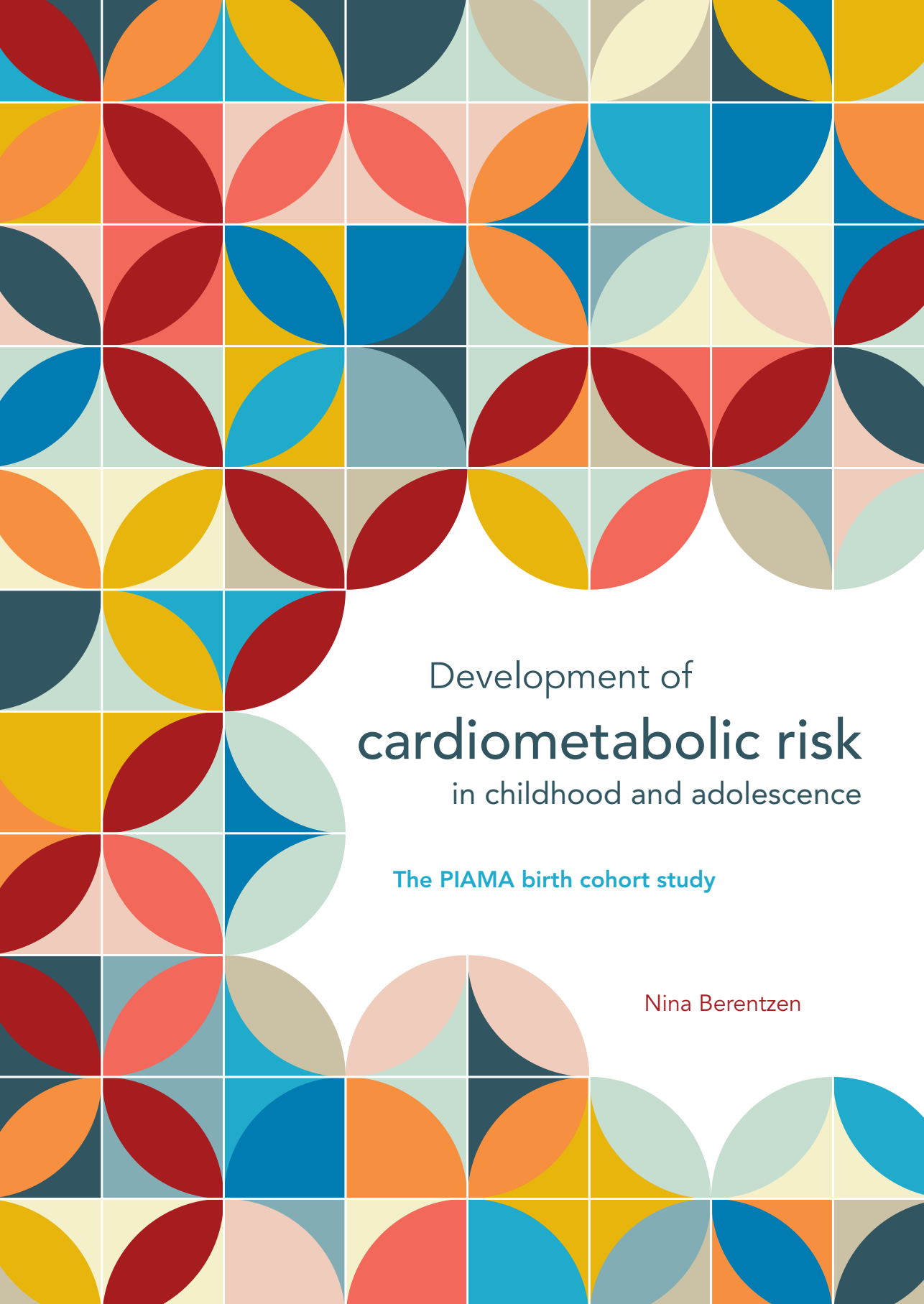

Development of cardiometabolic risk in childhood and adolescence

The PIAMA birth cohort study

Nina Berentzen

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Acknowledgements

The contribution of all children and parents or caregivers participating in the PIAMA study is gratefully acknowledged.

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Development of cardiometabolic risk in childhood and adolescence

The PIAMA birth cohort study

Ontwikkeling van het cardiometabool risico in de kindertijd en adolescentie

De PIAMA geboorte cohort studie
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties

in het openbaar te verdedigen
op dinsdag 19 april 2016 des middags te 12.45 uur

door

Nina Elise Berentzen

geboren op 21 september 1983 te Amsterdam

Promotor

Prof.dr. H.A. Smit

Copromotor

Dr. A.H. Wijga

Contents

Chapter 1

General introduction

The atherosclerotic process leading to cardiovascular disease begins early in life and is influenced over time by several risk factors. Investigating determinants that contribute to an unfavourable cardiometabolic profile in childhood and adolescence is important to improve cardiovascular health of the population.

Background

The atherosclerotic process over the life course

Atheroma (plaque) is a fatty deposit within the inside lining of arteries which, after decades of build-up, can lead to cardiovascular disease. Over time, the inner surface of the arteries becomes irregular and narrower, reducing blood flow through the arteries. Eventually, the plaque may rupture and cause the formation of blood clots. The blood clots may block the coronary or cerebral arteries and cause a heart attack or stroke many decades later[1,2]. The atherosclerotic process starts before symptoms of cardiovascular disease occur, already in the first decade of life. Fatty streaks have been found in the aorta of children as young as 2 years old[3-6] and even in premature foetuses of mothers with hypercholesterolemia during pregnancy[7]. During the second and third decades of life, atherosclerosis progresses into clinical atherosclerotic events[8-10]. From around age 45 years onwards, a steep increase in cardiovascular event rates occurs, with increasing risks for major cardiovascular events such as coronary heart disease, myocardial infarction, heart failure and non-haemorrhagic stroke (see Figure 1 for a schematic representation)[11-13].

Cardiometabolic markers for assessing future cardiovascular disease risk

Although cardiovascular disease events do not generally present before adulthood, the levels of some of the well-known markers of cardiovascular disease such as blood cholesterol, triglycerides, glucose, and blood pressure start increasing in childhood and adolescence[14]. Together, these markers contribute to a person's cardiometabolic profile. A landmark article from the Bogalusa Heart study showed that in young people, the severity of atherosclerosis increases with a higher number of elevated cardiometabolic markers[9]. Adults with cardiovascular disease had developed elevated levels of cardiometabolic markers during decades before the disease manifested[15]. This suggests that elevated cardiometabolic markers in young people indicate progression of atherosclerosis and an increased risk for future cardiovascular disease events.

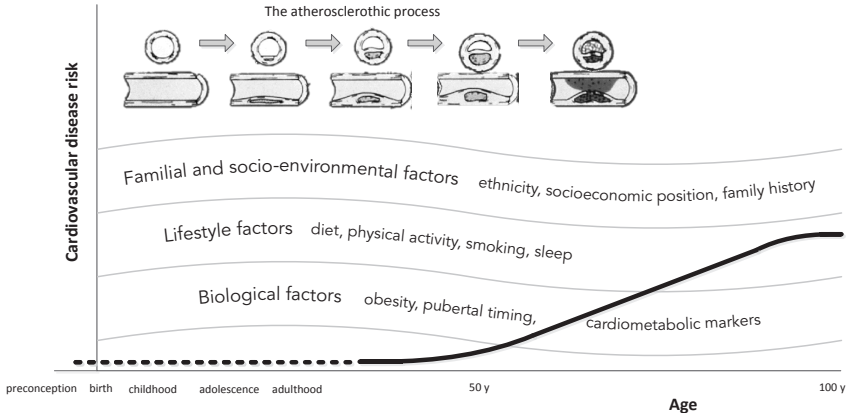

Figure 1. *The progression of atherosclerosis across the life course. This figure shows the progression of atherosclerosis (build-up of plaque in the arteries) with aging, and the concomitant increasing risk for overt cardiovascular disease. The dashed black line indicates subclinical atherosclerotic disease (the first two to three decades of life) whereas the solid black line indicates the period during which clinical events may occur. Several factors influence the risk of cardiovascular disease across the life course. Adapted from[11] and[8] (with permission).*

Tracking of cardiometabolic markers from childhood to adulthood

Levels of cardiometabolic markers during childhood are correlated with adult levels, referred to as 'tracking'. For example, childhood blood pressure, serum lipid levels, and body mass index (BMI) correlate strongly with levels in middle age[16] and with atherosclerosis in adulthood[17]. Tracking from childhood into adulthood has been observed not only for individual cardiometabolic markers but also for combinations of multiple cardiometabolic markers[18,19]. Tracking is important because it implies that children with high levels of cardiometabolic markers are likely to continue with high levels into adulthood and subsequently have a higher risk of cardiovascular disease.

Determinants of the cardiometabolic profile

Familial and socio-environmental factors, lifestyle factors, and biological factors play a key role in the aetiology of atherosclerosis and contribute to cardiovascular disease in adults (see Figure 1)[8-10,20]. It is important to identify and characterize determinants of the cardiometabolic profile in children and adolescents for specifying time windows suitable for prevention or intervention early in the process of cardiovascular disease development[21]. In this thesis, several relevant risk factors for developing cardiovascular disease in adults will be studied in relation to children's cardiometabolic profile.

Aim and outline of this thesis

The overall aim of this thesis is to identify and characterize determinants of an unfavourable cardiometabolic profile in childhood and adolescence. This thesis addresses determinants in each of the three domains mentioned in Figure 1: familial factors, lifestyle factors, and biological factors.

Familial factors

The risk of cardiovascular disease is higher among persons with a family history of cardiovascular disease or type 2 diabetes. Cardiovascular disease and type 2 diabetes often co-occur[22-24] and share risk factors such as hypertension, overweight, unhealthy diet and lack of physical activity. Type 2 diabetes increases the risk for cardiovascular disease by 2 to 3-fold in adults[23,25]. Children with a family history of cardiovascular disease or a family history of type 2 diabetes have elevated cardiometabolic markers such as cholesterol and blood pressure[22,26-31]. Despite the overlap in occurrence of cardiovascular disease and type 2 diabetes and their risk factors, it is unknown how a family history of cardiovascular disease and/or diabetes relates to cardiometabolic markers in children, and to what extent cardiovascular disease and diabetes history are independently associated with cardiometabolic markers. Thus, chapter 2 describes how the disease burden of cardiovascular disease and/or diabetes that runs within families is associated with the child's cardiometabolic profile, and examines disease specific associations with cardiometabolic markers.

Lifestyle factors

Several lifestyle factors are well-known determinants of cardiovascular disease. Physical activity, sedentary behaviour and dietary behaviour have been studied most frequently, and are included in current guidelines for cardiovascular health promotion and risk reduction in youth, for example those issued by the American Heart Association (AHA)[32].

Sleep has recently emerged as a potential determinant of cardiometabolic health in youth. Observational studies have shown that children with shorter sleep duration were more often overweight[33]; had higher waist circumference[34]; insulin resistance[35] and blood pressure[36]. In addition to short duration of sleep, low quality of sleep contributed to cardiovascular disease risk in adults[37]. However, in children, studies on sleep quality are scarce and they reported conflicting results[35,36,38]. Investigating both sleep duration

and sleep quality in children may clarify which sleep characteristics are most important for improving population-wide cardiovascular health. Chapter 3 describes how several aspects of sleep contribute to the child's cardiometabolic profile: sleep duration, sleep-wake pattern and sleep quality (night-time awakenings and daytime outcomes).

According to the US guidelines for cardiovascular health promotion and risk reduction in youth, children should limit their sedentary behaviour to less than 2 hours per day[32]. TV viewing and computer use are the most common measures of sedentary behaviour. Children with more than 2 hours of TV viewing were observed to have higher adiposity and decreased fitness[39]. Decreasing TV time led to reductions in BMI[39]. Until recently, not much attention has been devoted to explanatory factors for the association of sedentary behaviour with adiposity and cardiometabolic risk, although it is likely that this is not a direct causal relationship. Instead, screen time (TV and computer time) may lead to overweight through indirect mechanisms such as more snacking or less physical activity. For example, adolescents with higher levels of sedentary behaviour had a higher consumption of energy-dense snacks, drinks, and fast foods; and higher total energy intake[40]. Sedentary time seemed to occur at the expense of time spent in moderate-to-vigorous physical activity[41]. These results suggest two mechanisms by which screen time may contribute to obesity: increased energy intake and less time for physical activity[42]. However, the role of these potential mediators has not been formally tested. It is important to clarify these potential pathways and identify the targets—time spent sedentary, time spent physically active or snack consumption—that are most promising for prevention. Chapter 4 describes pathways by which screen time may contribute to higher adiposity (via more snacking or less physical activity) and to the cardiometabolic profile.

Biological factors

Many studies have attempted to understand the pathways from infant growth to overweight and to subsequent cardiovascular disease. Adults with coronary heart disease and diabetes more often experienced a specific pattern of weight gain, characterized by relative thinness at birth and subsequent accelerated weight gain during childhood, than adults who do not develop these diseases[43-47]. This specific pattern of growth may result in unfavourable levels of cardiometabolic markers already at a young age. However, previous studies focused on weight gain in infancy or in other specific periods and lacked repeated assessment of weight status with small time-intervals throughout childhood. A better understanding of how childhood overweight patterns contribute to an unfavourable

cardiometabolic profile will help to determine which stages of childhood are crucial for overweight prevention[48,49]. Chapter 5 describes the association between patterns of overweight development from 3 months to 11 years and the child's cardiometabolic profile at age 12 years.

Adolescence is characterized by rapid growth and development, and marks an important transition to an adult cardiometabolic profile[50]. This suggests that puberty may be a sensitive period for later health. Some evidence for this suggestion is based on associations of age at menarche with cardiovascular disease. Women with early menarche (<12 years) have an increased risk in adulthood of overweight and cardiometabolic disorders such as hypertension, hypercholesterolemia, and coronary heart disease[51-54]. This may be due at least partly to excess adiposity preceding puberty, since childhood overweight has been associated with earlier menarche[55,56]. However, adult studies lacked data on body composition preceding menarche, and the role of childhood overweight in the association of pubertal timing with adult cardiometabolic outcomes is still unclear[51]. In addition, in younger adolescents, and especially in males, evidence is lacking on potential impact of early or late pubertal timing on cardiometabolic markers. From an etiological perspective, it is important to clarify adiposity-related and adiposity-independent mechanisms that link pubertal timing to cardiometabolic health. Chapter 6 describes whether boys and girls with early or late pubertal timing have different levels of cardiometabolic markers than those with intermediate pubertal timing.

In summary, this thesis is outlined as follows:

- In Chapter 2, as a familial factor, family history of cardiovascular disease and/or diabetes is investigated in relation to the child's cardiometabolic profile.
- Chapters 3 and 4 address lifestyle factors. Several aspects of sleep (sleep duration, sleep-wake pattern and sleep quality) are investigated in relation to child's cardiometabolic profile; and pathways from screen time as an indicator of sedentary behaviour are investigated in relation to child's adiposity (via snacking and physical activity) and cardiometabolic profile.
- Chapters 5 and 6 address biological factors. Patterns of overweight development from infancy into childhood; and early, intermediate or late pubertal timing are investigated in relation to child's cardiometabolic profile.

The timing of the exposure and outcome variables studied in this thesis is shown in Figure 2.

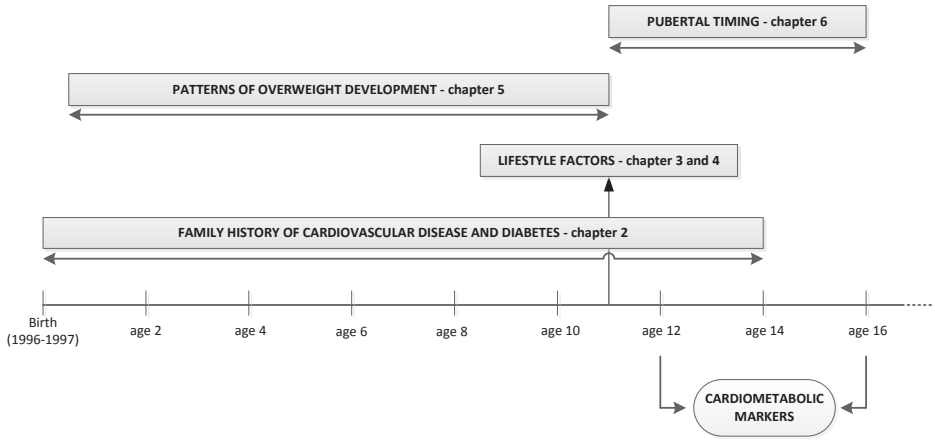

Figure 2. *Timing of the exposure and outcome variables studied in several chapters within this thesis, from birth to adolescence, among children participating in the PIAMA study.*

Study design

The individual studies presented in this thesis are embedded in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, a population based prospective study in the Netherlands that has followed children from before birth until young adulthood and is still ongoing. The PIAMA study was originally set up to identify early environmental and lifestyle determinants of asthma and allergy in childhood. Pregnant women were recruited from 1996 through 1997 in 52 antenatal clinics in three different regions of The Netherlands (see Figure 3): North (provinces Groningen, Friesland, Drenthe); Central (Utrecht, Gelderland); and West (Rotterdam and surrounding municipalities). In total, 3963 children were included in the study. Self-administered questionnaires were completed by parents during pregnancy, at the child's age 3 months and 1 year and then annually up to the child's age of 8 years. At age 11 and 14 years, besides the parents, the children were also invited to complete a questionnaire themselves. Clinical assessments were performed in subsamples of the population at ages 4, 8, 12, and 16 years.

Studies in this thesis have been restricted to participants who attended a clinical assessment at age 12 or 16 years. At age 12 years, all children still in the study (n=3380; 85% of the population at baseline) were invited to the clinical assessment, and 45% of them participated. At age 16 years, due to budget restraints not all children could be invited to the clinical assessment. Those invited were a random subsample of the children

still in the study at that time, excluding the Rotterdam area. Of the children invited to the clinical assessment at age 16 years (n=2159; 54% of the population at baseline), 37% participated. The cardiometabolic profile was characterized by indicators of adiposity, total and HDL cholesterol levels, total/HDL cholesterol ratio, blood pressure and glycated haemoglobin (HbA1c). In this thesis, BMI, waist circumference and waist-to-height ratio were used as indicators of adiposity (fat mass).

Figure 3. *Birthplaces within the Netherlands of participants in the PIAMA birth cohort.*

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Chapter 2

Family history of cardiovascular disease and diabetes and cardiometabolic markers in offspring

Nina E. Berentzen
Alet H. Wijga
Lenie van Rossem
Gerard H. Koppelman
Bo van Nieuwenhuizen
Ulrike Gehring
Annemieke M.W. Spijkerman
Henriëtte A. Smit

Submitted

Abstract

Aims

Despite the overlap in occurrence of cardiovascular disease (CVD) and type 2 diabetes and their risk factors, family history of these diseases has not yet been investigated simultaneously in relation to cardiometabolic markers in offspring. We examined whether family history of CVD and/or diabetes is associated with the cardiometabolic profile in offspring, and to what extent CVD and diabetes history are independently associated with cardiometabolic markers.

