the results might have been different if studying both the present participants and non-participants had been possible.

However, the general populations studied usually were older at adulthood, and in most of the studies childhood CV risk factors were measured before the rise of the obesity epidemic; these differences need to be considered, and may be significant. In the present study, subjects who previously attended childhood obesity treatment were studied in young adulthood.

About 40% of subjects were in early puberty at the time of childhood measurements, a status associated with a worsened metabolic state (for example, insulin resistance); this may have adversely influenced CV risk factors and contributed to the prevalence of CV risk factors observed. On the other hand, obesity itself, independent of pubertal influence, is associated with these risk factors, and they probably would have been present even if all the subjects had been in late puberty. One factor that has been shown in Study I and others (208, 218) to influence results in the obese state is the duration of obesity. This was not considered in this longitudinal study, however, but it would have been interesting to adjust for, especially if the population had been larger, to allow for more confounding factors in the statistical analyses.

Even though 29% of subjects decreased their BMI SDS at a clinically significant level from childhood to young adulthood, the distribution of BMI classes did not change. Severe obesity was present in 74% in young adulthood, despite participation in more than 3 years of childhood obesity treatment. Only 6% of subjects became normal weight. A high prevalence of persistent obesity has also been seen among young Japanese adults who previously participated in childhood obesity treatment (146). However, the follow-up in that study was performed via questionnaire, and analyses of childhood predictors were not performed. Nevertheless, evaluations showed that those with persistent obesity had higher prevalences of chronic diseases. The trend for obesity to persist among so many adolescents into adulthood must be decreased. This may partially be addressed by initiating treatment earlier, which has been shown to be effective, but something must also be done for those who do not receive treatment in time.

The lower prevalence of CV risk factors in adulthood compared to childhood may be in part influenced by the different cut-offs used for adults, since the mean values did not change drastically during the time between measurements. The prevalence of increased cIMT (223-225) and low CRF (154, 226) in young adulthood was high and concerning, since several studies have predicted CVD in adulthood using these risk factors. The level of cIMT in these young adults with a high persistence of severe obesity was about the same as the level in adults 10–15 years older, which is worrying. In addition, MetS, hypertension and low HDL cholesterol levels were present in 30%–40% of the individuals in young adulthood, which adds to their future risk of CVD, as seen among adults (223, 227).

The levels of childhood CV risk factors may be more predictive for future adverse end-organ function if a follow-up of this cohort were to be done in another 10 years; as of now, however, the cohort may still be too young.

5.5 THE EFFECT OF SUPERVISED EXERCISE ON CARDIORESPIRATORY FITNESS AND CARDIOVASCULAR RISK FACTORS

Treatment of adolescents is challenging for childhood obesity clinicians. It has been shown that BMI SDS in adolescents with obesity does not improve to the same extent as in younger children in treatment (153), and therefore the CV risk factors present cannot be improved via weight reduction. Clinicians need other approaches in order to handle the prevalence of CV risk factors among adolescents with obesity so as to reduce the future risk of CVD. Numerous CV risk factors in both children and adolescents with overweight or obesity have been improved by aerobic exercise without weight loss (158-160, 162, 164, 165), and therefore exercise may be one alternative as a complement to obesity treatment in adolescents. However, challenges regarding how to get adolescents with obesity to start exercising and to feel comfortable exercising need to be considered. Therefore, the 3-month period of a supervised individualised aerobic exercise regimen in Study IV was carefully designed according to the following criteria to increase the odds of exercise maintenance after the end of the supervised period:

- In respomce to meet requests from participants in an unpublished pilotstudy and the results from a published study (174), supervision by a personal coach was provided to give the needed guidance and support instead of group sessions.
- The exercise activity was chosen by the coach and adolescent together in order to reach the heart rate goal, but at the same time find an activity that might be joyful in order to increase interest and improve the attitude towards exercise (172, 173).
- The activity was performed close to the adolescent's home or school in order to be time-efficient (173).
- The intensity was adjusted to the adolescent's capacity at baseline, and was slowly increased so that the adolescent would feel more comfortable with the exercise (176).

This exercise regimen was not enough to implement physical activity habits that would improve long-term CRF. However, short-term CRF improved compared to a non-exercise group during the supervised period, but without changes or differences in anthropometric measurements throughout the study. CRF can be used as an indicator of compliance with the exercise regimen, and the impairment in CRF seen during the 9 months following the supervised period therefore indicate poor compliance during this time period. This indicates that a personal coach is needed to ensure the performance of exercise on a regular basis, and is needed for a longer time period than that provided in this study.

Even though short-term CRF improved, no effects on health-related quality of life were seen; this might be because the adolescents rated their health-related quality of life higher at baseline compared to others who improved their health-related quality of life after aerobic exercise (173), or the exercise period was simply too short to improve quality of life.

In addition, measured objectively, total physical activity did not change after the supervised exercise, which is in line with other studies (228), and in line with the theory that total

physical activity level is hard to change in children (166). If the adolescents performed exercise 3 times a week for 3 months and did not reduce their physical activity during the remainder of the day, total physical activity would have increased. However, when physical activity was objectively measured for 7 consecutive days after the supervised period, exercise might accidentally have been performed outside the 7 days of measurement, or have been performed on a day that was later excluded because of too little total time registered, thereby contributing to the absence of increased total physical activity.

No effects were seen on CV risk factors after the 3 months of supervised aerobic exercise, despite improvements in CRF without weight loss. However, arterial stiffness, measured as AIx@HR75, improved after the 9 months following the period of supervised exercise. Others have found an association between CRF and arterial stiffness (99, 100), but in healthy children and adolescents. In a study of children with overweight or obesity, an exercise regimen including resistance training with a duration of exercise that was almost double that in Study IV resulted in an improvement in endothelial cell function measured as the level of endothelial progenitor cells in the blood (229).

One reason for the absent effect on CV risk factors may be that the subjects were too healthy, though others found no effect from exercise on FMD in those already having normal levels (155). Or, it may be that the supervised exercise intervention period was too short to have an effect on the CV risk factors measured.

Although factors of known importance for participation and compliance were included, this was not sufficient for long-term exercise habits to be implemented among adolescents with obesity.

5.5.1 What is needed to induce long-term performance of exercise in adolescents with obesity?

In months 3 to 6, the adolescents were contacted by telephone to provide support after the supervised period. However, this support does not seem to have been enough to induce them to continue the exercise.

Perhaps an exercise app could be used in smartphones as a complement to supervised exercise in obesity treatment. The app should have an exercise schedule/diary to be filled in after the period of exercise, with some kind of feedback afterwards and reminders when exercise has not been performed. However, smart phones and the use of apps were not as available at the time Study IV was begun as they are today.

My experiences in Study IV are that several of the adolescents seemed to have lost hope for the future and of being able to make changes; instead, some seemed to "buy into the situation" and shut themselves in. The stigma around obesity probably also contributed to the fact that these individuals did not dare go to public places to perform exercise. Even though some adolescents reported that they performed the supervised exercise in Study IV because it was fun, others reported participating so as not to disappoint the coach and leave him or her

waiting unnecessarily for them to arrive. To perform exercise because of it is fun is an intrinsic motivation, however, to perform exercise so as not to disappoint the coach is an extrinsic motivation, and of disadvantage for maintaining long-term exercise.

In a previous study, adolescents with obesity reported more extrinsic motivations (e.g. losing weight and looking better) than intrinsic motivations (e.g. pleasure and satisfaction) for being physically active compared to normal-weight adolescents (172). Intrinsic factors are known to be more effective in motivating long-term participation (230), and may therefore be something to focus more upon in future interventions aimed at inducing regular exercise in subjects with obesity. However, expectations for the goal that are too high to be achieved when performing exercise have also been shown to result in failure (231). Adolescents in Study IV did not reduce their weight but only improved their CRF, which may have contributed to a reduction in motivation for continuing the exercise after the supervised period, even though the goal of the study was not to reduce their weight. Setting clear, realistic and reachable goals seems important for success.

5.6 METHODOLOGICAL CONSIDERATIONS NOT PREVIOUSLY DISCUSSED

In this section I will discuss methodological considerations that have not previously been noted.

5.6.1 BMI and BMI SDS

BMI used for the evaluation of body composition is highly dependent on height and cannot distinguish between muscle mass, subcutaneous and abdominal, or visceral adiposity, but is very easy to use and inexpensive. DXA is a more reliable and precise method, but is more expensive and/or complicated to perform in a clinical or research setting; therefore, BMI is still frequently used to evaluate body composition.

In order to be able to compare BMI between ages or genders, or to follow BMI over time (especially in childhood), BMI SDS is calculated. There are different references used, and the degree of obesity will differ depending on the reference population used. In this thesis, it is important to remember that three different references were used. Roland-Cachera et al. (17) and Karlberg et al. (18) are traditionally used in clinical settings, and were thus used in the first two studies. To be able to make comparisons with other studies, the IOTF reference (16), more commonly used internationally, was used in Studies III and IV.

The cut-off used in Study III for a reduction in BMI SDS was chosen because of studies showing a clinically significant effect on CV risk factors with this reduction (149). However, a greater reduction has a greater effect on CV risk factors (147-149).

5.6.2 Biochemical analyses

In cases in which analytical methods changed during the study period and were needed for comparison, results from one analytic method used at one time point were recalculated into units/levels of another method used at another time point in the study.

For example, the analytical method changed during data collection for insulin in Study II, so results for affected subjects were recalculated into the units/levels of the newer method used by the laboratory.