Methods

We used the data of 1374 children aged 12 years and their parents who participated in a birth cohort study in the Netherlands. Family history of CVD (myocardial infarction (MI) and stroke) and diabetes were parent-reported. Children were classified either as 'no'; 'moderate'; or 'strong' family history, based on early/late disease onset in parents and grandparents. Cardiometabolic markers were measured at age 12: waist circumference, cholesterol, blood pressure, glycated haemoglobin (HbA1c).

Results

Children with a strong family history of MI, and/or stroke, and/or diabetes (29% of the study population), compared to those with no family history, had 0.14 mmol/l higher total cholesterol (TC) (95% CI 0.04-0.24) and 0.20 higher total/high-density-lipoprotein cholesterol ratio (TC/HDLC ratio) (95% CI 0.07-0.34). Strong family history of MI and diabetes were independently associated with unfavourable cardiometabolic markers specific to those diseases. These associations remained after adjusting for BMI. Children with a moderate family history had no unfavourable cardiometabolic markers.

Conclusions

One third of the children had a strong family history of CVD and/or diabetes. These children had higher levels of TC and TC/HDLC ratio than children with no family history. A strong family history of cardiovascular disease and diabetes were independently associated with unfavourable cardiometabolic markers specific to those diseases. Family history of cardiovascular disease was important for children's cholesterol levels, whereas family history of diabetes was important for children's waist circumference and HbA1c.

Introduction

Family history reflects both genetic susceptibility as well as environmental- and lifestyle characteristics shared within families, e.g. socioeconomic position and health behaviours[1]. The association of cardiovascular disease (CVD) in first- or second degree relatives with the presence of risk factors for atherosclerosis such as high cholesterol and blood pressure (BP) in children has been described[2-4]. Other studies showed that familial aggregation of type 2 diabetes is also associated with the presence of cardiometabolic abnormalities in children[5-8]. CVD and type 2 diabetes often co-occur[3,9,10] and share risk factors such as hypertension, overweight, unhealthy diet and lack of physical activity. However, previous studies assessing cardiometabolic markers in children lacked data on family history of both CVD and diabetes, and it is unclear whether CVD and diabetes history are independently associated with cardiometabolic markers in children. Lifestyle modification may reduce the risk of both CVD and type 2 diabetes[11-14]. Individuals and families with a history of these diseases may be more motivated to change their lifestyle, because they are more aware of their increased risk for future disease[15]. Therefore, clarifying the cardiometabolic risk in children with a family history of CVD and/or diabetes is important for families at increased risk for future disease. Furthermore, when assessing associations of family history with children's cardiometabolic markers, few studies have accounted for the number of affected relatives, or have used a broader definition than early-onset disease to indicate a positive family history. In the current study, we examined differences in cardiometabolic markers among children with no family history, a moderate or a strong family history of CVD (myocardial infarction (MI) and stroke) and/or diabetes, in a contemporary Dutch population.

Methods

We used data from a population-based Dutch birth cohort study: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study with prenatal inclusion of 3,963 children in 1996/1997. A detailed description of the study design has been published previously[16]. At age 11, 3,541 children (89.4%) were still in the study. Of those children, 3,202 were invited for a clinical assessment at age 12, and 1,511 participated (47.2%). Around age 14, parents completed questionnaires including items on family history of

MI, stroke and diabetes. The study population for the current study consisted of 1374 children (704 girls, 670 boys) who had parental reports on family history, and had a clinical assessment at age 12. Children diagnosed with diabetes mellitus were excluded (n=5). The study protocol was approved by the medical ethics committees of the participating institutes. All parents gave written informed consent for participation in the PIAMA birth cohort and separately for the clinical assessment; additionally, children themselves gave written informed consent for the clinical assessment.

Classification of family history

Parents reported history of MI, stroke, and diabetes for the biological parents and grandparents of the child, and age at onset. Response categories for each disease history were 'yes', 'no' and 'unknown'. To define the severity of family history, we classified children into three categories depending on the number of affected relatives, age at onset and degree of kinship to the child. We did this separately for MI, stroke, and diabetes. Children with no affected parents or grandparents were classified as *'no family history'*. Children with 1-2 grandparents with late disease onset were classified as *'moderate family history'*. Children with at least one affected parent, or at least one grandparent with early disease onset, or 3-4 grandparents with late disease onset, were classified as *'strong family history'*. Further subdivision between parents and grandparents led to small numbers in each category and was therefore not feasible. Classification for family history of CVD and/or diabetes followed classification for individual disease. Thus, children classified as *'strong family history'* for at least one of MI, stroke or diabetes, were classified as *'strong family history of CVD and/or diabetes'*; the remaining children were classified as *'moderate'* (when they were classified as such for at least one of MI, stroke or diabetes) or *'no'* family history.

Early onset was defined as <55 y in grandfathers and <65 y in grandmothers for MI and stroke, in agreement with the 2011 integrated guidelines for cardiovascular health and risk reduction in youth by the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel[17]. For diabetes, early onset was before 50 y in grandfathers and grandmothers, a cut-off used in previous studies[18,19].

The questionnaire inquired about history of diabetes, but not type 2 diabetes explicitly. A small proportion (12/693, 1.7%) of all relatives with diabetes developed the disease before age 20; these were likely to have type 1 diabetes. We classified these relatives as not having diabetes, allowing us to interpret the results for children with a moderate or strong family history of diabetes as for type 2 diabetes.

Supplemental table S1 provides insight into the prevalence of MI, stroke, and diabetes for the study population, by disease and by family member. Prevalence of MI, stroke and diabetes in PIAMA parents were comparable to rates in the general (age-matched) Dutch population (see Supplemental information S1). We could not compare disease prevalence in grandparents to the Dutch population because ages of the grandparents were unknown.

The proportion of children with one or more unknown or missing items of family history was 15% (n=208). Missing values mostly concerned presence of disease (n=154) and sometimes the age at disease onset (n=39), or both (n=15). The proportion of diseases reported as unknown or missing, relative to the total number of family members (6 per child) was small; 2.5% for MI, 2.3% for stroke, and 2.6% for diabetes, respectively. We classified relatives with missing age at onset for CVD or diabetes as late-onset, to avoid misclassifying those with late-onset into the highest risk category. If a specific disease in a specific relative was missing or 'unknown', we categorized this relative as not having that disease, since this was most likely considering the young age of the children and their parents. Otherwise, excluding the entire family history for this child would lead to loss of information. In addition, it reflects the situation in general practice: people may not always be aware of diseases running in their (extended) families. We addressed this in a sensitivity analysis (see Supplemental information S2).

Assessment of anthropometry and cardiometabolic markers

Clinical assessments at age 12 were performed by trained staff during home visits according to standardized protocols. Height, weight and waist circumference were measured while children were wearing underwear. Body mass index (BMI, kg/m^2) was used in the analyses as age and sex specific standard deviation scores (z-scores) using the reference growth curves of the Dutch Fourth Nation-wide Growth Study carried out in 1997[20]. Overweight (including obesity) was defined based on international defined cut-off points[21]. Waist circumference was measured twice and the mean of the two measurements was used in analyses.

Systolic and diastolic BP were measured according to the recommendations of the American Heart Association Council on High Blood Pressure Research[22]. BP readings were obtained from the non-dominant upper arm using an Omron M6 monitor while the child was seated. The first measurement was taken after ≥5 minutes of rest, without talking. Depending on arm circumference, 17-22 cm or 22-32 cm cuffs were used. BP was

measured at least twice with 5 minutes intervals. If two consecutive measures differed by >5 mm Hg, another measurement was taken. The means of (2 or 3) systolic and diastolic measurements were used in analyses.

Blood was drawn for measurement of cholesterol and HbA1c. Serum total and high-density lipoprotein (HDL) cholesterol were determined enzymatically using Roche automated clinical chemistry analyzers (Roche Diagnostics, Indianapolis). The ratio between total and HDL cholesterol was calculated (TC/HDLC ratio). For analysis of HbA1c, erythrocytes from blood samples were stored, a 5 µl cell mass was lysated and HbA1c was measured by ion-exchange chromatography using the Adams A1c, HA-8160 HPLC Auto analyser (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial (DCCT) standards.

Other characteristics

Characteristics used to describe the study population were the child's sex, ethnicity, age, pubertal development, exposure to second-hand smoking, and parental age, education and BMI. Ethnicity was based on country of birth of the child's parents, and was categorized as Dutch, Non-Dutch western and nonwestern. Pubertal development (puberty development scale; 1-4)[23] was reported by the child at age 11 y and used as a continuous variable in the analysis. Exposure to second-hand smoking at child's age 11 y was reported by parents, and defined as smoking within the child's home once a week or more. Mother's and father's educational level were categorized as low (primary school, lower vocational or lower secondary education), intermediate (intermediate vocational education or intermediate/higher secondary education) and high (higher vocational education and university). Mother's and father's BMI were calculated from reported weight and height when the child's was 14 years old and categorized as overweight (BMI $\geq$ 25 (kg/m^2)) or normal weight (BMI < 25 kg/m^2)).

Statistical methods

Differences in cardiometabolic markers in children with moderate or strong family history were assessed by multiple linear regression analyses, treating no family history as reference. P-values for trend across categories were estimated by treating the categorical variables as continuous variables in the model. We adjusted for child's sex, age at clinical assessment, ethnicity, height, pubertal development, maternal and paternal education, and maternal and paternal age in the first adjusted model. These confounders were selected based

on prior knowledge and their associations with the outcome of interest. Parents and grandparents who were older at the time of family history assessment were more likely to have developed the disease. We adjusted for this by including maternal and paternal age in the regression models, considering these as a proxy for grandparental age. Analyses with BP were additionally adjusted for cuff size. In the second model, we added maternal and paternal BMI to investigate whether associations could be explained by BMI levels shared within families. In the third model, we further added the child's BMI (measured at age 12). We investigated whether family history of MI, stroke, or diabetes was independently associated with cardiometabolic markers, by mutually adjusting for the other two diseases. Differences by sex have been observed in the association between family history and offspring cardiometabolic markers[24]. We investigated this by including interaction terms of sex with family history categories in the regression models. We conducted two sensitivity analyses to determine the robustness of the associations (see Supplemental information S2). We used SAS version 9.3 for all analyses (SAS Institute, Cary, NC).

Table 1. Categories of family history of myocardial infarction, stroke and diabetes and number of children in each category

	N	%
Myocardial infarction[1]		
No family history	760	55.3
Moderate family history	387	28.2
Strong family history	227	16.5
Stroke[1]		
No family history	968	70.5
Moderate family history	300	21.8
Strong family history	106	7.7
Diabetes[1]		
No family history	844	61.4
Moderate family history	397	28.9
Strong family history	133	9.7
CVD and/or diabetes[2]		
No family history	375	27.3
Moderate family history	598	43.5
Strong family history	401	29.2

[1]Family history of a single disease did not exclude having a family history of another disease.
[2]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.

Results

History of parental MI was present for 1.3% of the children, 0.8% had a history of parental stroke, and 3.5% had a history of parental diabetes. More children had grandfathers than grandmothers with MI or stroke (respectively 43% vs 11% for MI, and 19% vs 14% for stroke), whereas history of diabetes was similar in grandfathers and grandmothers (24% vs 23%) (Supplemental table S1).

Based on family history of individual diseases, 17% of the children had a strong family history of MI, 8% of stroke and 10% of diabetes (Table 1). Considering a combination of family history of MI, and/or stroke, and/or diabetes, 29% of the children (n=401) had a strong family history. Of these children, 84% had a strong family history of one disease only; 43% (n=173) of MI only; 17% (n=69) of stroke only; and 24% (n=96) of diabetes only (not tabulated)). Children with a strong family history of CVD and/or diabetes were more often overweight, of non-western ethnicity, and had parents with a lower education and with a higher BMI, compared to children with no family history (Table 2). There were no large differences in age, sex, and puberty development.

Overall, children with a moderate family history of CVD and/or diabetes had no unfavourable cardiometabolic markers whereas children with a strong family history did, when compared to children with no family history (Table 3). P-values for trend from moderate to strong family history were significant only for the associations hereafter (Table 3). Children with a strong family history of MI, compared to those with no family history, had 0.12 mmol/l higher TC (95% CI 0.01; 0.23), 0.05 mmol/l lower HDL (95% CI -0.10; -0.003), and 0.24 higher TC/HDLC ratio (95% CI 0.09; 0.39) after adjustment for confounders (Table 4). Children with a strong family history of diabetes had 2.25 cm larger waist circumference (95% CI 1.08; 3.41), 0.20 mmol/l higher TC (95% CI 0.07; 0.34), 0.22 higher TC/HDLC ratio (95% CI 0.03; 0.40), and 0.57 mmol/mol higher HbA1c (95% CI 0.06; 1.09). A strong family history of stroke was not associated with cardiometabolic markers. Children with a strong family history of CVD and/or diabetes, compared to those with no family history had 0.13 mmol/l higher TC (95% CI 0.03; 0.23) and 0.18 higher TC/HDLC ratio (95% CI 0.04; 0.32). For most of the cardiometabolic markers, adjustment for BMI of the parents, and subsequent adjustment for BMI of the child hardly changed the effect estimates in children with a strong family history (Table 4). Effect estimates and interpretation of the results did not change after adjusting for waist circumference of the child instead of BMI (data not shown). The associations remained when mutually adjusting

Table 2. Characteristics of the study population within categories of family history of CVD[1] and/or diabetes

	No family history (n=375)		Moderate family history (n=598)		Strong family history (n=401)	
	N	Mean (std) or %	N	Mean (std) or %	N	Mean (std) or %
Child's characteristics						
Sex (boy)	375	49.1	598	49.0	401	48.1
Dutch	371	93.3	590	92.2	395	91.4
Non-western ethnicity	371	4.3	590	3.1	395	5.6
Age at clinical assessment (years)	375	12.7 (0.4)	598	12.7 (0.4)	401	12.7 (0.4)
Puberty development scale at age 11	371	1.5 (0.5)	585	1.5 (0.5)	394	1.6 (0.5)
Parental characteristics						
Mother's education						
Low	375	14.4	597	16.6	400	19.0
High	375	47.5	597	44.7	400	33.8
Father's education						
Low	372	15.6	596	17.8	395	27.3
High	372	55.4	596	48.0	395	38.5
Mother's BMI (kg/m2) at child's age 14	369	24.3 (3.4)	581	24.5 (3.8)	392	25.0 (4.3)
Father's BMI (kg/m2) at child's age 14	358	25.5 (3.1)	569	25.8 (3.3)	389	26.3 (3.5)
Mother overweight/obese at child's age 14	369	32.0	581	38.2	392	38.5
Father overweight/obese at child's age 14	358	50.8	569	53.6	389	59.6
Family history reported by						
Mother	374	82.4	597	85.1	401	87.8
Father	374	9.6	597	5.4	401	4.5
Both parents together	374	7.5	597	9.4	401	7.5
Mother's age at time of reporting family history	369	45.9 (3.6)	594	46.6 (3.7)	397	45.9 (3.8)
Father's age at time of reporting family history	365	47.9 (4.6)	589	48.8 (4.5)	395	48.6 (5.0)
Child's adiposity at age 12						
Child overweight/obese	375	11.7	597	12.7	400	13.5
BMI (z-score)	375	0.1 (1.0)	597	0.1 (1.1)	400	0.2 (1.1)
Cardiometabolic markers at age 12						
Waist circumference (cm)	375	66.0 (6.3)	595	66.3 (6.5)	399	67.2 (7.4)
Total cholesterol (mmol/L)	319	4.0 (0.7)	513	4.0 (0.6)	340	4.2 (0.7)
HDL cholesterol (mmol/L)	319	1.4 (0.3)	513	1.4 (0.3)	340	1.3 (0.3)
Total/HDL cholesterol ratio	319	3.1 (0.8)	513	3.1 (0.9)	340	3.3 (0.9)
Systolic blood pressure (mmHg)	352	115.0 (9.2)	566	114.8 (9.4)	384	114.6 (8.9)
Diastolic blood pressure (mmHg)	352	66.7 (6.2)	566	66.6 (6.6)	384	66.9 (6.5)
HbA1c (mmol/mol)	313	32.3 (2.5)	509	32.3 (2.4)	335	32.6 (2.6)

[1]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.

Table 3. Differences[1] in levels of cardiometabolic markers for children with a moderate or strong family history, compared to children with no family history (n=1374)

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[2]	Diastolic blood pressure (mmHg)[2]	HbA1c (mmol/mol)
Myocardial infarction							
Moderate family history	0.13 (-0.65; 0.90)	0.06 (-0.03; 0.15)	-0.02 (-0.06; 0.02)	0.11 (-0.01; 0.24)	-0.15 (-1.30; 1.00)	0.69 (-0.15; 1.53)	0.08 (-0.26; 0.41)
Strong family history	0.55 (-0.39; 1.49)	**0.12 (0.01; 0.23)*†**	**-0.05 (-0.10; -0.003)*†**	**0.24 (0.09; 0.39)*†**	-1.13 (-2.52; 0.26)	0.17 (-0.84; 1.19)	0.29 (-0.11; 0.70)
Stroke							
Moderate family history	-0.30 (-1.12; 0.52)	-0.03 (-0.12; 0.07)	-0.03 (-0.07; 0.02)	0.001 (-0.13; 0.13)	**1.30 (0.09; 2.51)***	0.27 (-0.61; 1.15)	**-0.41 (-0.77; -0.05)***
Strong family history	-0.25 (-1.51; 1.00)	-0.03 (-0.17; 0.11)	-0.05 (-0.12; 0.01)	0.10 (-0.09; 0.30)	-0.41 (-2.27; 1.44)	-0.42 (-1.77; 0.94)	-0.05 (-0.59; 0.49)
Diabetes							
Moderate family history	0.52 (-0.23; 1.27)	-0.01 (-0.10; 0.07)	-0.01 (-0.05; 0.03)	0.05 (-0.06; 0.17)	-0.71 (-1.82; 0.40)	-0.73 (-1.54; 0.08)	0.12 (-0.20; 0.44)
Strong family history	**2.25 (1.08; 3.41)*†**	**0.20 (0.07; 0.34)*†**	-0.004 (-0.07; 0.05)	**0.22 (0.03; 0.40)*†**	0.89 (-0.82; 2.61)	0.59 (-0.66; 1.84)	**0.57 (0.06; 1.09)*†**
CVD[3] and/or diabetes							
Moderate family history	-0.07 (-0.88; 0.74)	0.02 (-0.08; 0.11)	0.003 (-0.04; 0.05)	0.001 (-0.13; 0.13)	0.18 (-1.03; 1.38)	0.03 (-0.85; 0.92)	-0.06 (-0.42; 0.29)
Strong family history	0.64 (-0.25; 1.53)	**0.13 (0.03; 0.23)*†**	-0.03 (-0.07; 0.02)	**0.18 (0.04; 0.32)*†**	-0.19 (-1.51; 1.13)	0.11 (-0.86; 1.07)	0.22 (-0.16; 0.61)