Note that the analytical method used for HbA1c in Studies I and II was older than that used in Study IV; the units therefore differ between the studies, and are not comparable. Results are presented in percentages in Studies I and II, and when the newer International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method began to be used in Study IV, the results were presented as mmol/mol, and are consequently at higher levels than in Studies I and II.

5.6.3 Pulse wave analysis

Pulse wave analysis (PWA) is a method for determining systemic arterial stiffness as the augmentation index (AIx), derived from the radial artery waveform (194) and by using a general transfer function (195) converted to an estimated aortic pressure waveform in Studies II and IV. The pulse waveforms measured with PWA are quite similar to those measured with catheterisation (194); however, the carotid-femoral pulse wave velocity (PWV) method is considered the gold standard for measuring arterial stiffness, but at the regional site (232). Briefly, PWV measures the speed of the pulse wave as it travels from the carotid artery to the femoral artery by measuring the distance between the two sites and the time the pulse wave takes to travel this distance. The speed is correlated to arterial stiffness. PWA, however, was the primary choice of measurement for arterial stiffness here for the following reasons:

- To increase compliance; PWA does not require the subject to remove clothing (as is the case with the PWV method), which may be perceived as uncomfortable by subjects, especially those with obesity.
- To make it easier to perform measurements: it is more difficult to measure the distance from the carotid artery to the femoral artery in subjects with obesity, since the belly is in the way.
- Because PWA may be more sensitive for subjects < 50 years old, since the AIx increases more in younger people than the PWV, which instead seems more suitable for older subjects.

In addition, PWA is portable, cost-effective, user-independent, and quick and easy to perform.

Consensus about cut-offs for adverse levels of arterial stiffness, measured as AIx or AIx@HR75 by PWA, is not available. In Study III, the mean level at young adulthood was therefore compared with a Danish population of comparable age but with a low risk of CVD (233). A negative value is advantageous, and is often seen among younger subjects.

5.6.4 Metabolic syndrome

Consensus about classification of MetS is still being debated, and several references have been available over the years. In Studies III and IV, the widely used references by Zimmet et al. for children and adolescents (182) and Alberti et al. for adults (183) were used. The greatest difference between these references, besides the cut-offs for some included variables, is that for adults, obesity or an elevated waist circumference is not one of the three required criteria for having MetS.

5.6.5 24-hour ambulatory blood pressure monitoring

In order to record 24-hour blood pressure and be able to calculate dipping, 24-hour ABPM, which is considered superior to in-office blood pressure, was included in all studies. Although 24-hour ABPM is not considered to be highly reproducible if using general sleep-wake hours (51), when the actual sleep-wake hours from the diary are taken into account, the sleep-induced SBP dipping is very reproducible (234). Having subjects in this patient group complete the measurement twice would have been hard to achieve, since most of the subjects felt uncomfortable with performing even one measurement. Therefore, the measurement was only reproduced if the participant felt that he or she had an abnormal day or night during the measurement, such as a bad night's sleep, since a bad night's sleep is associated with a non-representative measurement (235). The ideal, of course, would have been to repeat the measurement to confirm the first measurement.

5.6.6 Submaximal bicycle test

In order to follow CRF over time, the submaximal bicycle test by Åstrand (185) was performed, since this was also the test performed at admission to the National Childhood Obesity Centre, and hence had already been obtained in childhood for those included in Study III. The same test was performed in Studies I and IV. An alternative would have been a maximal oxygen consumption test, which correlates well with the submaximal test (236). However, since this group of children and adolescents with obesity is known to usually have a low degree of exercise experience and hence low CRF (237), the less advanced submaximal bicycle test was a more suitable choice for this group. The submaximal test is built on the assumption that heart rate, work load and oxygen consumption are linearly related. When heart rate increases during the test due to increased work load, oxygen consumption increases. The submaximal test does not require advanced laboratory equipment and the presence of a medical specialist, as does the VO_2 max test. However, it has its limitations, such as the extrapolation of maximal oxygen consumption from the heart rate obtained, or nervousness and emotions affecting the heart rate. The test was performed on two different bicycles (one electronically-braked and the other mechanically-braked) in Study III. In order for tests performed with the different bicycles to be comparable, maximum VO_2 estimates were made using the Siconolfi computation (238) of the Åstrand nomogram from 1960 after transformation of watt to kilopond meter (239), and thereafter, age and sex adjustments were performed (185). In Study III, where the degree of CRF was classified according to Anderson

et al. (188), CRF for children younger than 15 years old was obtained from extrapolated values used in clinical settings in Sweden.

5.6.7 Accelerometry

The accelerometer Actiwatch® (AW, Model 4; Cambridge Neurotechnology Ltd, Cambridge, United Kingdom) was used in Study IV because it is validated for measuring activity during sleep (240); however, sleep measurements were not included here, which is a limitation (190). This accelerometer is easy to wear on the wrist, which was believed would increase compliance among children and adolescents, and in addition register the sort of low-intensity activity which was suspected to be present among the adolescents with obesity, since it has been shown that they participate less in organised sports compared to normal-weight adolescents (237). The 15-second epoch length used is suitable for adolescents (241), and enables the registration of shorter boosts in physical activity.

The wrist-worn accelerometer used in Study III has been validated against the Actigraph hip-worn accelerometer. The cut-offs used for intensities of physical activity were obtained and used by others in Sweden (190) studying children aged 8–10, which is a limitation.

5.6.8 Glucose tolerance tests

Frequently sampled intravenous glucose tolerance tests (FSIVGTT) were performed at enrolment in the National Childhood Obesity Centre and were included in the BORIS register; as such, they were included in Studies I and II. In Study IV, an oral glucose tolerance test (OGTT) was performed. These two tests are not interchangeable, since they measure different aspects of insulin-glucose metabolism (193).

The gold standard for measuring insulin sensitivity is the invasive euglycemic hyperinsulinemic clamp, which measures β cell sensitivity to glucose and the sensitivity of body tissues to insulin. However, this method is very advanced, time consuming, expensive, and can be negatively perceived by the subject; it is therefore rarely used in clinical settings today.

The FSIVGTT is less advanced and expensive than the euglycemic hyperinsulinemic clamp. FSIVGTT is highly reliable and reproducible, and the results for insulin sensitivity using FSIVGTT and the euglycemic hyperinsulinemic clamp are highly correlated, at least among those who do not have extreme insulin resistance. An advantage of the FSIVGTT is its ability to identify and separate distinct components of glucose disposal.

The OGTT is used after fasting glucose sampling to confirm and diagnose T2DM or impaired glucose tolerance, and does not measure insulin sensitivity. It is very easy to perform and much cheaper than the euglycemic hyperinsulinemic clamp and the FSIVGTT, and is therefore often used in both research and clinical settings. OGTT does not require two nurses, as does the FSIVGTT, during the period of time before the glucose is injected. Limitations with this method, however, are that gastric emptying and glucose absorption from the

gastrointestinal tract vary between subjects, which affect reproducibility even with the same subjects. Further, it is a relatively imprecise measure of glucose tolerance, and does not measure specific components as with FSIVGTT.

5.6.9 Echocardiography

Absolute LVM varies with sex and ethnicity, and values need to account for physiological variations related to body size. It is possible to index LVM obtained via echocardiography in different ways, and indexing for body surface area or height with an allometric exponent of 2.7 may be the most common (82). It has been concluded, however, that indexing for body surface area underestimates LVH in subjects with obesity, and that indexing for height with an exponent of 2.7 is more suitable. This indexing also reduces the variability among normal-weight subjects. Indexing for height is thought to take into account growth during childhood and approximate for lean body mass.

It is also possible to measure LVM using cardiac magnetic resonance; however, echocardiography is the method preferred, since it is cheaper, more available and accepted for measuring LVM. The two methods are not interchangeable, and the absolute values obtained differ between methods; however, cardiac magnetic resonance provides a more accurate and precise measure of LVM (82).

The cut-off for an abnormal LVM index can differ depending on the reference; in this thesis, the references by Khoury et al. for children (201) and Lang et al. for adults (197) were used.

5.6.10 Ultrasonography of the carotid arteries

Carotid intima-media thickness is relatively simple to measure non-invasively with ultrasonography. The reference used for abnormal cIMT levels, according to sex and age, was Engelen et al. (202). Unfortunately, cIMT was not included in the extensive investigation at admission to the National Childhood Obesity Centre in Study III, and it is therefore not possible to follow it from childhood to young adulthood. If it had been available, it would have been interesting, since the prevalence of high cIMT turned out to be high in young adulthood. This raises questions about the levels of cIMT in childhood: Were the levels already elevated, and to what extent if so? Was the cIMT already associated with the same risk factors in childhood? Would childhood levels track to young adulthood?

5.6.11 Recruitment and study populations

In Studies I, II and III, the study populations were dependent on the information reported in the BORIS register and related medical records of the measurements focused upon. In Study IV, the population was dependent on the flux of adolescents meeting their medical doctors at the National Childhood Obesity Centre and the involved outpatient clinics, and the doctors' decisions about the adolescents' suitability to participate based on the inclusion and exclusion criteria. However, after the adolescents were identified, randomization to one of the three groups was performed at the visit for baseline examinations. Since the recruitment turned out to be more difficult than expected, it was decided to terminate the study. This was in part

because of the flux of adolescents at the clinics and their clinical situations, contraindicated to the inclusion and exclusion criteria, but also due to a high dropout rate among controls. Therefore, only the exercise regimen was evaluated in Study IV, and the four populations are consequently somewhat selected with regard to the above aspects.