[1]Differences are ß (95%CI), adjusted for child's sex, age, ethnicity, height, pubertal development, maternal and paternal education, and maternal and paternal age.
[2]Additionally adjusted for cuff size.
[3]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.
*P<.05
†P for trend <.05

Table 4. Differences[1] in levels of cardiometabolic markers for children with a strong family history, compared to children with no family history

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[2]	Diastolic blood pressure (mmHg)[2]	HbA1c (mmol/mol)
Myocardial infarction							
Adjusted	0.55 (-0.39; 1.49)	**0.12 (0.01; 0.23)***	**-0.05 (-0.10; -0.003)***	**0.24 (0.09; 0.39)***	-1.13 (-2.52; 0.26)	0.17 (-0.84; 1.19)	0.29 (-0.11; 0.70)
+ BMI parents	0.23 (-0.68; 1.14)	**0.12 (0.01; 0.23)***	**-0.05 (-0.10; -0.002)***	**0.24 (0.09; 0.39)***	-1.18 (-2.57; 0.22)	0.13 (-0.90; 1.16)	0.39 (-0.02; 0.80)
+ BMI child	-	**0.12 (0.005; 0.23)***	-0.05 (-0.10; 0.0003)	**0.23 (0.08; 0.37)***	-1.33 (-2.71; 0.05)	0.04 (-0.98; 1.06)	0.39 (-0.02; 0.80)
Stroke							
Adjusted	-0.25 (-1.51; 1.00)	-0.03 (-0.17; 0.11)	-0.05 (-0.12; 0.01)	0.10 (-0.09; 0.30)	-0.41 (-2.27; 1.44)	-0.42 (-1.77; 0.94)	-0.05 (-0.59; 0.49)
+ BMI parents	-0.19 (-1.40; 1.02)	-0.02 (-0.17; 0.12)	-0.06 (-0.12; 0.01)	0.13 (-0.07; 0.33)	-0.53 (-2.38; 1.33)	-0.47 (-1.84; 0.90)	-0.01 (-0.56; 0.53)
+ BMI child	-	-0.02 (-0.16; 0.13)	-0.05 (-0.12; 0.01)	0.12 (-0.07; 0.31)	-0.35 (-2.18; 1.49)	-0.35 (-1.70; 1.00)	-0.02 (-0.56; 0.53)
Diabetes							
Adjusted	**2.25 (1.08; 3.41)***	**0.20 (0.07; 0.34)***	-0.004 (-0.07; 0.05)	**0.22 (0.03; 0.40)***	0.89 (-0.82; 2.61)	0.59 (-0.66; 1.84)	**0.57 (0.06; 1.09)***
+ BMI parents	**1.42 (0.28; 2.56)***	**0.21 (0.07; 0.34)***	-0.003 (-0.07; 0.06)	**0.20 (0.01; 0.39)***	0.50 (-1.24; 2.23)	0.56 (-0.72; 1.84)	0.54 (0.01; 1.06)
+ BMI child	-	**0.19 (0.05; 0.33)***	0.01 (-0.05; 0.08)	0.15 (-0.04; 0.33)	0.01 (-1.71; 1.73)	0.33 (-0.93; 1.60)	0.54 (0.01; 1.07)
CVD[3] and/or diabetes							
Adjusted	0.64 (-0.25; 1.53)	**0.13 (0.03; 0.23)***	-0.03 (-0.07; 0.02)	**0.18 (0.04; 0.32)***	-0.19 (-1.51; 1.13)	0.11 (-0.86; 1.07)	0.22 (-0.16; 0.61)
+ BMI parents	0.24 (-0.63; 1.10)	**0.14 (0.03; 0.24)***	-0.03 (-0.07; 0.02)	**0.18 (0.04; 0.33)***	-0.42 (-1.75; 0.92)	0.04 (-0.94; 1.03)	0.21 (-0.18; 0.61)
+ BMI child	-	**0.14 (0.03; 0.24)***	-0.02 (-0.07; 0.02)	**0.18 (0.04; 0.32)***	-0.45 (-1.77; 0.87)	-0.002 (-0.97; 0.97)	0.21 (-0.18; 0.61)

[1]Differences are ß (95%CI). The adjusted model included child's sex, age, ethnicity, height, pubertal development, maternal and paternal education, and maternal and paternal age. Subsequently, models additionally adjusted for maternal and paternal BMI; and child's BMI z-score at age 12 (excluding child's height).
[2]Additionally adjusted for cuff size.
[3]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.
*P<.05

Table 5. Differences[1] in levels of cardiometabolic markers for children with a strong family history, compared to children with no family history, mutually adjusted for family history of the other diseases

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[2]	Diastolic blood pressure (mmHg)[2]	HbA1c (mmol/mol)
Myocardial infarction	0.44 (-0.55; 1.42)	**0.11 (0.003; 0.22)***	-0.05 (-0.10; 0.002)	**0.22 (0.07; 0.37)***	-1.18 (-2.60; 0.23)	0.23 (-0.79; 1.25)	0.26 (-0.15; 0.66)
Stroke	-0.49 (-1.80; 0.83)	-0.03 (-0.17; 0.12)	-0.04 (-0.11; 0.02)	0.08 (-0.12; 0.28)	-0.34 (-2.23; 1.56)	-0.37 (-1.73; 0.99)	-0.09 (-0.63; 0.46)
Diabetes	**2.40 (1.17; 3.63)***	**0.18 (0.05; 0.32)***	-0.01 (-0.07; 0.06)	0.19 (0.001; 0.37)	1.14 (-0.61; 2.88)	0.55 (-0.70; 1.80)	**0.53 (0.01; 1.05)***

[1]Differences are ß (95%CI) and adjusted for child's sex, age, ethnicity, height, pubertal development, maternal and paternal education, and maternal and paternal age. The family history variables for MI, stroke and diabetes were combined in one model; estimates are thus mutually adjusted for family history of the other two diseases.
[2]Additionally adjusted for cuff size.
*P<.05

for family history of the other diseases (Table 5); strong family history of MI and diabetes were independently associated with 0.11 and 0.18 mmol/l higher TC, respectively.

Associations of family history of CVD and/or diabetes with cardiometabolic markers were similar in boys and girls (p-values for interaction >.15). We therefore show results for boys and girls combined. Sensitivity analyses indicated that associations were robust and not affected by missing data or by reporting of disease history for the family in-law (Supplemental information S2 and supplemental tables S2 and S3).

Discussion

One third of the 12-year-olds in our study had a strong family history of CVD and/or diabetes, and these children had higher TC and TC/HDLC ratio than children with no family history. As compared with family history of CVD and/or diabetes, a strong family history of a single disease was present in fewer children, but showed stronger associations with disease specific cardiometabolic markers. Family history of MI may be most relevant for children's cholesterol levels (HDL, TC and TC/HDLC ratio), whereas family history of diabetes may be relevant for children's waist circumference and HbA1c, in addition to cholesterol levels. Importantly, the associations for individual diseases were independent of family history of the other diseases. Children with a moderate family history had no unfavourable cardiometabolic markers.

As far as we are aware, only three studies have previously investigated family history of both CVD and diabetes and cardiometabolic outcomes in children from the general population[24-26]. One study investigated cardiometabolic markers in the child as predictors of parents' subsequent CVD and diabetes; therefore our results are not directly comparable[25]. The other two studies were conducted two decades ago within the Bogalusa Heart Study, and lacked disease history of grandparents and age of the parents, thus were therefore not able to categorize the exposure into no, moderate, and strong family history. Bao et al observed that parental MI was associated with higher systolic BP and insulin in offspring, parental stroke was not associated with cardiometabolic markers, and parental diabetes was associated with higher systolic BP and glucose, in adolescents aged 11-17 y[24]. Shear et al further specified that among children aged 5-7 y, those with one parent with diabetes plus another parent with CVD or diabetes had the highest lipid levels[26]. Our study is the first to investigate both diabetes and cardiovascular

disease history in two generations. Our findings add to the previous findings that in 12-year-old children from a contemporary cohort, history of MI and diabetes in parents and grandparents may be a relevant and important risk factor for unfavourable waist circumference, levels of cholesterol and HbA1c, and potentially for future cardiometabolic disease, largely independent of parental and child BMI.

In our study, stroke was not consistently associated with cardiometabolic markers. This is consistent with results from other studies in children[24,26] and adults[27]. Although a family history of stroke has been associated with clinical CHD outcomes, such as heart attack in adult studies, history of stroke generally is not associated with outcomes of subclinical atherosclerosis, such as the intermediate risk factors investigated in the current study. Bao et al noted that the predictive nature of stroke may also be limited due to differences in etiologic origin of different types of stroke, i.e. ischemic or haemorrhagic[24]. Family history of CVD or diabetes was not associated with child's BP in our study. The Bogalusa Heart Study showed that associations with BP may emerge at older ages than our population of 12-year-olds and particularly in children whose parents have hypertension[24].

A key strength of this study is the availability of extensive family history information for both CVD and diabetes, which allowed us to estimate the combined disease burden that runs within families and to show disease specific associations. We obtained disease history and age at onset for parents as well as grandparents, which enabled us to define the family history burden in children more precisely. We adjusted for parental BMI and child BMI and showed that most associations were independent of these factors. Limitations include reliance on retrospectively parental-reported family history. Although we could not validate reported history of diabetes and CVD with medical records, evidence suggests that family history of diabetes and major CVD events are reported fairly accurately, because they are serious, but not stigmatized, diseases with clear case definitions[3,28]. Family history of diabetes may have been underreported due to under diagnosis[3] which may have led to underestimation of the true association. Mothers of the PIAMA children completed most questionnaires (85%), which is likely to explain the higher number of unknowns and missing reports of disease cases in the fathers' lineage. However, the total percentage of missing responses and unknowns in reports of family history was small (2-3%), and a systematic review and meta-analysis recently concluded that transmission of CVD risk is not different between fathers and mothers lineage[29]. This seems true for diabetes too[30]. In addition, when we restricted the main analysis to children who lived with both parents together, effect sizes hardly changed, indicating

that potential misreporting by single parents did not affect the results. For these reasons, we do not expect that the higher proportion of missing family history reports in father's lineage will have biased the associations.

The percentage of children with a strong family history in our study and the strengths of the associations we observed should not be interpreted in absolute terms. In the current study, strong family history of CVD and/or diabetes was defined as at least one affected parent, at least one grandparent with early disease onset, or 3-4 grandparents with late disease onset, but no gold standard exists for the definition of family history of disease. The incidence of type 2 diabetes and particularly of CVD increases with age, therefore future events in parents and grandparents are likely to reclassify children towards higher risk categories. This may affect the magnitude of the observed differences, which may be more pronounced with an increasing proportion of children with a strong family history.

The associations, in particular with waist circumference and TC/HDLC ratio, were strong and the differences (2.0 cm waist circumference and 0.20 TC/HDLC ratio) may be considered relevant at the population level[31,32]. Cardiometabolic markers track over time from childhood to adulthood[33,34]. When measured in adolescence or early adulthood, cardiometabolic markers relate to adult carotid intima media thickness and cardiovascular disease[34,35]. Therefore, also children with subclinical elevated cardiometabolic markers may be at risk for developing cardiovascular disease in adulthood.

Children with a strong family history of CVD and/or diabetes more often were overweight, and had parents who were born in a non-western country, who had a lower education and a higher BMI. Typically, these are priority groups for the prevention of overweight and cardiovascular disease, but also the groups that are not easily reached by preventive efforts. Awareness of the potentially increased risk for their children of diseases later in life, that is associated with their own family disease history, may increase families' motivation to follow healthy lifestyle guidelines[36]. Most associations persisted after adjusting for parental BMI, indicating that parental BMI as a proxy for shared lifestyle within families could not completely explain the associations between family history and child's cardiometabolic markers. Moreover, associations remained significant after additionally adjusting for child's BMI, indicating that even children with a healthy weight could be at risk for unfavourable levels of cardiometabolic markers if their parents or grandparents had MI or diabetes.

Conclusion

A large proportion of children in our study had a strong family history of CVD and/or diabetes, with cholesterol levels that were significantly higher than the levels in children with no family history of disease. Children with a strong family history of CVD or diabetes had unfavourable cardiometabolic markers that were specific to those diseases.

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Supplemental Tables

Table S1. History of myocardial infarction, stroke and diabetes in parents and grandparents of children in the study population (n=1374)

	Myocardial infarction				Stroke				Diabetes			
	Yes	No	Reported as 'unknown'	Missing	Yes	No	Reported as 'unknown'	Missing	Yes	No	Reported as 'unknown'	Missing
Mother (n, %)[a]	3 (0.2)	1367 (99.5)	1 (0.1)	3 (0.2)	4 (0.3)	1363 (99.2)	2 (0.2)	5 (0.4)	17 (1.2)	1349 (98.2)	2 (0.2)	6 (0.4)
Father (n, %)[a]	15 (1.1)	1344 (97.8)	12 (0.9)	3 (0.2)	7 (0.5)	1353 (98.5)	10 (0.7)	4 (0.3)	31 (2.3)	1318 (95.9)	12 (0.9)	13 (1.0)[b]
Maternal grandmother (n, %)[a]	80 (5.8)	1269 (92.4)	23 (1.7)	2 (0.2)	102 (7.4)	1250 (91.0)	18 (1.3)	4 (0.3)	170 (12.4)	1179 (85.8)	17 (1.2)	8 (0.6)[b]
Maternal grandfather (n, %)[a]	302 (22.0)	1033 (75.2)	36 (2.6)	3 (0.2)	146 (10.6)	1194 (86.9)	31 (2.3)	3 (0.2)	173 (12.6)	1161 (84.5)	30 (2.2)	10 (0.7)[b]
Paternal grandmother (n, %)[a]	73 (5.3)	1247 (90.8)	48 (3.5)	6 (0.4)	91 (6.6)	1237 (90.0)	41 (3.0)	5 (0.4)	148 (10.8)	1177 (85.7)	43 (3.1)	6 (0.4)
Paternal grandfather (n, %)[a]	291 (21.2)	1013 (73.7)	65 (4.7)	5 (0.4)	119 (8.7)	1192 (86.8)	59 (4.3)	4 (0.3)	154 (11.2)	1154 (84.0)	58 (4.2)	8 (0.6)[b]

[a]Percentages are relative to the total number of children in the study population (n=1374).
[b]Including 12 relatives with onset of diabetes < 20 y.

Table S2. Differences[1] in levels of cardiometabolic markers for children with a moderate or strong family history, compared to children with no family history, among those with complete data

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[2]	Diastolic blood pressure (mmHg)[2]	HbA1c (mmol/mol)
Myocardial infarction (n=1265)							
Moderate family history	0.12 (-0.72; 0.97)	0.07 (-0.02; 0.16)	-0.03 (-0.07; 0.02)	**0.15 (0.03; 0.27)***	-0.38 (-1.54; 0.78)	0.52 (-0.31; 1.35)	0.10 (-0.25; 0.44)
Strong family history	-0.02 (-1.05; 1.01)	**0.14 (0.03; 0.25)***	-0.04 (-0.09; 0.02)	**0.22 (0.07; 0.36)***	**-1.66 (-3.08; -0.25)***	0.07 (-0.94; 1.07)	0.38 (-0.04; 0.80)
Stroke (n=1272)							
Moderate family history	-0.70 (-1.60; 0.19)	-0.01 (-0.11; 0.09)	-0.01 (-0.05; 0.04)	-0.02 (-0.14; 0.11)	0.81 (-0.42; 2.04)	0.02 (-0.86; 0.90)	-0.29 (-0.66; 0.08)
Strong family history	0.57 (-0.84; 1.99)	0.01 (-0.15; 0.16)	-0.06 (-0.13; 0.01)	0.15 (-0.05; 0.35)	-0.27 (-2.22; 1.68)	-0.44 (-1.83; 0.95)	-0.08 (-0.66; 0.49)
Diabetes (n=1268)							
Moderate family history	0.57 (-0.24; 1.39)	-0.01 (-0.10; 0.08)	-0.03 (-0.07; 0.01)	0.10 (-0.02; 0.21)	-0.89 (-2.01; 0.23)	-0.71 (-1.51; 0.09)	0.20 (-0.13; 0.53)
Strong family history	**2.51 (1.21; 3.80)***	**0.21 (0.07; 0.35)***	-0.03 (-0.09; 0.04)	**0.25 (0.06; 0.44)***	0.43 (-1.34; 2.21)	0.72 (-0.55; 1.98)	**0.78 (0.23; 1.32)***
CVD[3] and/or diabetes (n=1205)							
Moderate family history	0.22 (-0.67; 1.12)	0.04 (-0.06; 0.13)	-0.01 (-0.05; 0.04)	0.02 (-0.11; 0.14)	-0.30 (-1.53; 0.93)	-0.05 (-0.92; 0.83)	-0.16 (-0.53; 0.20)
Strong family history	0.91 (-0.07; 1.89)	**0.17 (0.07; 0.28)***	-0.03 (-0.08; 0.02)	**0.21 (0.08; 0.34)***	-0.74 (-2.09; 0.61)	0.02 (-0.95; 0.98)	0.34 (-0.07; 0.74)

[1]Differences are crude ß (95%CI).
[2]Adjusted for cuff size.
[3]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.
*P<.05

Table S3. Differences[1] in levels of cardiometabolic markers for children with a moderate or strong family history, compared to children with no family history, among those who lived with both parents together (n=1688)

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[2]	Diastolic blood pressure (mmHg)[2]	HbA1c (mmol/mol)
Myocardial infarction							
Moderate family history	0.12 (-0.77; 1.02)	0.06 (-0.03; 0.16)	-0.02 (-0.06; 0.02)	0.12 (-0.004; 0.25)	-0.19 (-1.42; 1.03)	0.81 (-0.06; 1.67)	0.07 (-0.30; 0.43)
Strong family history	0.08 (-1.01; 1.16)	**0.14 (0.03; 0.26)***	-0.04 (-0.09; 0.01)	**0.23 (0.07; 0.38)***	-1.00 (-2.48; 0.48)	0.13 (-0.72; 1.17)	**0.47 (0.04; 0.91)***
Stroke							
Moderate family history	-0.35 (-1.30; 0.59)	-0.03 (-0.13; 0.07)	-0.03 (-0.07; 0.02)	0.02 (-0.12; 0.15)	**1.30 (0.11; 2.48)***	0.15 (-0.76; 1.07)	-0.35 (-0.73; 0.04)
Strong family history	0.44 (-1.03; 1.91)	0.005 (-0.15; 0.16)	-0.06 (-0.13; 0.01)	0.15 (-0.06; 0.36)	-0.10 (-1.94; 1.74)	-0.01 (-1.42; 1.40)	0.11 (-0.48; 0.70)
Diabetes							
Moderate family history	0.63 (-0.24; 1.49)	-0.01 (-0.11; 0.08)	-0.02 (-0.06; 0.02)	0.07 (-0.06; 0.19)	-0.55 (-1.73; 0.64)	-0.46 (-1.29; 0.38)	0.18 (-0.17; 0.53)
Strong family history	**2.91 (1.58; 4.23)***	**0.15 (0.01; 0.30)***	-0.03 (-0.10; 0.03)	**0.23 (0.04; 0.42)***	0.71 (-1.08; 2.52)	0.81 (-0.46; 2.08)	**0.63 (0.08; 1.19)***
CVD[3] and/or diabetes							
Moderate family history	0.62 (-0.35; 1.58)	0.03 (-0.07; 0.13)	-0.004 (-0.05; 0.04)	0.04 (-0.10; 0.17)	-0.07 (-1.39; 1.25)	0.16 (-0.77; 1.10)	-0.20 (-0.59; 0.19)
Strong family history	**1.35 (0.30; 2.40)***	**0.14 (0.03; 0.26)***	-0.03 (-0.09; 0.02)	**0.21 (0.06; 0.36)***	-0.18 (-1.61; 1.25)	0.26 (-0.76; 1.27)	0.30 (-0.13; 0.72)

[1]Differences are crude ß (95%CI).
[2]Adjusted for cuff size.
[3]Cardiovascular disease (CVD) was myocardial infarction and/or stroke.
*P<.05

Supplemental Information

Supplemental information S1. Prevalence of myocardial infarction, stroke and diabetes in the PIAMA study and in the general Dutch population.

Parents of the PIAMA study population were a median age of 46 y (mothers), and 48 y (fathers) at the time of reporting family history; the interquartile range in age was 44-49 y (mothers) and 45-51 y (fathers). Rates of myocardial infarction, stroke and diabetes in PIAMA parents were comparable to rates in the general Dutch population for age range 40-54 y (see table below).

Prevalence of myocardial infarction, stroke and diabetes in the PIAMA parents and in the (age-matched) general Dutch population

	General population Men (40-54 y)	PIAMA Men (45-51 y)	General population Women (40-54 y)	PIAMA Women (44-49 y)
Myocardial infarction[1]	0.8% to 3.7%	1.1%	0.3% to 1.4%	0.2%
Stroke[2]	0.2% to 0.7%	0.5%	0.3% to 0.7%	0.3%
Diabetes mellitus[3]	2.1% to 5.8%	2.3%	1.6% to 4.2%	1.2%

[1]In the general population, the following ICPC (International Classification of Primary Care) codes were used to classify coronary heart disease: K75 acute myocardial infarction, K74 angina pectoris or K76 other/chronic ischemic heart disease. In PIAMA, only myocardial infarction was reported.
[2]In the general population, the following ICPC codes were used to classify stroke: K90 stroke and K89 TIA (Transient Ischemic Attack). In PIAMA, only stroke was reported.
[3]In the general population, diabetes mellitus (DM) was DM1 and DM2 (ICPC code T90 diabetes). Approximately 90% of all DM cases is DM2. In PIAMA, only late onset DM was included (age ≥20 years)).

Explanatory information: PIAMA parents reported the lifetime prevalence of diseases in parents and grandparents in 2011-2012. Prevalence in PIAMA was the number of cases divided by the total number of cases and non-cases for a specific disease, multiplied by 100 to obtain percentages. Prevalence in the general Dutch population was point prevalence at January 1, 2011. Prevalence rates were retrieved from the Netherlands Information Network of General Practice (huisartsenregistratie - LINH, Landelijk Informatie Netwerk Huisartsenzorg).

Reference:

The Netherlands Information Network of General Practice (LINH). Available through https://www.volksgezondheidenzorg.info/ (accessed 1-9-2015).

Supplemental information S2. Sensitivity analyses.

Missing or unknown family history items

As a sensitivity analysis we studied the effect of potentially misclassifying children with 'unknown' and missing family history to 'no' family history. We restricted the main analysis to children with complete data ('yes' or 'no') for family history of MI (n=1265), stroke (n=1272), diabetes (n=1268), and CVD and/or diabetes (n=1205), to investigate whether associations were different from those including children with missing data for family history. Restricting the main analysis to children with complete data for family history resulted in comparable effect sizes, yet statistically less significant due to lower sample size (Supplemental table S2).

Reports of disease history by the family in-law

Mothers of the PIAMA children completed most questionnaires including the questions on family history (85% vs 6% fathers and 9% both parents). To explore whether reporting disease history of the family in-law (e.g. mothers' reports of paternal grandparents and fathers' reports of maternal grandparents) affected the associations, we restricted the main analysis to children whose parents were living together (n=1688). We assumed that for these children family history might have been reported more accurately, since the parents could communicate each other's family history more easily. We could not restrict analyses to children of single-parent families, due to too few children in this group (n=191). Restricting the main analysis to children who lived with both parents together resulted in similar effect sizes (Supplemental table S3).

Chapter 3

Time in bed, sleep quality and associations with cardiometabolic markers in children

Nina E. Berentzen
Henriëtte A. Smit
Marga B.M. Bekkers
Bert Brunekreef
Gerard H. Koppelman
Johan C. De Jongste
Marjan Kerkhof
Lenie van Rossem
Alet H. Wijga

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Summary

We investigated associations of time in bed and multiple sleep quality characteristics, with cardiometabolic markers in children. Data from the PIAMA study, a population-based prospective birth-cohort study started in 1996–1997 in the Netherlands, were analysed. In total 1481 children aged 11-12 years completed a questionnaire (including questions on sleep) and underwent a medical examination. We measured body mass index (BMI), waist circumference (WC), total- and HDL cholesterol (TC, HDLC), blood pressure (BP) and glycated haemoglobin (HbA1c). Results showed that in girls, some sleep characteristics were related to anthropometrics (BMI, WC) and cholesterol. Girls with long time in bed (11-12,5 hrs) had 0.16 lower BMI z-score (95% CI -0.31; -0.01) and 0.99 cm lower WC (95% CI -2.01; -0.13), compared to girls who spent 10-10,5 hours in bed. Girls who went to bed late and rose early had 0.16 mmol/L higher TC (95% CI 0.01; 0.31), and 0.08 mmol/L higher HDLC (95% CI 0.01; 0.14) than 'Early to bed/Early rise' girls. Girls with night-time awakenings had 0.14 mmol/L higher TC (95% CI 0.03; 0.25) than girls without night-time awakenings. Girls who felt sleepy/tired ≥1day/wk, had 0.10 mmol/L lower HDLC (95% CI -0.16; -0.04) and 0.17 mmol/L higher TC/HDLC ratio (95% CI 0.02; 0.32) than girls who did not feel sleepy. No associations were found for boys. Sleep characteristics were not related to BP and HbA1c and effect sizes of the associations in girls were small. Therefore, we consider it premature to propose that improved sleep could reduce cardiovascular risk during childhood.

Introduction

Studies in adults have shown that inadequate sleep is associated with the metabolic syndrome and diabetes[1] and with cardiometabolic risk factors including weight status[2], cholesterol, blood pressure[3], and glucose metabolism[4].

Since cardiometabolic risk factors track from adolescence into adulthood[5], it is important to investigate whether sleep behaviour is also related to cardiometabolic markers in children. In the paediatric population, associations have been described between inadequate sleep and overweight[6]; waist circumference[7]; insulin resistance[8] and systolic blood pressure[9]. However, other studies did not demonstrate associations between sleep and cardiometabolic markers[10-12]. Previous studies in children mainly looked at sleep duration. Recently, it was suggested that sleep quality, in addition to sleep duration, might also be important for cardiovascular disease risk in adults[13]. In children, studies on sleep parameters other than duration (sleep efficiency, sleep regularity, duration of slow-wave sleep) are scarce, used different sleep methodologies (actigraph or laboratory polysomnography) and reported conflicting results[8,9,12].

A sex difference in regular sleep has been acknowledged, with male adolescents sleeping less than females on both school- and non-schooldays[14]. The aetiological basis for less sleep in male adolescents is not fully understood and despite a growing recognition, little systematic research has been done regarding these sex differences in childhood.

We aimed to investigate whether sleep duration and sleep quality influence cardio-metabolic markers. For this aim we studied multiple cardiometabolic markers separately in boys and girls around the age of 12 years from the general population. To assess sleep, we examined time in bed (as an indicator of sleep duration), sleep-wake pattern, and characteristics of sleep quality (night-time awakenings; difficulty with getting up in the morning; feeling rested after waking up; and feeling sleepy/tired during the day).

Methods

We used the data from a population-based Dutch birth cohort study: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study. A detailed description of the study design has been published previously[15]. In short, pregnant women were recruited

from the general population during their first antenatal visit in 1996-1997; the number of children at baseline was 3963. Yearly, around the child's birthday, data were collected by parental questionnaires up to the age of 8 years, and again at the age of 11 years by separate parental and child questionnaires. Additionally, in subgroups of children, extensive medical examinations were performed at the ages of 4, 8, and 12 years. The study protocol was approved by the medical ethics committees of the participating institutes, and all parents gave written informed consent. In total 2651 children completed the 11 years child questionnaire (response rate higher than 70%), including for the first time questions on sleep. The parental questionnaire did not contain questions on child's sleep. All children still in the study (n=3202) were invited to the medical examination at the age of 12 years, and 1511 (47%) of them were willing to participate. Our study population consisted of 1481 children (759 girls and 722 boys) who completed the questionnaire at age 11 years and had data on any of the cardiometabolic markers.

Assessment of sleep characteristics

The 11 years questionnaire completed by the children contained 15 questions on different aspects of sleep, which we used to create indicators of time in bed, sleep-wake pattern and sleep quality (night-time awakenings and daytime outcomes). Questions were adopted from validated questionnaires that had been previously used; this is further described in the supplemental information.

Time in bed and sleep-wake pattern

Usual bedtimes and get up times on schooldays were reported in respectively nine (from "<18:30" to "22:30PM") and eight (from "<6:00" to ">8:30AM") answer options. From reported bedtimes and get up times at schooldays, time in bed at schooldays was calculated. Time in bed was categorized because of previously reported nonlinear associations. Since there is a lack of empirical evidence on optimal sleep time for children, we categorized time in bed based on our own data as short (7.5-9.5hrs), medium (10-10.5hrs, reference) and long (11-12.5 hrs). The majority of the children were in the reference category (60%).

Since timing of sleep has been considered important for cardiometabolic risk factors, independently of sleep duration[16], we classified children into one of four sleep-wake patterns; Early to bed/Early rise (EE, reference); Early to bed/Late rise (EL); Late to bed/Early rise (LE); and Late to bed/Late rise (LL). Sleep-wake patterns were constructed

using median-splits for early or late bedtimes and get up times (median=21:00 PM for bedtimes and median=7:50 AM for get up times). Medians were calculated for boys and girls combined because medians for bedtimes and get up times did not differ between genders. After recoding, means of bedtimes and get up times respectively, for each sleep-wake pattern were; EE 20:35PM - 7:29AM; EL 20:42PM - 7:53AM; LE 21:14PM - 7:32AM; LL 21:15PM - 7:54AM.

Sleep quality: night-time awakenings and daytime outcomes

We used four indicators of sleep quality: one variable on night-time awakenings and three variables on daytime outcomes. A composite variable on night-time awakenings was created from two (three category) questions. The original questions asked for "frequency of night-time awakenings" (almost never, sometimes, almost every night) and "how long does it take before you fall asleep again" (immediately, a while, very long). The resulting composite variable on night-time awakenings had two categories (yes/no). "Yes" was "Waking almost every night; or waking sometimes, in combination with falling asleep again after a while or after a very long time". "No" was "Waking almost never; or waking sometimes, in combination with falling asleep again immediately".

Three questions were used as daytime outcomes of sleep quality. "Difficulty with getting up in the morning" and "'Feeling rested after waking on a school day" was reported in two categories (yes, no). A third question informed on the frequency of feeling sleepy or tired during the day. This was reported in five categories (from "never" to "3 times a week or more") and subsequently grouped in two categories; never or sometimes (reference), and at least once a week.

Assessment of cardiometabolic markers

During home visits at age 12 years, anthropometry measurements and blood pressure readings were obtained; and blood was drawn for measurement of cholesterol and glycated haemoglobin (HbA1c) by trained staff. Details on these measurements are provided as supplemental information.

Other characteristics

Information included in the child's questionnaire was age at time of completing the questionnaire, puberty development scale (1 to 4), and two questions regarding computer time and TV time, which were used to create a composite variable for total screen time

(minutes/week). Mother's educational level (low (primary school, lower vocational or lower secondary education), intermediate (vocational education or intermediate/higher secondary education) and high (vocational education and university)) was reported when the child was 1 years old.

Statistical analyses

We performed separate analyses for boys and girls, since we expected *a priori* that associations might differ between genders. Multiple linear regression was conducted to estimate associations between sleep variables and cardiometabolic markers. Potential confounders were identified by literature research and by examining associations between these factors and the exposure variables. The crude model included age of the child at medical examination and at completion of the questionnaire. The full model was additionally adjusted for height, mother's education, puberty development, and screen time. Screen time was included as a measure of sedentary behaviour, and also because of its reported independent adverse effect on sleep[17]. Analyses of systolic and diastolic blood pressure were additionally adjusted for cuff size used in BP measurements. Analyses of HbA1c were also adjusted for storage time of the blood samples. We additionally included BMI z-score and WC separately in the fully adjusted models to investigate to which extent associations between sleep and cardiometabolic markers were mediated by these factors.

In an additional analysis, we investigated the association between time in bed and overweight, in order to be able to compare our results to those of previous studies, which often reported overweight (and not BMI). We conducted logistic regression, adjusting for the same set of variables as formerly mentioned. Overweight (including obesity) was defined based on international defined cut-off points[18]. Normal weight children (excluding those classified thin or extremely thin) served as the reference. For all statistical analyses SAS software version 9.3 (SAS Institute, Inc., Cary, NC) was used.

Table 1 General information, sleep characteristics and cardiometabolic markers of the study population (n=1481)

	All children	Girls (n = 759; 51%)	Boys (n = 722; 49%)
General information			
Age (y) at time of questionnaire completion[a]	11.4 (0.3)	11.4 (0.3)	11.4 (0.3)
Age (y) at medical examination[b]	12.7 (0.4)	12.7 (0.4)	12.7 (0.4)
Height[b] (cm)	160 (8)	161 (7)	160 (8)
Mothers' education at baseline, n (%)			
Low	264 (18)	125 (17)	133 (18)
Intermediate	622 (41)	325 (43)	286 (40)
High	621 (41)	307 (41)	303 (42)
Puberty Development Scale[a] (PDS)	1.5 (0.5)	1.8 (0.6)	1.3 (0.3)
Total screen time[a] (min/day)	109 (68)	96 (62)	122 (71)
Overweight or obese, n (%)[b]	180 (12)	87 (12)	93 (13)
Time in bed and sleep-wake pattern[a]			
Time in bed at schooldays, n (%)			
Short (7.5-9.5 hrs)	108 (7)	47 (6)	61 (9)
Medium (10-10.5 hrs)	887 (60)	455 (60)	432 (60)
Long (11-12.5 hrs)	482 (33)	257 (34)	225 (31)
Sleep-wake pattern at schooldays, n (%)			
Early to bed/Early rise (EE)	448 (30)	239 (31)	209 (29)
Early to bed/Late rise (EL)	385 (26)	205 (27)	180 (25)
Late to bed/Early rise (LE)	283 (19)	138 (18)	145 (20)
Late to bed/Late rise (LL)	361 (24)	177 (23)	184 (26)
Sleep quality[a]			
Night-time awakenings, n (%)			
No	1080 (73)	551 (73)	529 (74)
Yes	396 (27)	207 (27)	189 (26)
Daytime outcomes			
Getting up in the morning, n (%)			
Not difficult to get up	725 (49)	331 (44)	394 (55)
Difficult to get up	749 (51)	427 (56)	322 (45)
Feeling rested after waking on a schoolday, n (%)			
Yes	1145 (78)	593 (78)	552 (77)
No	329 (22)	165 (22)	164 (23)
Feeling sleepy/tired during the day, n (%)			
Never or sometimes	1228 (83)	631 (83)	597 (84)
At least once a week	243 (17)	126 (17)	117 (16)
Cardiometabolic markers[b]			
Body mass index for age Z-score	0.12 (1.06)	0.05 (1.05)	0.19 (1.07)
Waist circumference (cm)	66.4 (6.7)	66.1 (6.5)	66.8 (6.9)
Total cholesterol (mmol/L)	4.1 (0.7)	4.1 (0.6)	4.0 (0.7)
HDL cholesterol (mmol/L)	1.4 (0.3)	1.4 (0.3)	1.4 (0.3)
TC/HDLC ratio	3.1 (0.9)	3.1 (0.8)	3.1 (1.0)
Systolic blood pressure (mmHg)	115 (9)	114 (10)	115 (9)
Diastolic blood pressure (mmHg)	67 (6)	67 (6)	67 (7)
HbA1c (%)	5.1 (0.2)	5.1 (0.3)	5.1 (0.2)

Data are mean(SD) unless otherwise noted.
[a]Around the age of 11 years [b]Around the age of 12 years

Results

Table 1 shows the general characteristics of the participants according to gender. Mean age at completion of the questionnaire was 11.4 (± 0.3) years, while mean age at the moment of medical examination was 12.7 (± 0.4) years. The majority of the mothers of the children completed intermediate or higher level of education (82%). This percentage was somewhat higher than in the total PIAMA study population (77%, *n*=3807). There was a difference in pubertal development between the genders, with girls being more progressed (puberty development scale 1.8 ± 0.6) than boys (pds 1.3 ± 0.3). Children on average spent 109 (± 68) minutes in front of a screen (TV or computer) daily, boys 26 (0-30) minutes more than girls. The prevalence of overweight (including obesity) was 12%. The total group of children who completed the 11 year questionnaire (*n*=2651) was comparable to the group of children who, in addition to completing the questionnaire, also participated in the medical examination at age 12 (*n*=1511), with respect to mothers educational level, puberty development, and prevalence of overweight. The smaller group spent less time (-20 minutes/wk.) in front of a screen, compared with the larger group.

Sleep characteristics

The majority of children (60%) reported 10-10.5 hours in bed at night; 33% longer and 7% shorter (table 1). Of all children, 27% had night-time awakenings, 22% did not feel rested after waking on a school day, and 17% felt sleepy/tired during the day at least once a week. About half of the children reported difficulty with getting up in the morning (51%), girls more than boys (56% versus 45%).

Table 2 shows the prevalence of children who reported daytime outcomes ("difficult to get up in the morning"; "not feeling rested after waking on a school day"; or "sleepy/tired at least once a week"), for categories of time in bed, sleep-wake pattern and night-time awakenings. Night-time awakenings correlated with daytime outcomes, for girls as well for boys (P<.01 for all daytime outcomes). For example; 40% of the boys and 34% of the girls reporting night-time awakenings, felt sleepy/tired at least one day a week, while this was 17% for boys and girls without night-time awakenings (P<.0001).

In girls, time in bed seemed to be correlated with daytime outcomes. Girls with short time in bed reported unfavourable daytime outcomes more frequently than girls with medium or long time in bed. For example, 40% of girls with short time in bed

reported not to "feel rested after waking on a school day", while this was 15% for girls with medium time in bed.

In boys, sleep-wake pattern seemed to be correlated with daytime outcomes. Boys with a late to bed/late rise pattern (LL) more often had unfavourable daytime outcomes than boys with the early to bed/early rise (EE) pattern. Among boys with a LL pattern, 54% reported "difficulty to get up in the morning", and 21% not to "feel rested after waking on a school day", while this was 38% and 12% respectively, for boys with a EE pattern.