Factors such as the stigmatisation of subjects with obesity probably contributed to the difficulties with recruitment and with performing studies in the patient group with obesity.

The results may have been affected by missing data due to the unwillingness of this group to be examined, especially the adolescents, who can be hard to deal with even when they are of normal weight.

In Study III, a large part of the eligible subjects declined to participate or had undergone bariatric surgery. Perhaps failed childhood obesity treatment contributed to unwillingness to participate and be re-examined. Maybe those who were included in Study III were healthier and more motivated, and therefore more successful than those who declined to participate or had undergone bariatric surgery.

In Study IV, most of the subjects hoped to be randomised to the exercise intervention. However, those randomised to metformin therapy were fairly satisfied, while the controls were disappointed at "getting nothing", which was one primary reason for dropouts in the control group. The control subjects were therefore offered the opportunity to perform supervised exercise for a 1-month period later on, after their participation had been completed.

Dropout rates among adolescents in treatment at the National Childhood Obesity Centre have been found to be as high as 70% after 3 years (242). In addition, treatment effects on BMI SDS have been low, which indicates there are general difficulties in working with adolescents. A high dropout rate is common in intervention studies. As a result, the combination of an intervention study among adolescents with obesity is hard to perform successfully.

Variations in individual childhood obesity treatments may have had an impact on the results.

5.6.12 Statistical implications

The statistical methods used and adjustments for confounding factors were limited by the size of the study populations. Non-parametric tests were used in some cases, primarily because of a limited study population.

In addition, missing data occurs in all four studies in different ways, which may therefore affect the results. However, this is also what clinicians experience when working with a population with obesity, and especially children and adolescents. Resistance to performing different physical examinations or to having blood samples taken, or even to showing up at an appointment are daily obstacles the clinicians must face.

In Study II, adjustments for pubertal status were made; this was not possible, however, in the other studies, and pubertal status may therefore have influenced the results.

5.7 CLINICAL IMPLICATIONS

It is important for clinicians working with childhood obesity to be aware of the comorbidities present, know which of them are important to treat, and know how to treat them. In Studies I and II, the obesity comorbidities microvascular endothelial dysfunction and non-dipping were present in children and adolescents with obesity attending childhood obesity treatment.

In Study III, several CV risk factors were present in childhood, but many prevalences also decreased in young adulthood; this may not always have been due to improved values, but rather causes such as cut-offs that differed from those for abnormal levels in childhood. The subjects were in a pubertal stage that is related to poorer insulin sensitivity, which is linked to the presence of several other risk factors; this may have contributed to the improvements seen in young adults, although this was to some extent a natural development.

Since the CV risk factors present among children and adolescents with obesity resolve to a high extent in young adulthood, one may wonder about the significance of testing methods to improve these risk factors in children and adolescents. However, since obesity in childhood and adolescence persist into young adulthood and thereafter at a high level, and CV risk factors in adults with obesity are highly present and predict CVD and mortality, it still feels meaningful to try to improve CV risk factors present in childhood and adolescence if weight loss is not effective. If weight loss is not effective, these subjects are almost doomed to have obesity in adulthood, and therefore will be at high risk for CVD. And if some improvements during childhood and adolescence make a difference in future CV health, they are worth trying.

The exercise regimen tested in Study IV turned out not to be very effective in improving CV risk factors as hypothesised, and cannot be recommended for implementation in clinical practice as a complement to obesity treatment in adolescents. However, clinicians should continue to encourage exercise, since it has shown positive effects in larger studies, although implementation of the particular regimen covered in this study might not be economically justified at clinics. Possible ways to minimise CV risk factors of importance for CV health in young adulthood might instead be pharmacological treatment of the high childhood levels of blood lipids, and maybe also blood pressure, which was found to be predictive of cIMT in Study III.

5.8 FUTURE RESEARCH

If possible, of course, weight loss would be the focus in childhood obesity treatment, since it is effective in reducing common CV risk factors. However, where weight loss is hard to achieve, as in adolescents, the effects and sustainability of lifestyle changes—such as increased physical activity/exercise, perhaps in combination with diet changes—should be studied. Emphasizing intrinsic motivations for change and having realistic and achievable expectations are important factors for lifestyle changes, and hence would be a focus as a

complement to the struggles with losing weight for children and, especially, adolescents. However, until it is possible to perform larger studies of how lifestyle changes implemented in childhood affect CV risk factors and CV health in adulthood among those who have participated in childhood obesity treatment, clinicians have to keep on struggling with what CV risk factors should be treated during childhood to decrease future CV risk in this group.

6 CONCLUSIONS

CV risk factors were already present in childhood in this group of severely obese patients. Disturbed endothelial function (measured as microvascular acetylcholine-induced endothelium-dependent dilatation) was present at an early age, and was affected by the duration of obesity in children with obesity without comorbidities compared to normal-weight controls.

Blunted nocturnal blood pressure dipping was twice as prevalent in prepubertal and early pubertal children with severe obesity compared to results from previous studies on children in general. Dyslipidemia and low CRF were highly prevalent, whereas a high LVM index and MetS were present among 20%–24% of the children. Hypertension was uncommon in childhood.

Endothelial function and nocturnal blood pressure dipping in children with severe obesity were not associated with insulin-glucose metabolism, as has been observed by others among adolescents with obesity.

Few childhood CV risk factors were predictive of adverse end-organ function in young adulthood; it does seem important, however, to monitor blood pressure, triglycerides and total cholesterol.

Clinicians should pay attention to the treatment of high childhood triglycerides, levels of total cholesterol and systolic daytime blood pressure, since these were found to be predictive of cIMT in young adults who had been in childhood obesity treatment. CRF can be improved via 3 months of supervised aerobic exercise. However, CRF decreased to baseline levels during the 9 months following the supervised exercise, and only small effects on CV risk factors were seen. As such, this exercise regimen cannot be recommended for implementation in clinical settings.

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8 REFERENCES

1. WHO. Obesity and overweight. [cited 2019 11th of april]; Fact sheet]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

2. Han JC, Lawlor DA, Kimm SY. Childhood obesity. Lancet. 2010;375(9727):1737-48. Epub 2010/05/11.

3. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. Int J Obes (Lond). 2011;35(7):891-8. Epub 2010/10/27.

4. WHO. Noncommunicable diseases. World Health Organization, WHO; 2018 [updated June 1st 2018; cited 2019 June 13th]; Available from: https://www.who.int/en/news-room/fact-sheets/detail/noncommunicable-diseases.

5. Socialstyrelsen. Statistik om dödsorsaker 2017. www.socialstyrelsen.se: 2018 Art.nr 2018-10-17.

6. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. Jama. 2003;289(2):187-93. Epub 2003/01/09.

7. Daniels SR. Cardiovascular disease risk factors and atherosclerosis in children and adolescents. Current atherosclerosis reports. 2001;3(6):479-85. Epub 2001/10/17.

8. McGill HC, Jr., McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of atherosclerosis in childhood and adolescence. The American journal of clinical nutrition. 2000;72(5 Suppl):1307S-15S. Epub 2000/11/04.

9. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. The New England journal of medicine. 1998;338(23):1650-6. Epub 1998/06/06.

10. Dhingra R, Vasan RS. Age as a risk factor. The Medical clinics of North America. 2012;96(1):87-91. Epub 2012/03/07.

11. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41. Epub 2000/09/23.

12. Herouvi D, Karanasios E, Karayianni C, Karavanaki K. Cardiovascular disease in childhood: the role of obesity. European journal of pediatrics. 2013;172(6):721-32. Epub 2013/01/24.

13. Kim DD, Basu A. Estimating the Medical Care Costs of Obesity in the United States: Systematic Review, Meta-Analysis, and Empirical Analysis. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2016;19(5):602-13. Epub 2016/08/28.

14. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization technical report series. 2000;894:i-xii, 1-253. Epub 2001/03/10.

15. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320(7244):1240-3. Epub 2000/05/08.

16. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity. 2012;7(4):284-94. Epub 2012/06/21.

17. Rolland-Cachera MF, Sempe M, Guilloud-Bataille M, Patois E, Pequignot-Guggenbuhl F, Fautrad V. Adiposity indices in children. The American journal of clinical nutrition. 1982;36(1):178-84. Epub 1982/07/01.

18. Karlberg J, Luo ZC, Albertsson-Wikland K. Body mass index reference values (mean and SD) for Swedish children. Acta Paediatr. 2001;90(12):1427-34. Epub 2002/02/21.

19. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627-42. Epub 2017/10/17.

20. Lissner L, Sohlstrom A, Sundblom E, Sjoberg A. Trends in overweight and obesity in Swedish schoolchildren 1999-2005: has the epidemic reached a plateau? Obesity reviews : an official journal of the International Association for the Study of Obesity. 2010;11(8):553-9. Epub 2009/12/23.

21. Moraeus L, Lissner L, Sjoberg A. Stable prevalence of obesity in Swedish schoolchildren from 2008 to 2013 but widening socio-economic gap in girls. Acta Paediatr. 2014;103(12):1277-84. Epub 2014/08/29.

22. Warensjö Lemming E ML, Petrelius Sipinen J, Londroos AK. Riksmaten ungdom 2016-2017. Livsmedelskonsumtion bland ungdomar i Sverige. Resultat från en matvaneundersökning bland ungdomar i årskurserna 5, 8 och 2 på gymnasiet. Rapportserie. www.livsmedelsverket.se/publicerat-material/: 2018 No. 14 Contract No.: ISSN 1104-7089.

23. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2016;17(2):95-107. Epub 2015/12/24.

24. Burt BA. Definitions of risk. Journal of dental education. 2001;65(10):1007-8. Epub 2001/11/09.

25. Balagopal PB, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, et al. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: a scientific statement from the American Heart Association. Circulation. 2011;123(23):2749-69. Epub 2011/05/11.

26. Twig G, Yaniv G, Levine H, Leiba A, Goldberger N, Derazne E, et al. Body-Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. The New England journal of medicine. 2016;374(25):2430-40. Epub 2016/04/14.

27. van Emmerik NM, Renders CM, van de Veer M, van Buuren S, van der Baan-Slootweg OH, Kist-van Holthe JE, et al. High cardiovascular risk in severely obese young children and adolescents. Archives of disease in childhood. 2012;97(9):818-21. Epub 2012/07/25.

28. Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet. 2001;358(9291):1400-4. Epub 2001/11/14.

29. Ayer J, Charakida M, Deanfield JE, Celermajer DS. Lifetime risk: childhood obesity and cardiovascular risk. European heart journal. 2015;36(22):1371-6. Epub 2015/03/27.

30. McCrindle BW. Cardiovascular consequences of childhood obesity. The Canadian journal of cardiology. 2015;31(2):124-30. Epub 2015/02/11.

31. Daniels SR. Complications of obesity in children and adolescents. Int J Obes (Lond). 2009;33 Suppl 1:S60-5. Epub 2009/04/14.

32. Li S, Chen W, Srinivasan SR, Xu J, Berenson GS. Relation of childhood obesity/cardiometabolic phenotypes to adult cardiometabolic profile: the Bogalusa Heart Study. American journal of epidemiology. 2012;176 Suppl 7:S142-9. Epub 2012/10/17.

33. Davison KK, Susman EJ, Birch LL. Percent body fat at age 5 predicts earlier pubertal development among girls at age 9. Pediatrics. 2003;111(4 Pt 1):815-21. Epub 2003/04/03.

34. Li W, Liu Q, Deng X, Chen Y, Liu S, Story M. Association between Obesity and Puberty Timing: A Systematic Review and Meta-Analysis. International journal of environmental research and public health. 2017;14(10). Epub 2017/10/25.

35. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty. World review of nutrition and dietetics. 2013;106:135-41. Epub 2013/02/23.

36. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. The Journal of clinical endocrinology and metabolism. 2015;100(1):301-8. Epub 2014/09/23.

37. Tanner JM. Growth at adolescence. 2nd ed. Oxford, United Kingdom: Blackwell; 1962.

38. Reinehr T, Toschke AM. Onset of puberty and cardiovascular risk factors in untreated obese children and adolescents: a 1-year follow-up study. Archives of pediatrics & adolescent medicine. 2009;163(8):709-15. Epub 2009/08/05.

39. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics. 2011;128 Suppl 5:S213-56. Epub 2011/11/16.

40. Juonala M, Viikari JS, Kahonen M, Solakivi T, Helenius H, Jula A, et al. Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood: the cardiovascular risk in young Finns study. Journal of the American College of Cardiology. 2008;52(4):293-9. Epub 2008/07/19.

41. Goran MI, Bergman RN, Gower BA. Influence of total vs. visceral fat on insulin action and secretion in African American and white children. Obesity research. 2001;9(8):423-31. Epub 2001/08/14.

42. Weiss R, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. Diabetes care. 2008;31 Suppl 2:S310-6. Epub 2008/02/15.

43. Ferreira SR, Cesarini PR, Vivolo MA, Zanella MT. Abnormal nocturnal blood pressure fall in normotensive adolescents with insulin-dependent diabetes is ameliorated following glycemic improvement. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1998;31(4):523-8. Epub 1998/08/12.

44. Bacha F, Saad R, Gungor N, Arslanian SA. Are obesity-related metabolic risk factors modulated by the degree of insulin resistance in adolescents? Diabetes care. 2006;29(7):1599-604. Epub 2006/06/28.

45. Giannini C, Caprio S. Islet function in obese adolescents. Diabetes, obesity & metabolism. 2012;14 Suppl 3:40-5. Epub 2012/09/07.

46. Reinehr T, Kiess W, de Sousa G, Stoffel-Wagner B, Wunsch R. Intima media thickness in childhood obesity: relations to inflammatory marker, glucose metabolism, and blood pressure. Metabolism: clinical and experimental. 2006;55(1):113-8. Epub 2005/12/06.

47. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England journal of medicine. 2005;352(16):1685-95. Epub 2005/04/22.

48. Kapiotis S, Holzer G, Schaller G, Haumer M, Widhalm H, Weghuber D, et al. A proinflammatory state is detectable in obese children and is accompanied by functional and morphological vascular changes. Arteriosclerosis, thrombosis, and vascular biology. 2006;26(11):2541-6. Epub 2006/09/16.

49. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arteriosclerosis, thrombosis, and vascular biology. 2002;22(8):1323-8. Epub 2002/08/13.

50. Wuhl E, Witte K, Soergel M, Mehls O, Schaefer F. Distribution of 24-h ambulatory blood pressure in children: normalized reference values and role of body dimensions. Journal of hypertension. 2002;20(10):1995-2007. Epub 2002/10/03.

51. Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. Journal of hypertension. 2016;34(10):1887-920. Epub 2016/07/29.

52. Muntner P, He J, Cutler JA, Wildman RP, Whelton PK. Trends in blood pressure among children and adolescents. Jama. 2004;291(17):2107-13. Epub 2004/05/06.

53. Kelly RK, Magnussen CG, Sabin MA, Cheung M, Juonala M. Development of hypertension in overweight adolescents: a review. Adolescent health, medicine and therapeutics. 2015;6:171-87. Epub 2015/11/07.

54. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. The New England journal of medicine. 2015;373(14):1307-17. Epub 2015/10/01.

55. Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization, 2011.

56. Yano Y, Kario K. Nocturnal blood pressure and cardiovascular disease: a review of recent advances. Hypertension research : official journal of the Japanese Society of Hypertension. 2012;35(7):695-701. Epub 2012/03/02.

57. Pieruzzi F, Antolini L, Salerno FR, Giussani M, Brambilla P, Galbiati S, et al. The role of blood pressure, body weight and fat distribution on left ventricular mass, diastolic function and cardiac geometry in children. Journal of hypertension. 2015;33(6):1182-92. Epub 2015/02/26.

58. Soergel M, Kirschstein M, Busch C, Danne T, Gellermann J, Holl R, et al. Oscillometric twenty-four-hour ambulatory blood pressure values in healthy children and adolescents: a multicenter trial including 1141 subjects. The Journal of pediatrics. 1997;130(2):178-84. Epub 1997/02/01.

59. Framme J, Dangardt F, Marild S, Osika W, Wahrborg P, Friberg P. 24-h Systolic blood pressure and heart rate recordings in lean and obese adolescents. Clinical physiology and functional imaging. 2006;26(4):235-9. Epub 2006/07/14.

60. Westerstahl M, Marcus C. Association between nocturnal blood pressure dipping and insulin metabolism in obese adolescents. Int J Obes (Lond). 2010;34(3):472-7. Epub 2009/09/16.

61. Mancia G, Parati G. The role of blood pressure variability in end-organ damage. Journal of hypertension Supplement : official journal of the International Society of Hypertension. 2003;21(6):S17-23. Epub 2003/09/30.

62. Hermida RC, Ayala DE, Mojon A, Fernandez JR. Blunted sleep-time relative blood pressure decline increases cardiovascular risk independent of blood pressure level--the "normotensive non-dipper" paradox. Chronobiology international. 2013;30(1-2):87-98. Epub 2012/10/09.

63. Hansen TW, Li Y, Boggia J, Thijs L, Richart T, Staessen JA. Predictive role of the nighttime blood pressure. Hypertension. 2011;57(1):3-10. Epub 2010/11/17.

64. Cuspidi C, Sala C, Tadic M, Gherbesi E, Grassi G, Mancia G. Nondipping pattern and carotid atherosclerosis: a systematic review and meta-analysis. Journal of hypertension. 2016;34(3):385-91; discussion 91-2. Epub 2016/01/29.

65. Marcovecchio ML, Patricelli L, Zito M, Capanna R, Ciampani M, Chiarelli F, et al. Ambulatory blood pressure monitoring in obese children: role of insulin resistance. Journal of hypertension. 2006;24(12):2431-6. Epub 2006/11/04.

66. Karavanaki K, Kazianis G, Konstantopoulos I, Tsouvalas E, Karayianni C. Early signs of left ventricular dysfunction in adolescents with type 1 diabetes mellitus: the importance of impaired circadian modulation of blood pressure and heart rate. Journal of endocrinological investigation. 2008;31(4):289-96. Epub 2008/05/14.

67. Lee SH, Kim JH, Kang MJ, Lee YA, Won Yang S, Shin CH. Implications of nocturnal hypertension in children and adolescents with type 1 diabetes. Diabetes care. 2011;34(10):2180-5. Epub 2011/09/14.

68. Macumber IR, Weiss NS, Halbach SM, Hanevold CD, Flynn JT. The Association of Pediatric Obesity With Nocturnal Non-Dipping on 24-Hour Ambulatory Blood Pressure Monitoring. American journal of hypertension. 2016;29(5):647-52. Epub 2015/08/28.