Table 2 Prevalence (%) of daytime sleepiness for categories of sleep duration, sleep-wake pattern and night-time awakenings

	Daytime outcomes					
	Difficult to get up in the morning		Feeling rested after waking		Sleepy/tired during the day	
	"Yes"		"No"		"At least once a week"	
	Girls	Boys	Girls	Boys	Girls	Boys
Time in bed at schooldays						
Short (7.5-9.5 hrs)	64	38	40	12	36	27
Medium (10-10.5 hrs)	55	43	15	16	20	22
Long (11-12.5 hrs)	58	50	15	19	23	23
P-value†	*0.57*	*0.34*	*<0.01**	*0.96*	*0.66*	*0.49*
Sleep-wake pattern at schooldays						
Early to bed/Early rise (EE)	53	38	14	12	23	19
Early to bed/Late rise (EL)	59	50	14	19	21	26
Late to bed/Early rise (LE)	54	38	20	13	20	26
Late to bed/Late rise (LL)	59	54	21	21	24	22
P-value†	*0.03**	*<.001**	*0.53*	*0.04**	*0.27*	*0.40*
Night-time awakenings						
No	53	43	13	12	17	17
Yes	64	52	26	30	34	40
P-value†	*<.001**	*<0.01**	*<.001**	*<.001**	*<.001**	*<.001**

†P-values were calculated for associations between sleep variables (rows) and daytime sleepiness (columns) using chi-square tests, within girls and boys separately. *P<0.05

Table 3a Multiple regression analysis of sleep characteristics and cardiometabolic markers, in girls (n=759)

	BMI z-score n=765	WC (cm) n=757	TC (mmol/L) n=627	HDLC (mmol/L) n=627	TC/HDLC ratio n=627	Systolic BP (mmHg), n=720	Diastolic BP (mmHg), n=720	HbA1c (%) n=620
	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1,2]	ß (95%CI)[1,2]	ß (95%CI)[1,3]
Time in bed and sleep-wake pattern								
Time in bed at schooldays								
Short (7.5-9.5 hrs)	-0.13 (-0.44; 0.18)	-0.42 (-2.23; 1.39)	-0.01 (-0.22; 0.21)	0.07 (-0.03; 0.17)	-0.18 (-0.44; 0.08)	1.25 (-1.76; 4.26)	-0.68 (-2.71; 1.36)	<-0.01 (-0.09; 0.09)
Medium (10-10.5 hrs) (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Long (11-12.5 hrs)	-0.16 (-0.31; -0.01)*	-0.99 (-1.87; -0.11)*	-0.06 (-0.16; 0.05)	<0.01 (-0.05; 0.05)	-0.04 (-0.17; 0.09)	-0.02 (-1.50; 1.48)	0.39 (-0.62; 1.41)	-0.01 (-0.06; 0.03)
Sleep-wake pattern at schooldays								
Early to bed/Early rise (EE) (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Early to bed/Late rise (EL)	-0.17 (-0.35; 0.01)	-1.00 (-2.08; 0.07)	0.05 (-0.08; 0.18)	0.03 (-0.03; 0.10)	-0.03 (-0.19; 0.12)	-0.90 (-2.71; 0.92)	-0.30 (-1.53; 0.93)	-0.01 (-0.07; 0.04)
Late to bed/Early rise (LE)	-0.03 (-0.24; 0.18)	-0.03 (-1.28; 1.21)	0.16 (0.01; 0.31)*	0.08 (0.01; 0.14)*	-0.10 (-0.28; 0.08)	0.65 (-1.43; 2.74)	-0.28 (-1.70; 1.13)	-0.01 (-0.07; 0.05)
Late to bed/Late rise (LL)	<0.01 (-0.19; 0.19)	-0.23 (-1.35; 0.89)	0.07 (-0.06; 0.20)	0.04 (-0.02; 0.11)	-0.03 (-0.19; 0.13)	-0.76 (-2.65; 1.13)	-0.32 (-1.61; 0.96)	-0.02 (-0.08; 0.03)
Sleep quality								
Night-time awakenings								
No (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Yes	0.03 (-0.13; 0.19)	0.44 (-0.49; 1.38)	0.14 (0.03; 0.25)*	0.02 (-0.03; 0.07)	0.05 (-0.09; 0.19)	-0.83 (-2.39; 0.74)	-0.44 (-1.50; 0.62)	-0.01 (-0.05; 0.04)
Daytime outcomes								
Getting up in the morning								
Not difficult to get up (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Difficult to get up	-0.01 (-0.16; 0.13)	0.02 (-0.81; 0.86)	-0.02 (-0.12; 0.08)	<0.01 (-0.04; 0.05)	-0.03 (-0.15; 0.10)	-0.45 (-1.84; 0.95)	-0.60 (-1.54; 0.35)	-0.02 (-0.06; 0.02)
Feeling rested after waking on a schoolday								
Yes (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
No	-0.29 (-0.48; -0.10)	-1.01 (-2.12; 0.10)	-0.04 (-0.17; 0.10)	0.01 (-0.05; 0.07)	-0.10 (-0.27; 0.06)	-1.43 (-3.30; 0.44)	-1.02 (-2.28; 0.24)	-0.01 (-0.06; 0.05)
Feeling sleepy/tired during the day								
Never or sometimes (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
At least once a week	0.09 (-0.08; 0.26)	0.76 (-0.25; 1.77)	-0.10 (-0.22; 0.03)	-0.10 (-0.16; -0.04)*	0.17 (0.02; 0.32)*	-1.19 (-2.88; 0.49)	-0.34 (-1.48; 0.80)	0.01 (-0.04; 0.06)

[1]All analyses were adjusted for age at completion of the questionnaire, age at medical examination, height, mother's education, puberty and screen time. [2]Analyses of blood pressure were additionally corrected for cuff size. [3]Analyses of HbA1c were additionally corrected for storage time. *p<.05

Table 3b Multiple regression analysis of sleep characteristics and cardiometabolic markers, in boys (n=722)

	BMI z-score n=722	WC (cm) n=718	TC (mmol/L) n=633	HDLC (mmol/L) n=633	TC/HDLC ratio n=633	Systolic BP (mmHg), n=683	Diastolic BP (mmHg), n=683	HbA1c (%) n=626
	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1]	ß (95%CI)[1,2]	ß (95%CI)[1,2]	ß (95%CI)[1,3]
Time in bed and sleep-wake pattern								
Time in bed at schooldays								
Short (7.5-9.5 hrs)	-0.06 (-0.36; 0.24)	-0.39 (-2.23; 1.45)	-0.15 (-0.35; 0.04)	0.03 (-0.07; 0.12)	-0.22 (-0.51; 0.08)	-0.81 (-3.34; 1.71)	0.08 (-1.87; 2.04)	0.03 (-0.04; 0.10)
Medium (10-10.5 hrs) (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Long (11-12.5 hrs)	-0.01 (-0.18; 0.16)	-0.04 (-1.09; 1.01)	-0.06 (-0.17; 0.05)	0.02 (-0.04; 0.07)	-0.14 (-0.31; 0.02)	0.92 (-0.49; 2.33)	0.22 (-0.87; 1.31)	0.03 (-0.01; 0.07)
Sleep-wake pattern at schooldays								
Early to bed/Early rise (EE) (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Early to bed/Late rise (EL)	0.02 (-0.19; 0.24)	-0.11 (-1.41; 1.19)	-0.06 (-0.20; 0.08)	0.02 (-0.05; 0.08)	-0.16 (-0.37; 0.05)	0.34 (-1.41; 2.10)	-0.09 (-1.45; 1.26)	0.03 (-0.02; 0.08)
Late to bed/Early rise (LE)	-0.05 (-0.28; 0.18)	-0.45 (-1.86; 0.97)	-0.08 (-0.23; 0.08)	0.01 (-0.05; 0.08)	-0.12 (-0.35; 0.11)	-0.70 (-2.60; 1.21)	0.26 (-1.21; 1.73)	-0.01 (-0.07; 0.04)
Late to bed/Late rise (LL)	0.07 (-0.14; 0.28)	-0.18 (-1.49; 1.13)	<0.01 (-0.14; 0.15)	-0.02 (-0.09; 0.04)	0.08 (-0.13; 0.29)	0.28 (-1.48; 2.04)	0.51 (-0.85; 1.87)	<-0.01 (-0.05; 0.05)
Sleep quality								
Night-time awakenings								
No (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Yes	0.08 (-0.09; 0.26)	0.54 (-0.56; 1.64)	0.08 (-0.04; 0.19)	0.02 (-0.04; 0.07)	0.01 (-0.17; 0.19)	-0.27 (-1.76; 1.21)	-0.17 (-1.31; 0.97)	-0.02 (-0.06; 0.03)
Daytime outcomes								
Getting up in the morning								
Not difficult to get up (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Difficult to get up	-0.06 (-0.22; 0.10)	-0.03 (-0.99; 0.93)	-0.07 (-0.17; 0.03)	<-0.01 (-0.05; 0.04)	-0.09 (-0.24; 0.07)	-0.61 (-1.91; 0.69)	-0.41 (-1.42; 0.59)	-0.01 (-0.05; 0.03)
Feeling rested after waking on a schoolday								
Yes (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
No	0.10 (-0.12; 0.31)	1.06 (-0.24; 2.36)	0.08 (-0.06; 0.22)	0.01 (-0.05; 0.07)	0.04 (-0.18; 0.25)	-1.26 (-3.04; 0.51)	-0.30 (-1.67; 1.08)	-0.02 (-0.07; 0.03)
Feeling sleepy/tired during the day								
Never or sometimes (ref.)	ref.	ref.	ref.	ref.	ref.	ref.	ref.	ref.
At least once a week	0.10 (-0.09; 0.29)	0.92 (-0.23; 2.07)	0.08 (-0.04; 0.21)	0.01 (-0.04; 0.07)	0.03 (-0.16; 0.21)	-1.06 (-2.61; 0.50)	0.27 (-0.93; 1.47)	-0.01 (-0.05; 0.04)

[1]All analyses were adjusted for age at completion of the questionnaire, age at medical examination, height, mother's education, puberty and screen time. [2]Analyses of blood pressure were additionally corrected for cuff size. [3]Analyses of HbA1c were additionally corrected for storage time. *p<.05

Associations between sleep and cardiometabolic markers

Time in bed, sleep-wake pattern, night-time awakenings or daytime outcomes did not significantly decrease, or increase, BP and HbA1c in the crude analysis, or after adjustment for other study factors in boys and girls (table 3a and table 3b). Among girls, after adjustment we observed significant associations between measures of sleep and anthropometric outcomes (BMI, WC) and cholesterol levels (table 3a). Girls with long time in bed (11-12.5 hours), had 0.16 lower BMI z-score (95% CI -0.31; -0.01) and 0.99 cm lower WC (95% CI -2.01; -0.13), compared to girls with medium time in bed (10-10.5 hours) at schooldays. Girls who went to bed late at night and rose early in the morning ('Late to bed/Early rise' pattern) had 0.16 mmol/L higher TC (95% CI 0.01; 0.31), and 0.08 mmol/L higher HDLC (95% CI 0.01; 0.14) compared with girls with an 'Early to bed/Early rise' pattern. Girls who had night-time awakenings had 0.14 mmol/L higher TC (95% CI 0.03; 0.25) than girls who did not have night-time awakenings. Girls who felt sleepy or tired at least once a week, had 0.10 mmol/L lower HDLC (95% CI -0.16; -0.04) and 0.17 mmol/L higher TC/HDLC ratio (95% CI 0.02; 0.32) than girls who were not feeling sleepy during the day. Among boys, time in bed, sleep-wake pattern, night-time awakenings or daytime outcomes did not significantly increase or decrease any of the cardiometabolic markers, in the crude analysis, or after adjustment for other study factors (table 3b).

Additionally we included BMI z-score and WC separately in the fully adjusted model, to investigate whether associations between sleep and cardiometabolic markers in girls were mediated by BMI or WC. Out of in total seven associations, two associations with cholesterol attenuated and were no longer significant after adjustment. The association with HDLC in girls with a 'Late to bed/Early rise' pattern was no longer significant after adjusting for WC (ß=0.07; 95% CI 0.00; 0.13). The association with TC/HDLC ratio in girls who felt sleepy at least once a week was no longer significant after adjusting for WC (ß=0.12; 95% CI -0.02; 0.26), or BMI (ß=0.14; 95% CI -0.00; 0.29). The other associations did not alter (data not shown). In an additional analysis with overweight (at age 12) instead of BMI as outcome variable, we found that odds of being overweight decreased for girls with long time in bed by 56% (OR=0.44, 95% CI 0.24; 0.79), compared with girls with medium time in bed. In boys, long time in bed was not associated with lower risk of overweight.

Discussion

This study in 11-12 year old children shows that sleep characteristics were not associated with blood pressure and HbA1c; and that in girls, but not in boys, some sleep characteristics were associated with anthropometric outcomes (BMI, WC) and cholesterol levels. More specifically, in girls, longer time in bed was associated with lower BMI and WC; night-time awakenings with higher TC, late to bed and early risers with higher TC and HDLC; and feeling sleepy/tired during daytime with lower HDLC and higher TC/HDLC ratio. It cannot be ruled out that the associations we found in girls were chance findings due to multiple comparisons. No specific sleep characteristic showed consistent associations with the cardiometabolic markers.

Strengths and limitations

Important strengths of our study include the large number of sleep parameters, multiple objectively measured cardiometabolic markers, ability to adjust simultaneously for several important confounders, and the large study population, allowing stratifying for gender. Our study offers insight not only in characteristics of sleep at night, but also in consequences of sleep during the day.

We used self-reported sleep data, which may be subject to misclassification due to reporting error. Some have suggested the use of objective sleep measurement (actigraphy or polysomnography) instead of self-report data. However, self-reported sleep duration was found to correlate reasonably well with actigraphy and performed better than parental report in estimating sleep duration[19]. In addition, actigraphy has low specificity for detecting night-time awakenings[20]; both methods are unfeasible for use in studies with many participants; and both methods cannot be used to asses experienced sleepiness during the day.

Time in bed was used as an indicator for sleep duration, and this probably overestimates actual sleep duration. Moreover, variation of sleep duration could exist within children who reported short or long time in bed; thus, this may be a source of random error. It is possible that some of the children may have or had sleep apnea or sleep disordered breathing (SDB), which would likely impact their sleep and cardiometabolic markers. However, the prevalence of moderate SDB (apnea-hypopnea index ≥ 5) was previously found to be low (1.2% in US children from the general population)[21]. We assume that in our population the prevalence of SDB was comparably low and did not substantially influence on our results.

The time lapse between completing the questionnaire and attending the medical examination was approximately 1 year. However for some children this lapse was smaller and 16 children completed the questionnaire only after undergoing the medical examination. Differences in time lapse could have introduced random error in the case that sleep affected the cardiometabolic markers *instantly.* We consider it more likely that sleep acts on these markers over a longer period of time.

Assessment of pubertal development was an important strength of our study. However, data on pubertal development were obtained at the same time as the sleep data and assessment of pubertal development was not repeated at the time of the medical examination. It is possible that some of the children entered puberty after assessment of sleep, and their progressed maturity may have influenced the outcomes.

Children in the PIAMA cohort were recruited from the general population, but children of ethnic and lower educated mothers were underrepresented. Loss to follow-up and selective no-show to the medical examination has occurred especially among participants of lower socio-economic status (SES). Mothers of children in the current study population were higher educated compared with mothers of the total PIAMA population. Studies that have investigated childhood sleep behaviour in relation to SES have shown that children of lower SES have later sleep onset and shorter sleep duration, and that the association between sleep duration and BMI or overweight was in the same direction, but stronger, for children of lower SES[22]. Although behavioural aspects of sleep (e.g. chosen bedtimes) may be related to SES, we consider it unlikely that the association between sleep and cardiometabolic markers would be markedly different in children from lower educated mothers.

Previous studies

Many previous studies in children specifically related sleep duration to weight status. Two meta-analyses found that children sleeping less than 10h had 58-89% greater odds of being obese[6,23]. However, these analyses were performed on mainly the same studies, and there is still considerable heterogeneity in effects reported by individual studies. In our study, long sleep duration decreased the risk of being overweight in girls, but not in boys. The number of children defined as short sleepers in our study sample was small, and it is possible that findings for these children were non-significant due to lack of power. However, since effect estimates were small we do not expect that associations would have become significant when we would have had a larger sample. Our results are

in line with several other studies that did not find a significant association, including two recent studies in very large populations; one US study of over 81,000 children aged 6-11 years[24], and one with a longitudinal design of 13,568 adolescents aged 12–18 years[25] which found short sleep duration was not significantly predictive of obesity. Both were not included in meta-analyses mentioned earlier.

Our findings are in line with one previous study in children of a wide age range, showing that sleep duration was not associated with BP, TC and HDLC[26]. Another study in obese adolescents measured sleep duration extensively by parental report, self-report and actigraphy and found no associations with BMI, WC, BP, and glucose with all methods[27].

Studies in children relating sleep characteristics, other than sleep duration, to cardiometabolic markers are scarce. One previous study related self-reported sleep-wake pattern to weight status in 9- to 16-year-olds[16]. This study found that late to bed/late rise adolescents had significantly higher BMI z-scores (0.66 vs. 0.45, P = 0.0015), and were 1.47 times more likely to be overweight or obese than Early to bed/Early rise adolescents. In our study, late to bed/late rise boys had higher BMI z-scores of 0.07, but not significantly. While the previous study adjusted for age, the study did not measure pubertal stage and did not stratify by sex; possibly explaining the difference in results. Another study comparing normal weight and overweight children found that they did not differ in self-reported sleep quality[28]. We could not identify other studies in children that assessed self-reported sleep quality in relation to cardiometabolic markers.

Although our results partly conflict with previous literature on associations among child and adolescent sleep duration and BMI, our findings are consistent with most studies on childhood sleep and other cardiometabolic risk factors. Moreover, the majority of previous studies only assessed sleep duration while other sleep parameters as in our study have been reported scarcely, especially daytime outcomes. It is therefore important for further studies to explore other sleep characteristics besides sleep duration, to gain insight into aspects of sleep most relevant to cardiometabolic risk. These studies should assess relations of sleep with multiple cardiometabolic outcomes and not overweight solely.

Conclusion

We report novel results on the relation between different sleep characteristics and multiple cardiometabolic markers. We observed some significant associations between indicators of sleep duration and quality and anthropometric outcomes (BMI, WC) and

cholesterol levels in girls. However, effect sizes were small and probably not clinically relevant, associations were not observed in boys, and were inconsistent across specific characteristics of sleep, or cardiometabolic markers. The associations do not point in the direction of an increased or decreased cardiovascular risk. Therefore, we consider it premature to propose that improved sleep could reduce cardiovascular risk during childhood.

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Supplemental Information

Assessment of sleep characteristics

The two questions concerning the usual bedtime and get up time during schooldays, from which sleep duration and sleep-wake pattern were calculated, were adopted from the Munich Chronotype Questionnaire. This questionnaire was validated against two sleep-logs. Correlations with sleep logs for sleep midpoint, were: Morningness-Eveningness Questionnaire, MEQ: r -.73; and Composite Scale of Morningness, CSM: r -.62[1].

The question on feeling sleepy or tired during the day was adopted from a validated questionnaire, including Likert-type response options (never; rarely; sometimes; often; very often)[2].

The questions on night-time awakenings, sleep latency after awakenings, and feeling rested after waking had been used in a previous study which showed good internal consistency; Cronbach's alphas for this study were .70, .77, and .77 at T1, T2 and T3 respectively[3]. These variables are also criteria for insomnia as provided in the DSM IV[4].

Assessment of cardiometabolic markers

During home visits, when the child was aged 12 years, trained staff measured height, weight, waist circumference, and blood pressure, and blood samples were drawn. Body weight was measured at the nearest 0.1 kg and height (cm) was measured at one decimal. Both anthropometric variables were measured while only wearing underwear. BMI for age and sex Standard Deviation Scores (SDS, z-scores) were calculated using the reference growth curves of the Dutch Fourth Nation-wide Growth Study carried out in 1997[5]. Waist circumference (cm) was measured twice and rounded to one decimal. The mean of the two measurements was calculated and used in analyses.

Blood pressure readings were obtained while children were asked to rest, without talking, for ≥5 minutes in the seated position before the first oscillometric BP measure was taken from the nondominant upper arm using an Omron M6 Monitor. Depending on arm circumference, 17 to 22 cm or 22 to 32 cm cuffs were used. The mean of two subsequent measures taken at 5-minute intervals was used. If two consecutive measures differed by >5 mm Hg, another measurement was taken. The averages of (2 or 3) systolic and diastolic measurements were calculated and used in analyses.

Blood samples in the tubes designated for serum cholesterol measurement were allowed to clot at room temperature for at least 30 minutes, and then cooled at 4°C until

maximum 24 hours after blood drawing. Samples were then cooled to -20°C, and stored at -80°C for a median 128 days (IQR 73-167) before they were centrifuged for 10 minutes and analysed. Total and high-density lipoprotein (HDL) cholesterol were measured and the ratio between total and HDL cholesterol was calculated (TC/HDLC ratio). Serum total and HDL cholesterol concentrations were determined enzymatically using Roche automated clinical chemistry analysers (Roche Diagnostics, Indianapolis).

For analysis of HbA1c, erythrocytes from blood samples were stored at -20°C for a mean period of 144 days (range 29-364) prior to assay. A 5 µl cell mass was lysated and HbA1c was measured by ion-exchange chromatography using the Adams A1c, HA-8160 HPLC Auto analyser (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial (DCCT) standards.

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Chapter 4

Screen time, adiposity and cardiometabolic markers: mediation by physical activity, not snacking, among 11-year-old children

Nina E. Berentzen
Henriëtte A. Smit
Lenie van Rossem
Ulrike Gehring
Marjan Kerkhof
Dirkje S. Postma
Hendriek C. Boshuizen
Alet H. Wijga

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Abstract

Background

There is evidence for a relation of TV viewing with adiposity and increased cardiometabolic risk factors in children and adolescents. It is unclear to what extent this relation is mediated by snacking and lack of physical activity. We determined whether associations of screen time with adiposity and cardiometabolic markers were mediated by these behaviours.

Methods

Children from a population-representative Dutch birth cohort (n=1447) reported screen time and other lifestyle factors by questionnaire around the age of 11 years (range 10-14), and had anthropometry and cardiometabolic markers measured around the age of 12 years (range 12-14). Adjusted associations of screen time with snacking, physical activity, adiposity and cardiometabolic markers (TC/HDLC ratio, blood pressure, glycated haemoglobin) were assessed by using formal mediation analysis. We tested the hypothesized paths by structural equation modelling, which allows quantification of the indirect effects associated with potential mediators.

Results

Children with ≥20 hours screen time per week, consumed more snacks (1.9 vs. 1.3 portions/day) and were less physically active (4.3 vs. 4.8 days/week), than children with maximum 6 hours screen time. Screen time was directly associated with higher adiposity (standardized β=0.10 to 0.12 depending on the outcome, P<.001), and indirectly through less physical activity. The association of screen time with TC/HDLC ratio was almost completely mediated by adiposity (β=0.39, P<.0001), and to a minor extent by physical activity (β=-0.06, P=.02). There was no direct association of screen time with TC/HDLC ratio.

Conclusions

The adverse association of screen time with adiposity was partly mediated by physical activity, but not by snacking. The association of screen time with TC/HDLC ratio was almost completely mediated by adiposity. Our results may suggest that future efforts in society and public health should be directed to replace screen time with physical activity for reducing children's adiposity and cardiometabolic risk.

Introduction

There is substantial evidence linking TV viewing to overweight and increased cardiometabolic risk in children and adolescents[1-3]. This relationship may be partly mediated by indirect mechanisms, such as less physical activity and increased consumption of energy-dense snacks, drinks, fast foods and higher total energy intake[3]. Consumption of these nutritionally questionable foods and drinks during TV viewing may be fuelled by TV commercials aimed at children advertising unhealthy foods[3].

The relative contribution of these mediators to associations is, however, unclear. It has been suggested that sedentariness increases metabolic risk independent of physical activity, especially when physical activity is defined as moderate to vigorous physical activity[4]. For example, the hypothesis that screen time (TV and computer time) replaces more active pursuits is called into question by randomized controlled interventions showing that reducing total screen time decreases adolescents' weight gain without increasing physical activity[5-7]. The hypothesis that the association of TV time with cardiometabolic markers can be explained by increased adiposity is refuted by studies showing that associations persisted after adjusting for body mass index or waist circumference[8,9]. From a public health point of view it is important to clarify these potential pathways and identify the targets –time spent sedentary, time spent physically active or snack consumption– that are most promising for overweight and cardiometabolic risk prevention.

It is important to consider and measure both TV time and computer time, as they add up to total screen time[10]. However, evidence for an association with adiposity is generally stronger for TV time than for computer time, and it may be that TV time is more passive, and more often combined with snacking as well as exposure to food marketing then computer time[3,8,10].

In the current study we investigate associations of total screen time (TV time and computer time) with adiposity and cardiometabolic markers, and address the question whether these associations are mediated by snack intake and physical activity. We tested these assumptions by formal mediation analysis, which allows quantification of the indirect effects associated with potential mediators. In addition, we examined whether associations with varied between TV time and computer time.

We hypothesized that the association of more screen time with higher adiposity and cardiometabolic risk (blood pressure, cholesterol, glycated haemoglobin), is mediated by higher consumption of snacks and less time spent physically active. In addition, we

hypothesized that the association of more screen time with higher cardiometabolic risk is mediated by higher adiposity.

Subjects and methods

Participants

We used data from a population-based Dutch birth cohort study: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study with prenatal inclusion of 3 963 children in 1996/1997. A detailed description of the study design has been published previously[11,12]. At age 11, 3 541 children were still in the study. Of those, 2 651 children completed the questionnaire at age 11 (response rate 74.9%). Of all children invited (*n*=3 202) for the clinical assessment at age 12, 1 511 participated (47.2%). The study population for the current study consisted of 1 447 children with self-reported data on screen time at age 11 as well as clinical assessment at age 12. The study protocol was approved by the medical ethics committees of the participating institutes, and all parents gave written informed consent.

Assessment of exposure and potential mediators: screen time, physical activity and snacking

TV viewing, physical activity and snacking were assessed by child questionnaire at age 11 years (range 11-14). Habitual computer and TV time were reported in days per week and hours per day. At the time of data collection (2008-2009), handheld and portable computers (such as minibooks and tablets) had not yet been introduced to the public. Therefore, in this study, computer time represented time spent at a desktop computer or laptop, thus time spent sedentary. For the analysis, the reported time spent on TV viewing (hours/week) and computer use (hours/week) were summed to compute a screen time variable (hours/week). In analyses with TV time and computer time separately, these variables were transformed by taking the square root as they were non-normally distributed. Habitual physical activity was reported as the number of days/week being active (e.g. sports, cycling, playing outside) for at least one full hour a day, and was used as such in the analysis. Distributing a food frequency questionnaire was unfeasible in this large cohort. Instead, the questionnaire inquired about children's habitual consumption of five different snacks: savoury snacks (e.g. crisps, nuts), cookies, cakes/pastries, candy and snacks/fast foods (e.g. hamburger, French fries). These were reported in number of days

per week and number of portions per day (with five response options, ranging from less than 1 to more than 3 portions per day). For the analysis the reported number of portions of snacks were multiplied and divided by 7 to represent number of portions per day. The five snack variables were subsequently summed to compute a daily snack score variable (portions/day). The score ranged from 0 to 7.4 portions per day.

Assessment of anthropometry and cardiometabolic markers

Clinical assessments at age 12 years (range 12-14) were performed by trained staff during home visits. Anthropometric measurements and blood pressure (BP) readings were obtained; and blood was drawn for measurement of cholesterol and glycated haemoglobin (HbA1c). All anthropometric variables were measured while only wearing underwear. Waist circumference (WC, cm) was measured twice and rounded to one decimal. The mean of the two measurements was used in analyses. Body weight was measured at the nearest 0.1kg and height (cm) was measured at one decimal. Waist-to-height ratio (WHtR) was used in the analysis. Body mass index (BMI, kg/m2) was used in the analysis as age and sex specific standard deviation scores (z-scores) using the reference growth curves of the Dutch Fourth Nation-wide Growth Study carried out in 1997[13]. Overweight (including obesity) was defined based on international defined cut-off points[14]. BP readings were obtained while children were asked to rest, without talking, for ≥5 minutes in the seated position before the first oscillometric BP measure was taken from the nondominant upper arm using an Omron M6 Monitor. Depending on arm circumference, 17-22cm or 22-32cm cuffs were used. If two consecutive measures, taken at 5-minute intervals, differed by >5 mm Hg, another measurement was taken. The mean of (2 or 3) systolic and diastolic measurements were calculated and used in analyses. Serum total and high-density lipoprotein (HDL) cholesterol were determined enzymatically using Roche automated clinical chemistry analysers (Roche Diagnostics, Indianapolis). The total-to-HDL cholesterol ratio (TC/HDLC ratio) was used in the analysis. For laboratory determination of HbA1c, erythrocytes from blood samples were stored at -20°C for a mean period of 144 days (range 29-364) prior to assay. A 5 µl cell mass was lysated and HbA1c was measured by ion-exchange chromatography using the Adams A1c, HA-8160 HPLC Auto analyser (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial standards. TC/HDLC ratio and HbA1c were log transformed because of their skewed distributions.

Covariate assessment

Based on previous literature, the main covariates considered in the statistical analysis were age, gender, puberty development and mother's education. Mother's education was used as a measure of socioeconomic position. Age and puberty development (puberty development scale[15]) were reported by the child at age 11 and were used as continuous variables in the analysis. Mother's educational level, reported when the child was 1 year old, was used as a categorical variable in the analyses, i.e. low (primary school, lower vocational or lower secondary education), intermediate (intermediate vocational education or intermediate/higher secondary education) and high (higher vocational education and university).

Statistical analysis

Means and percentages of characteristics and cardiometabolic markers (including BMI, WC, and WtHR) were compared for children in lower and upper quintiles of screen time. To test for the association of screen time with cardiometabolic markers, as a first step we used linear regression models, adjusted for gender because of observed differences between boys and girls in screen time and cardiometabolic markers. In order to test for interaction by gender and mothers' education in the association of screen time with cardiometabolic markers, multiple regression models were fitted including interaction terms (adjusting for gender, height, age, puberty and mothers' education).

Secondly, in order to estimate the proportion of mediation in the association of screen time with cardiometabolic markers, we used structural equation modelling (SEM). SEM provides estimates of the magnitude and significance of hypothesised connections between sets of variables. Compared to multiple regression, SEM has two major advantages. First, SEM allows the specification and testing of multiple mediators in one model. Another advantage of the SEM is its ability to use multiple indicators to measure a latent construct, which increases the reliability of the parameter estimates by controlling for measurement error[16] In a regression model, multiple indicators of the same construct cause collinearity problems. When using SEM it is important to pre-specify theoretical concepts and hypotheses of the associations that will be tested. We hypothesized (see Figure 1) that the association of screen time with adiposity would be (in part) mediated by more snacking and less physical activity; and that associations of screen time with cardiometabolic markers would be mediated by higher adiposity. We aimed only to test these associations and no other possible pathways. Adiposity

is frequently measured by BMI, WC or WHtR. In order to capture several aspects of adiposity relevant for cardiometabolic risk, we used all three measures and combined these into a latent (unmeasured) variable. In Figure 1, observed variables are displayed as boxes and the latent variable as a circle. SEM was conducted for each cardiometabolic marker, adjusting for the same set of possible confounders as in the multiple regression models by using the residual method[17]. Gender differences in the association between screen time and excess body weight or fat are commonly observed, but the directions are inconsistent[8,18]. Therefore we stratified the SEM analysis by gender, also in case of non-significant interaction, to identify which pathways in the mediation model would differ between boys and girls. In addition, we analysed TV time and computer time separately as predictor variables, for cardiometabolic markers that were associated with screen time or adiposity in the SEM analysis.

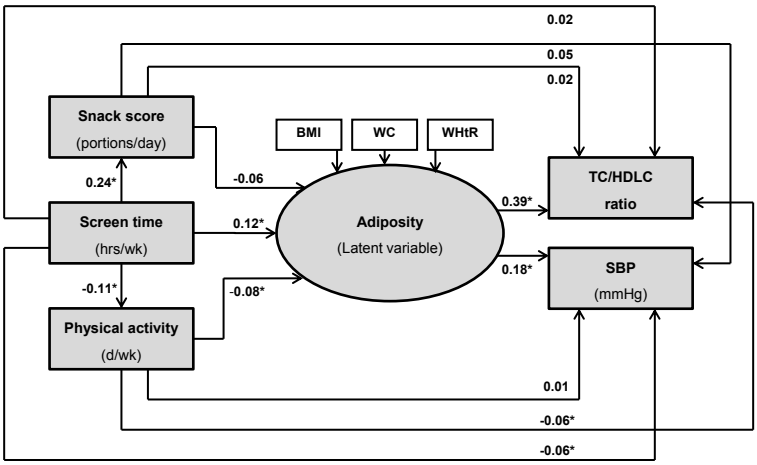

Figure 1. *Associations between screen time and cardiometabolic markers, accounting for mediation by snacking, physical activity and adiposity. Regression coefficients displayed are pathways of the model with outcome TC/HDLC ratio, except for the coefficients on pathways to SBP. The latent variable adiposity was represented by measured variables BMI, WC and WHtR. Regression coefficients are standardized and were adjusted for covariates using the residual method. *p<.05 after adjusting for age, pubertal status, height, and maternal education. Abbreviations: TC/HDLC ratio, total-to-high-density-lipoprotein cholesterol ratio; SBP, systolic blood pressure; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio.*

Table 1. Participant characteristics and cardiometabolic markers among the total study sample and among lowest and highest quintiles of screen time

	Total sample[1]				Lower Quintile of Screen Time (0-6 hours/wk)		Upper Quintile of Screen Time (20-49 hours/wk)		
	n	mean (sd) or %	lower 95% CL	upper 95% CL	*n*	mean (sd) or %	*n*	mean (sd) or %	*P* for difference[3]
Age at child questionnaire (years)	1446	11.3 (0.3)	11.3	11.4	319	11.3 (0.3)	253	11.4 (0.3)	.09
Age at clinical examination (years)	1447	12.7 (0.4)	12.6	12.7	319	12.7 (0.4)	253	12.6 (0.4)	.33
Puberty development scale	1438	1.5 (0.5)	1.5	1.6	318	1.5 (0.5)	253	1.5 (0.5)	.89
Gender (% boys)	703/1447	48.6%	-	-	112/319	35.1%[2]	160/253	63.2%[2]	<.0001
Mother's education (% low)	250/1445	17.3%	-	-	49/317	15.4%[2]	67/252	26.6%[2]	<.001
Overweight or obese (%)	177/1447	12.2%	-	-	30/319	9.4%[2]	43/253	17.0%[2]	<.001
Screen time (hours/wk)	1447	12.7 (7.9)	12.3	13.1	319	3.9 (1.7)	253	26.0 (6.1)	<.0001
Television time (hours/wk)	1447	7.5 (5.1)	7.2	7.8	319	2.3 (1.8)	253	14.5 (5.2)	<.0001
Computer time (hours/wk)	1447	5.2 (4.5)	4.9	5.4	319	1.6 (1.4)	253	11.4 (5.8)	<.0001
Snack score (portions/day)	1447	1.6 (0.9)	1.5	1.6	319	1.3 (0.8)	253	1.9 (1.0)	<.0001
Physical activity (days/wk)	1447	4.8 (1.8)	4.7	4.9	319	4.8 (1.8)	253	4.3 (1.8)	<.01
Height (cm)	1447	160.0 (7.7)	159.6	160.4	319	159.5 (7.3)	253	160.4 (8.1)	.15
Weight (kg)	1447	48.3 (9.3)	47.8	48.7	319	47.3 (8.4)	253	49.6 (10.3)	<.01
BMI (z-score)	1447	0.12 (1.1)	0.06	0.17	319	0.00 (1.0)	253	0.29 (1.2)	<.01
Waist circumference (cm)	1447	66.4 (6.7)	66.1	66.8	319	65.6 (5.9)	253	68.1 (7.9)	<.0001
Waist-to-height ratio	1447	0.42 (0.04)	0.41	0.42	319	0.41 (0.04)	253	0.42 (0.05)	<.001
TC/HDLC ratio	1235	3.1 (0.8)	3.1	3.2	271	3.1 (0.7)	209	3.3 (0.9)	<.01
Systolic BP (mmHg)	1415	115.1 (9.6)	114.6	115.6	315	115.9 (9.9)	250	114.7 (9.0)	.12
Diastolic BP (mmHg)	1415	66.7 (6.6)	66.4	67.0	315	67.1 (6.2)	250	66.7 (6.6)	.50
HbA1c (mmol/mol)	1221	32.4 (2.6)	32.3	32.6	262	32.5 (2.2)	209	32.2 (2.5)	.15

[1]Total (n=1,447): children with data on all lifestyle variables and adiposity variables, and data on cholesterol and/or BP.
[2]Percentage of children among the indicated quintile.
[3]P-value for difference between lower and upper quintiles.
Abbreviations: BMI, body mass index; BP, blood pressure; CL, confidence limits; sd, standard deviation; TC/HDLC ratio, total-to-high-density-lipoprotein cholesterol ratio.

We displayed standardized regression coefficients (range from -1 to 1), which express a difference in the outcome variable corresponding to a difference of 1 standard deviation in the exposure variable. Standardized regression coefficients are estimates that result when all variables are standardized to a mean of 0 and a variance of 1. This permits direct comparison of the regression coefficients between different variables. To assess model fit we used the root-mean-squared error of approximation (RMSEA), and Bentler's Comparative Fit Index (CFI). Typically, a RMSEA value of <0.05 and a CFI value close to 1 are indicative of good fit. The $\chi 2$-test of overall fit is very sensitive to large sample size and has therefore not been used in our models. We used the PROC CALIS procedure in SAS version 9.3 (SAS Institute, Cary, NC), using covariance matrices and the maximum-likelihood estimation method.