69. Seeman T, Hradsky O, Gilik J. Nocturnal blood pressure non-dipping is not associated with increased left ventricular mass index in hypertensive children without end-stage renal failure. European journal of pediatrics. 2016;175(8):1091-7. Epub 2016/06/28.

70. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285-95. Epub 2007/03/14.

71. Houben AJ, Eringa EC, Jonk AM, Serne EH, Smulders YM, Stehouwer CD. Perivascular Fat and the Microcirculation: Relevance to Insulin Resistance, Diabetes, and Cardiovascular Disease. Current cardiovascular risk reports. 2012;6(1):80-90. Epub 2012/01/17.

72. Montero D, Walther G, Perez-Martin A, Roche E, Vinet A. Endothelial dysfunction, inflammation, and oxidative stress in obese children and adolescents: markers and effect of lifestyle intervention. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2012;13(5):441-55. Epub 2011/12/03.

73. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. European heart journal. 2010;31(15):1865-71. Epub 2010/03/04.

74. Urbina EM. Abnormalities of vascular structure and function in pediatric hypertension. Pediatr Nephrol. 2016;31(7):1061-70. Epub 2015/08/16.

75. Dangardt F, Chen Y, Berggren K, Osika W, Friberg P. Increased rate of arterial stiffening with obesity in adolescents: a five-year follow-up study. PloS one. 2013;8(2):e57454. Epub 2013/03/02.

76. Cote AT, Phillips AA, Harris KC, Sandor GG, Panagiotopoulos C, Devlin AM. Obesity and arterial stiffness in children: systematic review and meta-analysis. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(4):1038-44. Epub 2015/01/31.

77. Urbina EM, Kimball TR, Khoury PR, Daniels SR, Dolan LM. Increased arterial stiffness is found in adolescents with obesity or obesity-related type 2 diabetes mellitus. Journal of hypertension. 2010;28(8):1692-8. Epub 2010/07/22.

78. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2008;21(2):93-111; quiz 89-90. Epub 2008/02/12.

79. Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. Pediatrics. 2006;117(5):1560-7. Epub 2006/05/03.

80. Park MH, Skow A, De Matteis S, Kessel AS, Saxena S, Viner RM, et al. Adiposity and carotid-intima media thickness in children and adolescents: a systematic review. BMC pediatrics. 2015;15:161. Epub 2015/10/18.

81. Manco M, Nobili V, Alisi A, Panera N, Handberg A. Arterial Stiffness, Thickness and Association to Suitable Novel Markers of Risk at the Origin of Cardiovascular Disease in Obese Children. International journal of medical sciences. 2017;14(8):711-20. Epub 2017/08/22.

82. Armstrong AC, Gidding S, Gjesdal O, Wu C, Bluemke DA, Lima JA. LV mass assessed by echocardiography and CMR, cardiovascular outcomes, and medical practice. JACC Cardiovascular imaging. 2012;5(8):837-48. Epub 2012/08/18.

83. Friberg P, Allansdotter-Johnsson A, Ambring A, Ahl R, Arheden H, Framme J, et al. Increased left ventricular mass in obese adolescents. European heart journal. 2004;25(11):987-92. Epub 2004/06/03.

84. Di Bonito P, Capaldo B, Forziato C, Sanguigno E, Di Fraia T, Scilla C, et al. Central adiposity and left ventricular mass in obese children. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2008;18(9):613-7. Epub 2007/12/18.

85. Kharod AM, Ramlogan SR, Kumar S, Raghuveer T, Drake W, Dai H, et al. Childhood obesity increases left-ventricular mass irrespective of blood pressure status. Pediatric cardiology. 2014;35(2):353-60. Epub 2013/08/31.

86. Brady TM. The Role of Obesity in the Development of Left Ventricular Hypertrophy Among Children and Adolescents. Current hypertension reports. 2016;18(1):3. Epub 2015/12/25.

87. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. The New England journal of medicine. 2004;350(23):2362-74. Epub 2004/06/04.

88. Chinali M, de Simone G, Roman MJ, Best LG, Lee ET, Russell M, et al. Cardiac markers of pre-clinical disease in adolescents with the metabolic syndrome: the strong heart study. Journal of the American College of Cardiology. 2008;52(11):932-8. Epub 2008/09/06.

89. Blechner M, Williamson AA. Consequences of Obstructive Sleep Apnea in Children. Current problems in pediatric and adolescent health care. 2016;46(1):19-26. Epub 2015/12/04.

90. Partinen M, Jamieson A, Guilleminault C. Long-term outcome for obstructive sleep apnea syndrome patients. Mortality. Chest. 1988;94(6):1200-4. Epub 1988/12/01.

91. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126-31. Epub 1985/03/01.

92. Bassett DR, Jr., Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Medicine and science in sports and exercise. 2000;32(1):70-84. Epub 2000/01/27.

93. Ribeiro JC, Guerra S, Oliveira J, Andersen LB, Duarte JA, Mota J. Body fatness and clustering of cardiovascular disease risk factors in Portuguese children and adolescents. American journal of human biology : the official journal of the Human Biology Council. 2004;16(5):556-62. Epub 2004/09/16.

94. Myers J, McAuley P, Lavie CJ, Despres JP, Arena R, Kokkinos P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status. Progress in cardiovascular diseases. 2015;57(4):306-14. Epub 2014/10/01.

95. Thijssen DH, Cable NT, Green DJ. Impact of exercise training on arterial wall thickness in humans. Clin Sci (Lond). 2012;122(7):311-22. Epub 2011/12/14.

96. Eisenmann JC, Welk GJ, Wickel EE, Blair SN. Combined influence of cardiorespiratory fitness and body mass index on cardiovascular disease risk factors among 8-18 year old youth: The Aerobics Center Longitudinal Study. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2007;2(2):66-72. Epub 2007/09/01.

97. Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologia. 2007;50(9):1832-40. Epub 2007/07/21.

98. Hurtig-Wennlof A, Ruiz JR, Harro M, Sjostrom M. Cardiorespiratory fitness relates more strongly than physical activity to cardiovascular disease risk factors in healthy children and adolescents: the European Youth Heart Study. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2007;14(4):575-81. Epub 2007/08/02.

99. Meyer J, Elmenhorst J, Giegerich T, Oberhoffer R, Muller J. Controversies in the association of cardiorespiratory fitness and arterial stiffness in children and adolescents. Hypertension research : official journal of the Japanese Society of Hypertension. 2017;40(7):675-8. Epub 2017/02/17.

100. Ried-Larsen M, Grontved A, Froberg K, Ekelund U, Andersen LB. Physical activity intensity and subclinical atherosclerosis in Danish adolescents: the European Youth Heart

Study. Scandinavian journal of medicine & science in sports. 2013;23(3):e168-77. Epub 2013/01/23.

101. Nassis GP, Psarra G, Sidossis LS. Central and total adiposity are lower in overweight and obese children with high cardiorespiratory fitness. European journal of clinical nutrition. 2005;59(1):137-41. Epub 2004/09/30.

102. Eriksen MP, LeMaistre CA, Newell GR. Health hazards of passive smoking. Annual review of public health. 1988;9:47-70. Epub 1988/01/01.

103. Juonala M, Magnussen CG, Venn A, Gall S, Kahonen M, Laitinen T, et al. Parental smoking in childhood and brachial artery flow-mediated dilatation in young adults: the Cardiovascular Risk in Young Finns study and the Childhood Determinants of Adult Health study. Arteriosclerosis, thrombosis, and vascular biology. 2012;32(4):1024-31. Epub 2012/02/22.

104. Gall S, Huynh QL, Magnussen CG, Juonala M, Viikari JS, Kahonen M, et al. Exposure to parental smoking in childhood or adolescence is associated with increased carotid intima-media thickness in young adults: evidence from the Cardiovascular Risk in Young Finns study and the Childhood Determinants of Adult Health Study. European heart journal. 2014;35(36):2484-91. Epub 2014/03/07.

105. Li Z, Snieder H, Harshfield GA, Treiber FA, Wang X. A 15-year longitudinal study on ambulatory blood pressure tracking from childhood to early adulthood. Hypertension research : official journal of the Japanese Society of Hypertension. 2009;32(5):404-10. Epub 2009/03/28.

106. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation. 2008;117(25):3171-80. Epub 2008/06/19.

107. Juhola J, Magnussen CG, Viikari JS, Kahonen M, Hutri-Kahonen N, Jula A, et al. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. The Journal of pediatrics. 2011;159(4):584-90. Epub 2011/04/26.

108. Eisenmann JC, Welk GJ, Wickel EE, Blair SN. Stability of variables associated with the metabolic syndrome from adolescence to adulthood: the Aerobics Center Longitudinal Study. American journal of human biology : the official journal of the Human Biology Council. 2004;16(6):690-6. Epub 2004/10/21.

109. Malina RM. Physical activity and fitness: pathways from childhood to adulthood. American journal of human biology : the official journal of the Human Biology Council. 2001;13(2):162-72. Epub 2001/07/20.

110. Kelly AS, Steinberger J, Jacobs DR, Hong CP, Moran A, Sinaiko AR. Predicting cardiovascular risk in young adulthood from the metabolic syndrome, its component risk factors, and a cluster score in childhood. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2011;6(2-2):e283-9. Epub 2010/11/13.

111. Lloyd LJ, Langley-Evans SC, McMullen S. Childhood obesity and adult cardiovascular disease risk: a systematic review. Int J Obes (Lond). 2010;34(1):18-28. Epub 2009/05/13.

112. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. Circulation. 2001;104(23):2815-9. Epub 2001/12/06.

113. Berenson GS. Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. The American journal of cardiology. 2002;90(10C):3L-7L. Epub 2002/12/03.

114. Li S, Chen W, Srinivasan SR, Bond MG, Tang R, Urbina EM, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. Jama. 2003;290(17):2271-6. Epub 2003/11/06.

115. Li S, Chen W, Srinivasan SR, Berenson GS. Childhood blood pressure as a predictor of arterial stiffness in young adults: the bogalusa heart study. Hypertension. 2004;43(3):541-6. Epub 2004/01/28.

116. Toprak A, Wang H, Chen W, Paul T, Srinivasan S, Berenson G. Relation of childhood risk factors to left ventricular hypertrophy (eccentric or concentric) in relatively young adulthood (from the Bogalusa Heart Study). The American journal of cardiology. 2008;101(11):1621-5. Epub 2008/05/21.

117. Hartiala O, Magnussen CG, Kajander S, Knuuti J, Ukkonen H, Saraste A, et al. Adolescence risk factors are predictive of coronary artery calcification at middle age: the cardiovascular risk in young Finns study. Journal of the American College of Cardiology. 2012;60(15):1364-70. Epub 2012/09/18.

118. Juonala M, Jarvisalo MJ, Maki-Torkko N, Kahonen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. Circulation. 2005;112(10):1486-93. Epub 2005/09/01.

119. Juonala M, Viikari JS, Ronnemaa T, Helenius H, Taittonen L, Raitakari OT. Elevated blood pressure in adolescent boys predicts endothelial dysfunction: the cardiovascular risk in young Finns study. Hypertension. 2006;48(3):424-30. Epub 2006/08/02.

120. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Jama. 2003;290(17):2277-83. Epub 2003/11/06.

121. Raitakari OT, Juonala M, Viikari JS. Obesity in childhood and vascular changes in adulthood: insights into the Cardiovascular Risk in Young Finns Study. Int J Obes (Lond). 2005;29 Suppl 2:S101-4. Epub 2005/12/31.

122. Magnussen CG, Venn A, Thomson R, Juonala M, Srinivasan SR, Viikari JS, et al. The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in Young Finns study, the Bogalusa Heart study, and the CDAH (Childhood Determinants of Adult Health) study. Journal of the American College of Cardiology. 2009;53(10):860-9. Epub 2009/03/07.

123. Ried-Larsen M, Grontved A, Kristensen PL, Froberg K, Andersen LB. Moderate-and-vigorous physical activity from adolescence to adulthood and subclinical atherosclerosis in adulthood: prospective observations from the European Youth Heart Study. British journal of sports medicine. 2015;49(2):107-12. Epub 2013/04/16.

124. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. The New England journal of medicine. 2011;365(20):1876-85. Epub 2011/11/18.

125. Magnussen CG, Koskinen J, Chen W, Thomson R, Schmidt MD, Srinivasan SR, et al. Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone: the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. Circulation. 2010;122(16):1604-11. Epub 2010/10/06.

126. Magnussen CG, Smith KJ. Pediatric Blood Pressure and Adult Preclinical Markers of Cardiovascular Disease. Clinical medicine insights Blood disorders. 2016;9:1-8. Epub 2016/05/12.

127. Ferreira I, Twisk JW, Stehouwer CD, van Mechelen W, Kemper HC. Longitudinal changes in .VO2max: associations with carotid IMT and arterial stiffness. Medicine and science in sports and exercise. 2003;35(10):1670-8. Epub 2003/10/03.

128. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. Pediatrics. 2007;120(2):340-5. Epub 2007/08/03.

129. Ruiz JR, Castro-Pinero J, Artero EG, Ortega FB, Sjostrom M, Suni J, et al. Predictive validity of health-related fitness in youth: a systematic review. British journal of sports medicine. 2009;43(12):909-23. Epub 2009/01/23.

130. Baker JL, Olsen LW, Sorensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. The New England journal of medicine. 2007;357(23):2329-37. Epub 2007/12/07.

131. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. The New England journal of medicine. 2010;362(6):485-93. Epub 2010/02/12.

132. Magnussen CG, Smith KJ, Juonala M. What the Long Term Cohort Studies that Began in Childhood Have Taught Us about the Origins of Coronary Heart Disease. Current cardiovascular risk reports. 2014;8(373). Epub 2014/01/30.

133. Janssen I, Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C, et al. Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. Obesity research. 2005;13(6):1106-15. Epub 2005/06/25.

134. Freedman DS, Dietz WH, Tang R, Mensah GA, Bond MG, Urbina EM, et al. The relation of obesity throughout life to carotid intima-media thickness in adulthood: the Bogalusa Heart Study. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2004;28(1):159-66. Epub 2003/10/29.

135. Li X, Li S, Ulusoy E, Chen W, Srinivasan SR, Berenson GS. Childhood adiposity as a predictor of cardiac mass in adulthood: the Bogalusa Heart Study. Circulation. 2004;110(22):3488-92. Epub 2004/11/24.

136. Tapp RJ, Venn A, Huynh QL, Raitakari OT, Ukoumunne OC, Dwyer T, et al. Impact of adiposity on cardiac structure in adult life: the Childhood Determinants of Adult Health (CDAH) study. BMC cardiovascular disorders. 2014;14:79. Epub 2014/07/02.

137. Juhola J, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Combined effects of child and adult elevated blood pressure on subclinical atherosclerosis: the International Childhood Cardiovascular Cohort Consortium. Circulation. 2013;128(3):217-24. Epub 2013/06/20.

138. Cicek Y, Durakoglugil ME, Kocaman SA, Cetin M, Erdogan T, Dogan S, et al. Non-dipping pattern in untreated hypertensive patients is related to increased pulse wave velocity independent of raised nocturnal blood pressure. Blood pressure. 2013;22(1):34-8. Epub 2012/07/13.

139. Juonala M, Viikari JS, Ronnemaa T, Marniemi J, Jula A, Loo BM, et al. Associations of dyslipidemias from childhood to adulthood with carotid intima-media thickness, elasticity, and brachial flow-mediated dilatation in adulthood: the Cardiovascular Risk in Young Finns Study. Arteriosclerosis, thrombosis, and vascular biology. 2008;28(5):1012-7. Epub 2008/03/01.

140. Eisenmann JC, Wickel EE, Welk GJ, Blair SN. Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: the Aerobics Center Longitudinal Study (ACLS). American heart journal. 2005;149(1):46-53. Epub 2005/01/22.

141. Kvaavik E, Klepp KI, Tell GS, Meyer HE, Batty GD. Physical fitness and physical activity at age 13 years as predictors of cardiovascular disease risk factors at ages 15, 25, 33, and 40 years: extended follow-up of the Oslo Youth Study. Pediatrics. 2009;123(1):e80-6. Epub 2009/01/02.

142. Chen W, Srinivasan SR, Li S, Xu J, Berenson GS. Metabolic syndrome variables at low levels in childhood are beneficially associated with adulthood cardiovascular risk: the Bogalusa Heart Study. Diabetes care. 2005;28(1):126-31. Epub 2004/12/24.

143. Park MH, Falconer C, Viner RM, Kinra S. The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2012;13(11):985-1000. Epub 2012/06/27.

144. Bjerregaard LG, Jensen BW, Angquist L, Osler M, Sorensen TIA, Baker JL. Change in Overweight from Childhood to Early Adulthood and Risk of Type 2 Diabetes. The New England journal of medicine. 2018;378(14):1302-12. Epub 2018/04/05.

145. Juonala M, Magnussen CG, Venn A, Dwyer T, Burns TL, Davis PH, et al. Influence of age on associations between childhood risk factors and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study, the Childhood Determinants of Adult Health Study, the Bogalusa Heart Study, and the Muscatine Study for the International Childhood Cardiovascular Cohort (i3C) Consortium. Circulation. 2010;122(24):2514-20. Epub 2010/12/04.

146. Togashi K, Masuda H, Rankinen T, Tanaka S, Bouchard C, Kamiya H. A 12-year follow-up study of treated obese children in Japan. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2002;26(6):770-7. Epub 2002/05/31.

147. Reinehr T, Andler W. Changes in the atherogenic risk factor profile according to degree of weight loss. Archives of disease in childhood. 2004;89(5):419-22. Epub 2004/04/23.

148. Reinehr T, Kiess W, Kapellen T, Andler W. Insulin sensitivity among obese children and adolescents, according to degree of weight loss. Pediatrics. 2004;114(6):1569-73. Epub 2004/12/03.

149. Ford AL, Hunt LP, Cooper A, Shield JP. What reduction in BMI SDS is required in obese adolescents to improve body composition and cardiometabolic health? Archives of disease in childhood. 2010;95(4):256-61. Epub 2009/12/08.

150. Hvidt KN. Blood pressure and arterial stiffness in obese children and adolescents. Danish medical journal. 2015;62(3). Epub 2015/03/10.

151. Wunsch R, de Sousa G, Toschke AM, Reinehr T. Intima-media thickness in obese children before and after weight loss. Pediatrics. 2006;118(6):2334-40. Epub 2006/12/05.

152. Frye SS, Fernandez-Mendoza J, Calhoun SL, Gaines J, Vgontzas AN, Liao D, et al. Childhood obesity, weight loss and developmental trajectories predict the persistence and remission of childhood sleep-disordered breathing. Pediatric obesity. 2019;14(1). Epub 2018/09/27.