Results

On average, children spent 12.7 hours weekly (standard deviation 7.9) in front of a TV or computer (Table 1); boys more than girls (14.2 vs. 11.2 hours/week, data not shown in table). Children with at least 20 hours of screen time per week (upper quintile), consumed more snacks (1.9 vs. 1.3 portions/day) and were less physically active (4.3 vs. 4.8 days/week), as compared to children with a maximum of 6 hours screen time per week. They also more often had lower educated mothers (26.6%) than their peers with the least screen time (15.4%). Children who had 1 hour/week additional screen time had a significantly higher BMI of 0.01 z-score, 0.11 cm larger WC, and 0.0005 larger WtHR (Table 2). As for cardiometabolic markers, children with 1 hour/week additional screen time had a significantly higher TC/HDLC ratio (0.002 95%CI: 0.001; 0.04), but no significantly higher systolic or diastolic BP and HbA1c. No statistically significant interaction of screen time with gender or mother's education was found in relation to BMI, WC, WtHR, or cardiometabolic markers in multiple regression analyses (data not shown).

Table 2. Crude associations between screen time (hours/wk) and cardiometabolic markers

Pathway	Regression coefficient[1]	95% CI	Standardized regression coefficient[1]	*P*
Screen time → Waist circumference (cm)	0.11	0.06-0.15	0.13	<.0001
Screen time → BMI (z-score)	0.01	0.005-0.02	0.09	<.01
Screen time → Waist-to-height ratio	0.0005	0.0002-0.0007	0.10	<.001
Screen time → log TC/HDLC ratio	0.002	0.001-0.04	0.07	.01
Screen time → Systolic BP (mmHg)	-0.05	-0.11-0.02	-0.04	.13
Screen time → Diastolic BP (mmHg)	-0.02	-0.07-0.02	-0.03	.29
Screen time → log HbA1c (mmol/mol)	-0.00004	-0.001-0.001	-0.004	.88

[1]Adjusted for gender. Abbreviations: BMI, body mass index; TC/HDLC ratio, total-to-high-density-lipoprotein cholesterol ratio; 95% CI, 95% confidence interval; BP, blood pressure.

The relationships of screen time with cardiometabolic makers, mediated by snack consumption, physical activity and adiposity, are shown in Figure 1 and Table 3. Screen time was directly associated with higher adiposity (standardized β=0.10 to 0.12, P<.001), and also indirectly through less physical activity, as shown by the significant association between physical activity and adiposity (β=-0.07 to -0.08, P<.01). The association of screen time with adiposity was not mediated by more snacking. The association of screen time with TC/HDLC ratio was almost completely mediated by adiposity (β=0.39, P<.0001), and to a minor extent by physical activity (β=-0.06, P=.02); there was no direct association between screen time and TC/HDLC ratio (β=0.02, P=.41).

Although there was no statistically significant interaction with gender in the multiple linear regression model, we stratified the SEM analysis by gender to assess whether results were comparable for boys and girls. Associations were similar in boys and girls except for a difference in the association between screen time and adiposity. In boys, this association was strongly statistically significant; whereas in girls, the association was weak and not statistically significant (Table 3).

Table 3. Associations between screen time and cardiometabolic markers, accounting for mediation by snacking, physical activity and adiposity

	Total sample			Boys			Girls		
	Standardized regression coefficient[1]	s.e.	*P*	Standardized regression coefficient[2]	s.e.	*P*	Standardized regression coefficient[2]	s.e.	*P*
Outcome: log TC/HDLC ratio (n=1223)									
Screen time → Snack score	**0.24**	0.03	<.0001	**0.23**	0.04	<.0001	**0.25**	0.04	<.0001
Screen time → Physical activity	**-0.11**	0.03	<.001	**-0.11**	0.04	<.01	**-0.10**	0.04	.01
Screen time → Adiposity	**0.12**	0.03	<.001	**0.17**	0.04	<.0001	0.06	0.04	.16
Snack score → Adiposity	-0.06	0.03	.05	**-0.11**	0.04	<.01	0.001	0.04	.97
Physical activity → Adiposity	**-0.08**	0.03	<.01	-0.07	0.04	.07	-0.07	0.04	.10
Adiposity → TC/HDLC ratio (log)	**0.39**	0.02	<.0001	**0.45**	0.03	<.0001	**0.33**	0.04	<.0001
Screen time → TC/HDLC ratio (log)	0.02	0.03	.41	0.01	0.04	.87	0.03	0.04	.49
Snack score → TC/HDLC ratio (log)	0.02	0.03	.57	0.03	0.04	.43	0.01	0.04	.82
Physical activity → TC/HDLC ratio (log)	**-0.06**	0.03	.02	**-0.09**	0.04	.01	-0.03	0.04	.40
Outcome: Systolic BP (n=1404)									
Screen time → Snack score	**0.24**	0.03	<.0001	**0.25**	0.04	<.0001	**0.25**	0.03	<.0001
Screen time → Physical activity	**-0.11**	0.03	<.0001	**-0.12**	0.04	<.01	**-0.08**	0.04	.02
Screen time → Adiposity	**0.10**	0.03	<.001	**0.17**	0.04	<.0001	0.04	0.04	.31
Snack score → Adiposity	-0.05	0.03	.08	**-0.12**	0.04	<.01	0.03	0.04	.46
Physical activity → Adiposity	**-0.07**	0.03	<.01	-0.05	0.04	.21	**-0.09**	0.04	.02
Adiposity → Systolic BP	**0.18**	0.03	<.0001	**0.23**	0.04	<.0001	**0.13**	0.04	<.001
Screen time → Systolic BP	**-0.06**	0.03	.04	-0.04	0.04	.26	-0.07	0.04	.06
Snack score → Systolic BP	0.05	0.03	.06	0.07	0.04	.07	0.04	0.04	.32
Physical activity → Systolic BP	0.01	0.03	.85	-0.003	0.04	.94	0.01	0.04	.73

Table 3. continued

	Total sample			Boys			Girls		
	Standardized regression coefficient[1]	s.e.	*P*	Standardized regression coefficient[2]	s.e.	*P*	Standardized regression coefficient[2]	s.e.	*P*
Outcome: Diastolic BP (n=1404)									
Screen time → Snack score	**0.24**	0.03	<.0001	**0.25**	0.04	<.0001	**0.25**	0.03	<.0001
Screen time → Physical activity	**-0.11**	0.03	<.0001	**-0.12**	0.04	<.001	**-0.08**	0.04	.02
Screen time → Adiposity	**0.10**	0.03	<.001	**0.17**	0.04	<.0001	0.04	0.04	.31
Snack score → Adiposity	-0.05	0.03	.08	**-0.12**	0.04	<.01	0.03	0.04	.46
Physical activity → Adiposity	**-0.07**	0.03	<.01	-0.05	0.04	.21	**-0.09**	0.04	.02
Adiposity → Diastolic BP	**0.14**	0.03	<.0001	**0.17**	0.04	<.0001	**0.11**	0.04	<.01
Screen time → Diastolic BP	-0.05	0.03	.10	-0.02	0.04	.58	-0.07	0.04	.05
Snack score → Diastolic BP	0.02	0.03	.42	0.04	0.04	.29	0.005	0.04	.90
Physical activity → Diastolic BP	0.005	0.03	.86	0.02	0.04	.63	-0.01	0.04	.80
Outcome: log HbA1c (n=1210)									
Screen time → Snack score	**0.23**	0.03	<.0001	**0.22**	0.04	<.0001	**0.24**	0.04	<.0001
Screen time → Physical activity	**-0.12**	0.03	<.0001	**-0.12**	0.04	<.01	**-0.10**	0.04	.01
Screen time → Adiposity	**0.12**	0.03	<.001	**0.17**	0.04	<.0001	0.06	0.04	.16
Snack score → Adiposity	**-0.06**	0.03	.03	**-0.10**	0.04	.01	-0.02	0.04	.67
Physical activity → Adiposity	**-0.07**	0.03	.01	-0.07	0.04	.11	-0.07	0.04	.07
Adiposity → HbA1c (log)	-0.01	0.03	.80	-0.02	0.04	.65	0.01	0.04	.84
Screen time → HbA1c (log)	-0.01	0.03	.71	-0.04	0.04	.32	0.03	0.04	.55
Snack score → HbA1c (log)	0.03	0.03	.31	0.07	0.04	.10	-0.01	0.04	.82
Physical activity → HbA1c (log)	0.03	0.03	.23	0.02	0.04	.56	0.05	0.04	.27

Abbreviations: TC/HDLC ratio, total-to-high-density-lipoprotein cholesterol ratio; s.e., standard error; BP, blood pressure. Statistically significant results (p<.05) are indicated in bold.
[1]Adjusted regression models include gender, age during questionnaire, age during clinical examination, height, puberty, mother's education for all outcomes and the following additional covariates: cuff size for systolic and diastolic BP. [2]Adjusted as above but excluding gender.

Table 4. Associations of TV and computer time with cardiometabolic markers, accounting for mediation by snacking, physical activity and adiposity

	TV Time (squared)			Computer time (squared)		
	Standardized regression coefficient[1]	s.e.	*P*	Standardized regression coefficient[1]	s.e.	*P*
Outcome: log TC/HDLC ratio	n=1223			n=1224		
Screen time → Snack score	**0.18**	0.03	<.0001	**0.21**	0.03	<.0001
Screen time → Physical activity	-0.05	0.03	.09	**-0.10**	0.03	<.001
Screen time → Adiposity	**0.08**	0.03	.01	**0.09**	0.03	<.01
Snack score → Adiposity	-0.05	0.03	.12	-0.05	0.03	.08
Physical activity → Adiposity	**-0.09**	0.03	<.01	**-0.08**	0.03	<.01
Adiposity → TC/HDLC ratio (log)	**0.39**	0.02	<.0001	**0.40**	0.02	<.0001
Screen time → TC/HDLC ratio (log)	0.04	0.03	.16	-0.005	0.03	.86
Snack score → TC/HDLC ratio (log)	0.01	0.03	.60	0.02	0.03	.41
Physical activity → TC/HDLC ratio (log)	**-0.06**	0.03	.02	**-0.06**	0.03	.02
Outcome: Systolic BP	n=1404			n=1405		
Screen time → Snack score	**0.19**	0.03	<.0001	**0.22**	0.03	<.0001
Screen time → Physical activity	-0.05	0.03	.06	**-0.10**	0.03	<.001
Screen time → Adiposity	**0.07**	0.03	<.01	**0.08**	0.03	.01
Snack score → Adiposity	-0.04	0.03	.17	-0.04	0.03	.14
Physical activity → Adiposity	**-0.08**	0.03	<.01	**-0.08**	0.03	<.01
Adiposity → Systolic BP	**0.17**	0.03	<.0001	**0.17**	0.03	<.0001
Screen time → Systolic BP	-0.04	0.03	.09	-0.05	0.03	.05
Snack score → Systolic BP	0.04	0.03	.09	0.05	0.03	.07
Physical activity → Systolic BP	0.01	0.03	.74	0.01	0.03	.82
Outcome: Diastolic BP	n=1404			n=1405		
Screen time → Snack score	**0.19**	0.03	<.0001	**0.22**	0.03	<.01
Screen time → Physical activity	-0.05	0.03	.06	**-0.10**	0.03	<.001
Screen time → Adiposity	0.07	0.03	<.01	**0.08**	0.03	<.01
Snack score → Adiposity	-0.04	0.03	.17	-0.04	0.03	.14
Physical activity → Adiposity	**-0.08**	0.03	<.01	**-0.08**	0.03	<.01
Adiposity → Diastolic BP	**0.14**	0.03	<.0001	**0.14**	0.03	<.0001
Screen time → Diastolic BP	-0.02	0.03	.56	-0.05	0.03	.07
Snack score → Diastolic BP	0.01	0.03	.60	0.02	0.03	.42
Physical activity → Diastolic BP	0.01	0.03	.74	0.006	0.03	.83

Abbreviations: TC/HDLC ratio, total-to-high-density-lipoprotein cholesterol ratio; s.e., standard error; BP, blood pressure. Statistically significant results (p<.05) are indicated in bold. [1]Adjusted regression models include gender, age during questionnaire, age during clinical examination, height, puberty, mother's education for all outcomes and the following additional covariates: cuff size for systolic and diastolic BP.

Goodness of fit measures for the model with outcome TC/HDLC ratio (total sample), were: CFI; 0.996, and RMSEA 0.039 (90% confidence interval 0.022, 0.057). Fit measures for the model with outcome systolic BP were: CFI; 0.995, and RMSEA 0.040 (90% confidence interval 0.024, 0.057). These indicate good approximate model fit.The model with the outcome diastolic BP resulted in estimates that were slightly weaker compared to those of the model with the outcome systolic BP. The model with the outcome HbA1c resulted in no significant associations of screen time or adiposity with HbA1c. When we analysed TV time and computer time separately, estimates were similar to those for screen time and in the same direction (Table 4). Direct associations with higher adiposity were statistically significant for both TV time and computer time.

Discussion

This study shows that the adverse association of screen time with adiposity was partly mediated by less physical activity, but not by more snacking. The association of screen time with TC/HDLC ratio was almost completely mediated by adiposity and, to a minor extent, by physical activity.

A large number of studies have addressed the association of screen time with overweight or cardiometabolic risk. Few studies however, have investigated the pathways linking the two phenomena, using statistical methods adequately suited for this purpose[19-22]. The previous studies differed with regard to the exposure (TV viewing, TV in the bedroom, computer time or sedentary time), the outcome (two studies examined BMI/WC, two examined other cardiometabolic markers), and mediators (no study so far included snacking or physical activity in combination with adiposity).

One study found that the association of TV in the bedroom with BMI/WC was not mediated by physical activity[20]; while another study found that the association of TV time and BMI was not mediated by snacking during TV viewing and junk food consumption[21]. A third study found that the association of sedentary time with cardiometabolic markers was mediated by BMI or WC[22], while a fourth study could not replicate this[19]. To our knowledge, our study was the first to test three potential mediators (snacking, physical activity and adiposity) simultaneously.

Despite the strong association of screen time with snacking in the expected direction, snacking was not a mediator in the association with adiposity. Although this may

seem surprising, contrary to what is generally believed snacking has not been consistently associated with overweight or measures of body fat in previous observational studies[23-25]. It has been suggested that it is the remainder of the diet, not the snacks per se, that is responsible for overweight in children[25]. Regrettably, in the current study we could not adjust for energy intake.

Our stratified results indicated that in boys, there was a significant direct association of screen time with adiposity; while in girls, there was no direct association. The interaction of screen time with gender, however, was not statistically significant.

Children with more screen time had lower educated mothers. In addition, children in the study sample had higher educated mothers (intermediate or high: 82% vs. 77%) than children of the total PIAMA study population (*n*=3 807). The study sample may therefore underrepresent children of lower educated families or families with a lower socio-economic position. However, we found no significant interaction between mother's education and screen time, so we treated mother's education as a confounder and adjusted for it in the analysis. Although we cannot exclude the possibility of residual confounding by education or socio-economic position, we do not expect that the mechanisms linking screen time to adiposity would be very different between children of lower or higher socio-economic position.

Strengths of our study were application of structural equation modeling as a formal mediation analysis, which accounts for interrelationships among all analysed variables simultaneously; adjustment for a number of important confounders; and capturing several aspects of adiposity relevant for cardiometabolic risk by combining three measures of body composition in a latent adiposity variable. Some limitations need to be addressed. Diet and physical activity data were self-reported, which may have led to misclassification of these behaviors due to selective underreporting. In case this occurred, associations would be underestimated, but it is unlikely that the associations we found would not have been found in case of more objective measurements. TV time and computer time are indicators for sedentary behaviour, but may not accurately represent total sedentary time. Therefore, our results regarding the association with adiposity should be interpreted for screen time alone and not for total sedentary time. The correlation between self-reported and directly measured sedentary behaviour is generally small, suggesting that they measure different constructs[26,27]. Surprisingly, associations with markers of adiposity and cardiometabolic risk in children are stronger for self-reported screen time than for objectively measured total sedentary time[27]. It may be that other characteristics of

sedentary behaviour than total sedentary time are more important and recently patterns of sedentary behaviour have become a matter of interest. Evidence in adults suggests that prolonged bouts of uninterrupted sedentary behaviour have a deleterious impact on markers of insulin sensitivity and triglyceride levels[28]. Accelerometer-derived breaks in sedentary time have been associated with reduced cardiometabolic risk in children by some[29,30], but not all studies[31,32] and warrant further research.

A review of longitudinal studies in children concluded that there was insufficient evidence for a longitudinal association between sedentary time (mainly TV viewing) and markers of adiposity or metabolic risk[8]. The present study does not exclude the possibility that excess body weight may predispose children toward more time spent sedentary and less time spent physically active. Although screen time, physical activity and snacking were assessed prior to the measurement of cardiometabolic markers in our study, this was an observational study which does not allow identifying causal relationships. More research with longer follow-up or trials is important for a better understanding of the impact of sedentary behavior, physical activity and diet on cardiometabolic markers. Screen time is a potential modifiable factor and was already varying greatly (0-49 hours/week) in our study population at the age of 11 years. If limiting screen time would reduce adiposity directly, or indirectly through promoting physical activity, this could have important benefits at a population level. Our results may suggest that future efforts in society and public health should be directed to replace screen time with physical activity for reducing children's adiposity and cardiometabolic risk.

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Chapter 5

Overweight patterns throughout childhood and cardiometabolic markers in early adolescence

Nina E. Berentzen
Lenie van Rossem
Ulrike Gehring
Gerard H. Koppelman
Dirkje S. Postma
Johan C. De Jongste
Henriëtte A. Smit
Alet H. Wijga

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Abstract

Background

Risk of cardiovascular and metabolic disease is higher in adults who were relatively thin at birth and had subsequent accelerated weight gain. This specific pattern of weight gain may relate to unfavourable cardiometabolic markers already in childhood. We prospectively assessed whether children with different patterns of overweight development from age 3 months to 11 years had distinct levels of cardiometabolic markers at age 12 years.

Subjects/Methods

We used data of 1500 children participating in the PIAMA birth cohort that started in 1996/1997. Parents reported height and weight during 10 waves of follow-up from age 3 months to 11 years. Four distinct overweight development patterns were derived using longitudinal latent class analysis; 'never'; 'early transient'; 'gradually developing' and 'persistent' overweight. Cardiometabolic markers (total-to-high-density lipoprotein cholesterol (TC/HDLC) ratio, blood pressure (BP), glycated haemoglobin (HbA1c)) were assessed at age 12 years in 1500 children.

Results

Children who developed overweight gradually and children with persistent overweight throughout childhood, at age 12 had a 2-3 fold higher risk of having high (>90th centile) TC/HDLC ratio, systolic and diastolic BP, compared to children who were never overweight. In children who gradually developed overweight, TC/HDLC ratio was 0.75 higher (95%CI 0.54-0.96); systolic BP 4.90 mmHg higher (95%CI 2.45-7.36) and diastolic BP 1.78 mmHg higher (95%CI 0.07-3.49) than in children who never had overweight. Estimates for children with persistent overweight were similar.