153. Danielsson P, Kowalski J, Ekblom O, Marcus C. Response of severely obese children and adolescents to behavioral treatment. Archives of pediatrics & adolescent medicine. 2012;166(12):1103-8. Epub 2012/10/31.

154. McAuley PA, Beavers KM. Contribution of cardiorespiratory fitness to the obesity paradox. Progress in cardiovascular diseases. 2014;56(4):434-40. Epub 2014/01/21.

155. Hopkins ND, Stratton G, Tinken TM, McWhannell N, Ridgers ND, Graves LE, et al. Relationships between measures of fitness, physical activity, body composition and vascular function in children. Atherosclerosis. 2009;204(1):244-9. Epub 2008/10/22.

156. Stoner L, Rowlands D, Morrison A, Credeur D, Hamlin M, Gaffney K, et al. Efficacy of Exercise Intervention for Weight Loss in Overweight and Obese Adolescents: Meta-Analysis and Implications. Sports Med. 2016;46(11):1737-51. Epub 2016/05/04.

157. Vasconcellos F, Seabra A, Katzmarzyk PT, Kraemer-Aguiar LG, Bouskela E, Farinatti P. Physical activity in overweight and obese adolescents: systematic review of the effects on physical fitness components and cardiovascular risk factors. Sports Med. 2014;44(8):1139-52. Epub 2014/04/20.

158. Kelly AS, Wetzsteon RJ, Kaiser DR, Steinberger J, Bank AJ, Dengel DR. Inflammation, insulin, and endothelial function in overweight children and adolescents: the role of exercise. The Journal of pediatrics. 2004;145(6):731-6. Epub 2004/12/08.

159. Watts K, Beye P, Siafarikas A, O'Driscoll G, Jones TW, Davis EA, et al. Effects of exercise training on vascular function in obese children. The Journal of pediatrics. 2004;144(5):620-5. Epub 2004/05/06.

160. Mendelson M, Michallet AS, Monneret D, Perrin C, Esteve F, Lombard PR, et al. Impact of exercise training without caloric restriction on inflammation, insulin resistance and visceral fat mass in obese adolescents. Pediatric obesity. 2015;10(4):311-9. Epub 2014/08/05.

161. Ryder JR, Vega-Lopez S, Ortega R, Konopken Y, Shaibi GQ. Lifestyle intervention improves lipoprotein particle size and distribution without weight loss in obese Latino adolescents. Pediatric obesity. 2013;8(5):e59-63. Epub 2013/04/12.

162. Naylor LH, Davis EA, Kalic RJ, Paramalingam N, Abraham MB, Jones TW, et al. Exercise training improves vascular function in adolescents with type 2 diabetes. Physiological reports. 2016;4(4). Epub 2016/02/19.

163. Zhang H, Jiang L, Yang YJ, Ge RK, Zhou M, Hu H, et al. Aerobic exercise improves endothelial function and serum adropin levels in obese adolescents independent of body weight loss. Scientific reports. 2017;7(1):17717. Epub 2017/12/20.

164. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M, et al. Aerobic exercise training improves insulin sensitivity without changes

in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. Metabolism: clinical and experimental. 2005;54(11):1472-9. Epub 2005/10/29.

165. Brambilla P, Pozzobon G, Pietrobelli A. Physical activity as the main therapeutic tool for metabolic syndrome in childhood. Int J Obes (Lond). 2011;35(1):16-28. Epub 2010/12/09.

166. Wilkin TJ. Can we modulate physical activity in children? No. Int J Obesity. 2011;35(10):1270-6.

167. Gomersall SR, Rowlands AV, English C, Maher C, Olds TS. The ActivityStat hypothesis: the concept, the evidence and the methodologies. Sports Med. 2013;43(2):135-49. Epub 2013/01/19.

168. Hagstromer M, Elmberg K, Marild S, Sjostrom M. Participation in organized weekly physical exercise in obese adolescents reduced daily physical activity. Acta Paediatr. 2009;98(2):352-4. Epub 2008/11/14.

169. Ross R, de Lannoy L, Stotz PJ. Separate Effects of Intensity and Amount of Exercise on Interindividual Cardiorespiratory Fitness Response. Mayo Clinic proceedings. 2015;90(11):1506-14. Epub 2015/10/13.

170. Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. Jama. 2012;307(7):704-12. Epub 2012/02/18.

171. Hay J, Maximova K, Durksen A, Carson V, Rinaldi RL, Torrance B, et al. Physical activity intensity and cardiometabolic risk in youth. Archives of pediatrics & adolescent medicine. 2012;166(11):1022-9. Epub 2012/09/12.

172. Deforche BI, De Bourdeaudhuij IM, Tanghe AP. Attitude toward physical activity in normal-weight, overweight and obese adolescents. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2006;38(5):560-8. Epub 2006/04/26.

173. Goldfield GS, Kenny GP, Alberga AS, Tulloch HE, Doucette S, Cameron JD, et al. Effects of aerobic or resistance training or both on health-related quality of life in youth with obesity: the HEARTY Trial. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2017;42(4):361-70. Epub 2017/02/09.

174. Corder K, Atkin AJ, Ekelund U, van Sluijs EM. What do adolescents want in order to become more active? BMC public health. 2013;13:718. Epub 2013/08/07.

175. Cheng LA, Mendonca G, Farias Junior JC. Physical activity in adolescents: analysis of the social influence of parents and friends. Jornal de pediatria. 2014;90(1):35-41. Epub 2013/10/26.

176. Ekkekakis P, Lind E. Exercise does not feel the same when you are overweight: the impact of self-selected and imposed intensity on affect and exertion. Int J Obes (Lond). 2006;30(4):652-60. Epub 2005/09/01.

177. Martin H, Hu J, Gennser G, Norman M. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation. 2000;102(22):2739-44. Epub 2000/11/30.

178. Bonamy AK, Bendito A, Martin H, Andolf E, Sedin G, Norman M. Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. Pediatric research. 2005;58(5):845-9. Epub 2005/09/27.

179. Diagnosis and classification of diabetes mellitus. Diabetes care. 2011;34 Suppl 1:S62-9. Epub 2011/01/14.

180. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9. Epub 1985/07/01.

181. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama. 2001;285(19):2486-97. Epub 2001/05/23.

182. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatric diabetes. 2007;8(5):299-306. Epub 2007/09/14.

183. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5. Epub 2009/10/07.

184. Parati G, Stergiou G, O'Brien E, Asmar R, Beilin L, Bilo G, et al. European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring. Journal of hypertension. 2014;32(7):1359-66. Epub 2014/06/03.

185. Astrand I. Aerobic work capacity in men and women with special reference to age. Acta physiologica Scandinavica Supplementum. 1960;49(169):1-92. Epub 1960/01/01.

186. Borg G. Perceived exertion as an indicator of somatic stress. Scandinavian journal of rehabilitation medicine. 1970;2(2):92-8. Epub 1970/01/01.

187. Morinder G, Larsson UE, Norgren S, Marcus C. Insulin sensitivity, VO2max and body composition in severely obese Swedish children and adolescents. Acta Paediatr. 2009;98(1):132-8. Epub 2008/09/24.

188. Andersson G, Forsberg, A., Malmgren, S. Nya konditionstest på cykel: testledarutbildning. Tabeller. Stockholm: SISU Idrottsböcker; 2014.

189. Ekelund U, Sardinha LB, Anderssen SA, Harro M, Franks PW, Brage S, et al. Associations between objectively assessed physical activity and indicators of body fatness in 9- to 10-y-old European children: a population-based study from 4 distinct regions in Europe (the European Youth Heart Study). The American journal of clinical nutrition. 2004;80(3):584-90. Epub 2004/08/24.

190. Ekblom O, Nyberg G, Bak EE, Ekelund U, Marcus C. Validity and comparability of a wrist-worn accelerometer in children. Journal of physical activity & health. 2012;9(3):389-93. Epub 2012/03/29.

191. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes. 1989;38(12):1512-27. Epub 1989/12/01.

192. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes technology & therapeutics. 2003;5(6):1003-15. Epub 2004/01/08.

193. Trout KK, Homko C, Tkacs NC. Methods of measuring insulin sensitivity. Biological research for nursing. 2007;8(4):305-18. Epub 2007/04/26.

194. O'Rourke MF, Gallagher DE. Pulse wave analysis. Journal of hypertension Supplement : official journal of the International Society of Hypertension. 1996;14(5):S147-57. Epub 1996/12/01.

195. Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation. 1997;95(7):1827-36. Epub 1997/04/01.

196. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. The Journal of physiology. 2000;525 Pt 1:263-70. Epub 2000/05/16.

197. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification. European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology. 2006;7(2):79-108. Epub 2006/02/07.

198. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2010;23(5):465-95; quiz 576-7. Epub 2010/05/11.

199. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. The American journal of cardiology. 1986;57(6):450-8. Epub 1986/02/15.

200. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH. Effect of growth on variability of left ventricular mass: assessment of allometric signals in adults and children and their capacity to predict cardiovascular risk. Journal of the American College of Cardiology. 1995;25(5):1056-62. Epub 1995/04/01.

201. Khoury PR, Mitsnefes M, Daniels SR, Kimball TR. Age-specific reference intervals for indexed left ventricular mass in children. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2009;22(6):709-14. Epub 2009/05/09.

202. Engelen L, Ferreira I, Stehouwer CD, Boutouyrie P, Laurent S. Reference intervals for common carotid intima-media thickness measured with echotracking: relation with risk factors. European heart journal. 2013;34(30):2368-80. Epub 2012/11/29.

203. Varni JW, Burwinkle TM, Seid M, Skarr D. The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. Ambulatory pediatrics : the official journal of the Ambulatory Pediatric Association. 2003;3(6):329-41. Epub 2003/11/18.

204. Hansell J, Henareh L, Agewall S, Norman M. Non-invasive assessment of endothelial function - relation between vasodilatory responses in skin microcirculation and brachial artery. Clinical physiology and functional imaging. 2004;24(6):317-22. Epub 2004/11/04.

205. Henricson J, Tesselaar E, Persson K, Nilsson G, Sjoberg F. Assessment of microvascular function by study of the dose-response effects of iontophoretically applied

drugs (acetylcholine and sodium nitroprusside)--methods and comparison with in vitro studies. Microvascular research. 2007;73(2):143-9. Epub 2006/12/13.

206. Khan F, Green FC, Forsyth JS, Greene SA, Morris AD, Belch JJ. Impaired microvascular function in normal children: effects of adiposity and poor glucose handling. The Journal of physiology. 2003;551(Pt 2):705-11. Epub 2003/06/26.

207. Monostori P, Barath A, Fazekas I, Hodi E, Mate A, Farkas I, et al. Microvascular reactivity in lean, overweight, and obese hypertensive adolescents. European journal of pediatrics. 2010;169(11):1369-74. Epub 2010/06/17.

208. Chin LC, Huang TY, Yu CL, Wu CH, Hsu CC, Yu HS. Increased cutaneous blood flow but impaired post-ischemic response of nutritional flow in obese children. Atherosclerosis. 1999;146(1):179-85. Epub 1999/09/16.

209. Bhattacharjee R, Alotaibi WH, Kheirandish-Gozal L, Capdevila OS, Gozal D. Endothelial dysfunction in obese non-hypertensive children without evidence of sleep disordered breathing. BMC pediatrics. 2010;10:8. Epub 2010/02/17.

210. Schlager O, Willfort-Ehringer A, Hammer A, Steiner S, Fritsch M, Giurgea A, et al. Microvascular function is impaired in children with morbid obesity. Vasc Med. 2011;16(2):97-102. Epub 2011/03/12.

211. Tryggestad JB, Thompson DM, Copeland KC, Short KR. Obese children have higher arterial elasticity without a difference in endothelial function: the role of body composition. Obesity (Silver Spring). 2012;20(1):165-71. Epub 2011/10/15.

212. Bhattacharjee R, Kim J, Alotaibi WH, Kheirandish-Gozal L, Capdevila OS, Gozal D. Endothelial dysfunction in children without hypertension: potential contributions of obesity and obstructive sleep apnea. Chest. 2012;141(3):682-91. Epub 2011/10/28.

213. Tobler K, Freudenthaler A, Baumgartner-Parzer SM, Wolzt M, Ludvik B, Nansalmaa E, et al. Reduction of both number and proliferative activity of human endothelial progenitor cells in obesity. Int J Obes (Lond). 2010;34(4):687-700. Epub 2010/01/13.

214. Cubbon RM, Mercer BN, Sengupta A, Kearney MT. Importance of insulin resistance to vascular repair and regeneration. Free radical biology & medicine. 2013;60:246-63. Epub 2013/03/08.

215. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. The New England journal of medicine. 2003;348(7):593-600. Epub 2003/02/14.

216. Jie KE, Goossens MH, van Oostrom O, Lilien MR, Verhaar MC. Circulating endothelial progenitor cell levels are higher during childhood than in adult life. Atherosclerosis. 2009;202(2):345-7. Epub 2008/06/24.

217. Saladini F, Palatini P. Isolated Systolic Hypertension in Young Individuals: Pathophysiological Mechanisms, Prognostic Significance, and Clinical Implications. High blood pressure & cardiovascular prevention : the official journal of the Italian Society of Hypertension. 2017;24(2):133-9. Epub 2017/04/05.

218. Rabbia F, Silke B, Conterno A, Grosso T, De Vito B, Rabbone I, et al. Assessment of cardiac autonomic modulation during adolescent obesity. Obesity research. 2003;11(4):541-8. Epub 2003/04/12.

219. Dangardt F, Volkmann R, Chen Y, Osika W, Marild S, Friberg P. Reduced cardiac vagal activity in obese children and adolescents. Clinical physiology and functional imaging. 2011;31(2):108-13. Epub 2010/11/23.

220. Petrova M, Townsend R, Teff KL. Prolonged (48-hour) modest hyperinsulinemia decreases nocturnal heart rate variability and attenuates the nocturnal decrease in blood pressure in lean, normotensive humans. The Journal of clinical endocrinology and metabolism. 2006;91(3):851-9. Epub 2006/01/06.

221. O'Connor EA, Evans CV, Burda BU, Walsh ES, Eder M, Lozano P. Screening for Obesity and Intervention for Weight Management in Children and Adolescents: Evidence Report and Systematic Review for the US Preventive Services Task Force. Jama. 2017;317(23):2427-44. Epub 2017/06/21.

222. Dangardt F, Charakida M, Georgiopoulos G, Chiesa ST, Rapala A, Wade KH, et al. Association between fat mass through adolescence and arterial stiffness: a population-based study from The Avon Longitudinal Study of Parents and Children. The Lancet Child & adolescent health. 2019;3(7):474-81. Epub 2019/05/28.

223. Niiranen TJ, Vasan RS. Epidemiology of cardiovascular disease: recent novel outlooks on risk factors and clinical approaches. Expert review of cardiovascular therapy. 2016;14(7):855-69. Epub 2016/04/09.

224. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. American journal of epidemiology. 1997;146(6):483-94. Epub 1997/09/18.

225. Carpenter M, Sinclair H, Kunadian V. Carotid Intima Media Thickness and Its Utility as a Predictor of Cardiovascular Disease: A Review of Evidence. Cardiology in review. 2016;24(2):70-5. Epub 2016/01/31.

226. Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. Jama. 1989;262(17):2395-401. Epub 1989/11/03.

227. Wannamethee SG, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Archives of internal medicine. 2005;165(22):2644-50. Epub 2005/12/14.

228. Ruotsalainen H, Kyngas H, Tammelin T, Kaariainen M. Systematic review of physical activity and exercise interventions on body mass indices, subsequent physical activity and psychological symptoms in overweight and obese adolescents. Journal of advanced nursing. 2015;71(11):2461-77. Epub 2015/06/03.

229. Park JH, Miyashita M, Kwon YC, Park HT, Kim EH, Park JK, et al. A 12-week after-school physical activity programme improves endothelial cell function in overweight and obese children: a randomised controlled study. BMC pediatrics. 2012;12:111. Epub 2012/08/02.

230. Ryan RM, Deci EL. Intrinsic and Extrinsic Motivations: Classic Definitions and New Directions. Contemporary educational psychology. 2000;25(1):54-67. Epub 2000/01/06.

231. Trottier K, Polivy J, Herman CP. Effects of resolving to change one's own behavior: expectations vs. experience. Behavior therapy. 2009;40(2):164-70. Epub 2009/05/13.

232. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice

using carotid-femoral pulse wave velocity. Journal of hypertension. 2012;30(3):445-8. Epub 2012/01/27.

233. Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. Aortic augmentation index: reference values in a large unselected population by means of the SphygmoCor device. American journal of hypertension. 2010;23(2):180-5. Epub 2009/12/05.

234. Ben-Dov IZ, Ben-Arieh L, Mekler J, Bursztyn M. Blood pressure dipping is reproducible in clinical practice. Blood pressure monitoring. 2005;10(2):79-84. Epub 2005/04/07.

235. Manning G, Rushton L, Donnelly R, Millar-Craig MW. Variability of diurnal changes in ambulatory blood pressure and nocturnal dipping status in untreated hypertensive and normotensive subjects. American journal of hypertension. 2000;13(9):1035-8. Epub 2000/09/12.

236. Ekblom B, Engstrom LM, Ekblom O. Secular trends of physical fitness in Swedish adults. Scandinavian journal of medicine & science in sports. 2007;17(3):267-73. Epub 2007/05/16.

237. Berndtsson G, Mattsson E, Marcus C, Larsson UE. Age and gender differences in VO2max in Swedish obese children and adolescents. Acta Paediatr. 2007;96(4):567-71. Epub 2007/03/30.

238. Cullinane EM, Siconolfi S, Carleton RA, Thompson PD. Modification of the Astrand-Rhyming sub-maximal bicycle test for estimating VO2max of inactive men and women. Medicine and science in sports and exercise. 1988;20(3):317-8. Epub 1988/06/01.

239. Ekblom-Bak E, Bjorkman F, Hellenius ML, Ekblom B. A new submaximal cycle ergometer test for prediction of VO2max. Scandinavian journal of medicine & science in sports. 2014;24(2):319-26. Epub 2012/11/07.

240. Sadeh A. The role and validity of actigraphy in sleep medicine: an update. Sleep medicine reviews. 2011;15(4):259-67. Epub 2011/01/18.

241. Aibar A, Bois JE, Zaragoza J, Generelo E, Julian JA, Paillard T. Do epoch lengths affect adolescents' compliance with physical activity guidelines? The Journal of sports medicine and physical fitness. 2014;54(3):326-34. Epub 2014/04/18.

242. Danielsson P, Svensson V, Kowalski J, Nyberg G, Ekblom O, Marcus C. Importance of age for 3-year continuous behavioral obesity treatment success and dropout rate. Obesity facts. 2012;5(1):34-44. Epub 2012/03/22.