Conclusions

Children with gradually developing overweight, and those with persistent overweight had unfavourable cholesterol and blood pressure levels already at age 12, whereas children with early transient overweight avoided these unfavourable outcomes. Our results support the hypothesis that specific overweight patterns predispose to an adverse cardiometabolic profile, which is already apparent in early adolescence before progressing to adult cardiometabolic disease.

Introduction

Adults with coronary heart disease and diabetes more often experienced a specific pattern of weight gain, characterized by relative thinness at birth and subsequent accelerated weight gain during childhood, than adults who do not develop these diseases[1-5]. The observed association between growth in early life and disease many decades later raises the question whether this increased cardiometabolic risk already builds up during childhood and adolescence. Prospective studies showed that children with accelerated weight gain have higher levels of cardiometabolic markers, such as blood pressure and cholesterol, than those who follow normal curves[6,7]. However, most of these studies focused on weight gain in infancy or in other specific periods and lacked repeated assessment of weight status with small time-intervals throughout childhood. A better understanding of how patterns of overweight in childhood contribute to an unfavourable cardiometabolic profile will help to determine which stages of childhood are crucial for overweight prevention[7,8].

An important strength of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study is the availability of 10 assessments of body mass index (BMI) between birth and 11 years of age, and measurements of multiple cardiometabolic markers; cholesterol, blood pressure and HbA1c at age 12 years. In a previous study using data from this cohort[9], we showed that distinct patterns of overweight exist from 3 months to 11 years. In the current study, we examined whether children following these patterns had distinct levels of cardiometabolic markers at age 12.

Methods

Study participants

We used the data from a population-based ongoing Dutch birth cohort study: the PIAMA study, with prenatal inclusion of 3963 children in 1996/1997. A detailed description of the study design was published previously[10]. At age 11 years, 3541 children (89%) were still in the study. At age 12, 3202 children were invited to a clinical assessment, and 1511 of them were willing to participate (response rate 47%). The study population for the current study consisted of 1500 children of whom data were available for at least 4 observations of body mass index between the ages of 3 months to 11 years, and for any of the cardiometabolic

markers at age 12. The study protocol was approved by the medical ethics committees of the participating institutes. All parents gave written informed consent for the general study and separately for the clinical assessment; additionally, children themselves gave written informed consent for the clinical assessment.

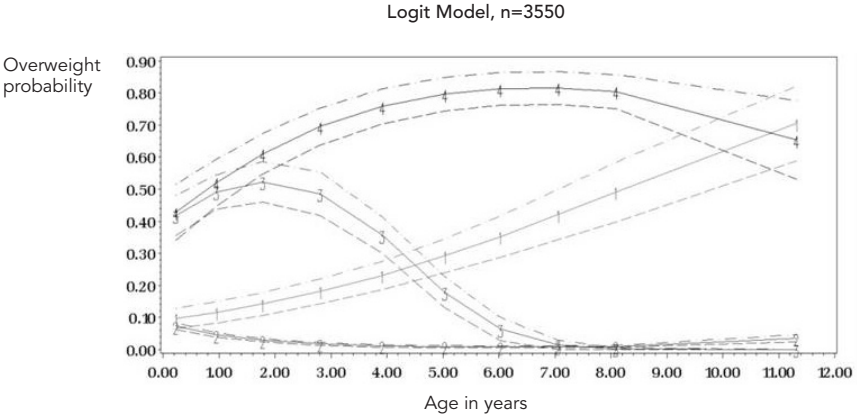
Assessment of childhood BMI between birth and 11 years

Parents reported child's height (in cm) and weight (in kg) at the age of 3 months, annually from age 1 to 8 years, and again at 11 years. Parents were asked to report child's weight and height measured by a medical professional during the regular scheduled visits to a youth health centre if this measurement was within the last 3 months. Otherwise parents were asked to measure weight and height themselves without shoes and heavy clothes. BMI was calculated as weight/height2, and percentiles were calculated according to national growth curves, derived from the National Growth Study[11]. A child was considered overweight when BMI for age and sex was above the 90th percentile of the reference population. Previously we reported on the validity of self-reported versus measured height and weight, and found that BMI (as measured by height and weight) reported by the parents underestimates the absolute prevalence of overweight. Since the difference between self-reported and measured BMI was systematic, the use of parental-reported height and weight is a valid method for identifying children in the highest decile for BMI[12,13].

Patterns of overweight development

In a previous study, longitudinal latent class analysis (LLCA) was used to identify patterns of overweight development from age 3 months to 11 years[9]. These patterns were included as independent variables in the current study. Four overweight patterns were derived from 90% of the population at baseline (3550/3963): never overweight, early transient overweight, gradually developing overweight, and persistent overweight. Each pattern describes the risk of being overweight (i.e. ≥90th percentile) from the age of 3 months to 11 years. The patterns represent distinct typical patterns of overweight development that exist in the population. They do not represent distinct groups of individual children: rather, individual children have a certain probability of belonging to each of the four patterns (posterior membership probabilities). The never overweight pattern is characterized by a low probability of being overweight (<5%) at every age (Figure 1). The early transient overweight pattern is characterized by a relatively high probability (around 45%) of

being overweight at age 3 months, followed by a decrease from age 2 onwards, and reaching a probability near 0% around the age of 7. The gradually developing overweight pattern is characterized by a 10% probability of being overweight at age 3 months, gradually increasing to around 70% at the age of 11. The persistent overweight pattern is characterized by an already high (40%) probability of being overweight early in life that reaches a peak (about 80%) around the age of 7 (see supplemental text S1 for further description of the overweight patterns).



1. Gradually developing overweight pattern
2. Never overweight pattern
3. Early transient overweight pattern
4. Persistent overweight pattern

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Figure 1. *Four patterns of overweight development in the PIAMA birth cohort study*

Assessment of cardiometabolic markers at age 12 years

Clinical assessments around age 12 (range 12–14y) were performed by trained staff during home visits. Systolic and diastolic blood pressures (BP) were measured according to the recommendations of the American Heart Association Council on High Blood Pressure Research[14]. BP readings were obtained from the non-dominant upper arm using an Omron M6 monitor while the child was seated. The first measurement was taken after≥5 minutes of rest, without talking. Depending on arm circumference, 17 to 22 cm or 22 to 32 cm cuffs were used. BP was measured at least twice with 5 minutes intervals. If two consecutive measures differed by >5 mm Hg, another measurement was taken. The means of (2 or 3) systolic and diastolic measurements were used in analyses.

Blood was drawn for measurement of cholesterol and glycated haemoglobin (HbA1c). Serum total and high-density lipoprotein (HDL) cholesterol were determined enzymatically using Roche automated clinical chemistry analyzers (Roche Diagnostics, Indianapolis). The ratio between total and HDL cholesterol was calculated (TC/HDLC ratio). For analysis of HbA1c, erythrocytes from blood samples were stored, a 5 µl cell mass was lysated and HbA1c was measured by ion-exchange chromatography using the Adams A1c, HA-8160 HPLC Auto analyser (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial (DCCT) standards.

Characteristics of the study population

Characteristics that were used to describe the study population were child's sex, ethnicity, age, pubertal development, overweight, waist circumference, waist-to-height ratio, maternal weight gain during pregnancy, preterm birth, birth weight, breastfeeding, exposure to in-utero smoking and to second-hand smoking, maternal and paternal education and parental BMI. Child's weight, height and waist circumference were measured during the clinical assessment at age 12. Waist-to-height ratio and BMI for age and sex were calculated and overweight (including obesity) was defined based on international cut-off points[15]. Preterm birth was defined as gestational age <37 weeks. Breastfeeding was categorized as no breastfeeding, ≤16 weeks, and >16 weeks. Ethnicity was based on country of birth of the child's parents, and was categorized as Dutch, Non-Dutch western and nonwestern. Mother's and father's educational level were categorized as low (primary school, lower vocational or lower secondary education), intermediate (intermediate vocational education or intermediate/higher secondary education) and high (higher vocational education and university). Maternal smoking during pregnancy was defined as smoking at least 4 weeks after onset of pregnancy. Exposure to second-hand smoking at home was defined as smoking within the child's home once a week or more. Mother's and father's BMI were calculated from reported weight and height and categorized as overweight (BMI ≥ 25 (kg/m2)) or not overweight (BMI < 25 kg/m2)). Pubertal development (puberty development scale; 1-4)[16] was reported by the child at age 11 and used as a continuous variable in the analysis.

Statistical analyses

In order to describe children of the total study population and in each of the patterns separately we computed frequencies and means, weighted according to children's probability of belonging to each pattern. We examined the association of overweight patterns with cardiometabolic markers in two ways. Firstly, we applied multiple linear regression analysis for each cardiometabolic marker separately, treating the never overweight pattern as reference. Posterior probabilities for three patterns (excluding the never overweight pattern) were included as independent variables in the regression models. Secondly, besides the analysis with cardiometabolic markers as continuous outcome variables, we investigated associations of overweight patterns with the risk of having unfavourable levels of cardiometabolic markers. Using Poisson regression with robust error variance we estimated relative risks (RRs) of having unfavourable levels of cardiometabolic markers. We used >90th percentile (<10th percentile for HDLC) as cut-off value to indicate unfavourable levels. Cut-offs were: TC >4.9 mmol/L; HDLC <1.0 mmol/L; TC/HDLC ratio >4.2; systolic BP >127 mmHg; diastolic BP >75 mmHg; and HbA1c >35.5 mmol/mol. We assessed clustering of cardiometabolic markers to define children with the metabolic syndrome phenotype, as described and applied in previous studies[17-19]. We estimated RRs of having clustering of cardiometabolic markers; two or more of unfavourable cholesterol (TC, HDLC, or TC/HDLC ratio); BP (systolic or diastolic BP); or HbA1c.

Factors that differed between overweight patterns (see Table 1) were examined for their role as potential confounders. The confounders included in the statistical analysis were sex, ethnicity, maternal and paternal education. We selected these confounders based on prior knowledge and their associations (p<.20) with the outcome of interest or a change in effect estimate of more than 10%. Analyses with blood pressure were additionally adjusted for cuff size.

Overweight may cluster within families due to shared familial factors (genetics and environment). In addition, maternal overweight during pregnancy may predispose the child for overweight by intrauterine programming of the child's endocrine system[20,21]. Maternal prepregnancy BMI captures all of these aspects in one variable; therefore, we additionally adjusted for this variable in separate regression models. Sex has been shown to affect cardiovascular risk[22,23]. We therefore included interaction terms between sex and overweight pattern in the regression models to estimate associations between overweight patterns and cardiometabolic markers for boys and girls separately.

Table 1. Characteristics of the total study population and for each of the overweight patterns

	Total study population (n=1500)		Never overweight pattern (n=1220)[1]	Early transient overweight pattern (n=130)[1]	Gradually increasing overweight pattern (n=95)[1]	Persistent overweight pattern (n=55)[1]
	n/N	Mean (SD) or %	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)
Characteristics in childhood						
Boy (%)	734/1500	48.9	48.1 (1.4)	49.2 (3.2)	56.6 (3.7)	48.7 (6.0)
Firstborn (%)	778/1500	51.9	52.9 (1.4)	50.7 (3.2)	47.7 (3.8)	42.3 (5.9)
Preterm birth (<37 weeks, %)	67/1498	4.5	4.7 (0.6)	3.7 (1.2)	5.2 (1.8)	0.8 (0.4)
Birth weight (kg)	1498	3532.3 (526.4)	3487.1 (463.0)	3670.8 (162.2)	3640.8 (151.2)	3848.9 (72.3)
No breastfeeding (%)	218/1497	14.6	13.4 (1.0)	18.0 (2.6)	21.5 (3.2)	14.3 (4.0)
Breastfeeding for more than 16 weeks (%)	595/1497	39.8	40.9 (1.4)	37.3 (3.1)	32.7 (3.5)	38.3 (5.8)
Maternal weight gain during pregnancy (kg)	1458	13.6 (4.8)	13.4 (4.2)	14.0 (1.6)	13.9 (1.4)	14.6 (1.1)
Maternal age at child's birth (years)	1485	31.3 (3.7)	31.3 (3.3)	31.4 (1.2)	31.3 (1.0)	31.9 (0.7)
Low maternal education (%)	261/1498	17.4	17.1 (1.1)	15.8 (2.3)	22.7 (3.2)	17.3 (4.5)
High maternal education (%)	618/1498	41.3	41.7 (1.4)	43.3 (3.2)	33.8 (3.5)	41.7 (5.9)
Low paternal education (%)	307/1488	20.6	20.1 (1.1)	17.9 (2.5)	27.9 (3.5)	24.4 (5.1)
High paternal education (%)	692/1488	46.5	48.8 (1.4)	43.9 (3.2)	30.4 (3.4)	39.9 (5.9)
Non-Dutch western ethnicity (%)	52/1479	3.5	3.4 (0.5)	5.7 (1.6)	2.0 (1.0)	4.0 (2.5)
Nonwestern ethnicity (%)	68/1479	4.6	4.5 (0.6)	5.1 (1.4)	5.6 (1.8)	2.8 (1.8)
Exposure to intrauterine smoking (%)	212/1491	14.2	14.4 (1.0)	10.9 (1.9)	19.5 (3.1)	9.5 (3.3)
Exposure to second-hand smoking age 11 (%)	162/1462	11.1	10.4 (0.9)	8.1 (1.7)	18.2 (3.1)	19.3 (4.8)
Maternal overweight before pregnancy (%)	250/1407	17.8	15.4 (1.1)	15.8 (2.5)	33.5 (3.8)	39.9 (6.2)
Maternal overweight child's age 1 (%)	324/1425	22.7	19.4 (1.1)	23.8 (2.9)	40.6 (3.9)	51.9 (6.1)
Maternal overweight child's age 8 (%)	445/1433	31.1	28.3 (1.3)	26.8 (3.0)	50.5 (3.9)	61.9 (5.9)
Paternal overweight child's age 8 (%)	671/1382	48.6	45.0 (1.5)	46.9 (3.4)	72.8 (3.4)	80.8 (4.8)
Age at clinical assessment (years)	1500	12.7 (0.4)	12.7 (0.3)	12.7 (0.1)	12.6 (0.1)	12.6 (0.1)
Puberty development scale (1-4)	1456	1.5 (0.5)	1.5 (0.5)	1.5 (0.2)	1.7 (0.2)	1.6 (0.1)

Table 1. continued

	Total study population (n=1500)		Never overweight pattern (n=1220)[1]	Early transient overweight pattern (n=130)[1]	Gradually increasing overweight pattern (n=95)[1]	Persistent overweight pattern (n=55)[1]
	n/N	Mean (SD) or %	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)	Weighted mean (SD) or weighted % (SE)
Adiposity at age 12 y						
Overweight/obesity (%)	184/1497	12.3	5.8 (0.6)	5.1 (1.2)	59.5 (3.5)	68.1 (5.4)
Waist circumference (cm)	1494	66.4 (6.6)	65.0 (4.7)	66.6 (1.6)	75.2 (2.5)	76.4 (1.5)
Waist-to-height ratio	1494	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)	0.5 (0.0)	0.5 (0.0)
Cardiometabolic markers at age 12 y						
Total cholesterol (mmol/L)	1276	4.1 (0.6)	4.0 (0.6)	4.1 (0.2)	4.1 (0.2)	4.1 (0.1)
HDL cholesterol (mmol/L)	1275	1.4 (0.3)	1.4 (0.3)	1.4 (0.1)	1.2 (0.1)	1.2 (0.1)
TC/HDLC ratio	1275	3.1 (0.8)	3.1 (0.7)	3.0 (0.2)	3.6 (0.3)	3.6 (0.2)
Systolic blood pressure (mmHg)	1460	115.1 (9.5)	115.0 (8.4)	114.5 (2.9)	115.8 (2.7)	116.3 (2.0)
Diastolic blood pressure (mmHg)	1460	66.7 (6.5)	66.6 (5.7)	66.3 (2.1)	66.9 (1.9)	68.4 (1.4)
HbA1c (mmol/mol)	1261	32.4 (2.6)	32.4 (2.3)	32.4 (0.8)	32.4 (0.8)	32.3 (0.5)

[1]Based on children's highest posterior probability

Childhood overweight is linked to earlier pubertal development in girls[24], and possibly in boys[25], and pubertal development affects levels of cardiometabolic markers[24]. We therefore considered pubertal development a potential mediator, hypothesizing that the gradually developing and persistent overweight patterns would be associated with advanced pubertal development at age 11 years, and that associations between these overweight patterns and cardiometabolic markers could be partly mediated by more advanced pubertal development. We investigated this in a sensitivity analysis by conducting path analysis (CALIS procedure in SAS) using covariance matrices and the maximum-likelihood estimation method. All analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC).

Results

Based on pattern of the highest posterior probability, 81% of the total study population (1220/1500) belonged to the never overweight pattern; 9% (n=130) to the early transient overweight pattern; 6% (n=95) to the gradually developing overweight pattern; and 4% (n=55) to the persistent overweight pattern. The current prevalence of overweight at age 12 was 12.3% in the total study population (Table 1). The prevalence of overweight at age 12 was similar in the never overweight pattern and the early transient overweight pattern (5.8% and 5.1%, respectively), and it was highest in the persistent overweight pattern (68.1%). Children in the gradually developing overweight pattern more often had lower educated parents, were more often boys, exposed to intra-uterine smoking, and of nonwestern ethnicity compared with those in the never overweight pattern. Children in the persistent overweight pattern had the highest prevalence of parental overweight compared with those in the never overweight pattern.

The levels of cardiometabolic markers in the early transient overweight pattern were similar to those in the never overweight pattern (Table 2). In contrast, in both the gradually developing and persistent overweight patterns, levels of HDLC were lower, TC/HDLC ratios were higher and levels of systolic BP were higher than in the never overweight pattern (Table 2). In the gradually developing overweight pattern, TC was 0.21 mmol/L higher (95% CI 0.03; 0.39), HDLC was -0.21 mmol/L lower (95% CI -0.30; -0.13); TC/HDLC ratio 0.75 higher (95% CI 0.54; 0.96); systolic BP 4.90 mmHg higher (95% CI 2.45; 7.36) and diastolic BP 1.78 mmHg higher (95% CI 0.07; 3.49) than in the

never overweight pattern. Estimates for the persistent overweight pattern were similar to those for the gradually developing overweight pattern. HbA1c was similar in all of the overweight patterns.

Besides mean differences of absolute levels of cardiometabolic markers between patterns, we estimated relative risks of having unfavourable levels of cardiometabolic markers. In both the gradually developing and persistent overweight patterns, relative risks of unfavourable levels of HDLC, TC, TC/HDLC ratio, systolic and diastolic blood pressure were increased and ranged between 2.04-3.32 (Table 3). The RR for clustering of two or more cardiometabolic markers was elevated in the gradually developing overweight pattern (RR 2.34 (95% CI 1.16-4.73)), and was increased but not statistically significant in the persistent overweight pattern (RR 1.96 (95% CI 0.82-4.66)).

Point estimates were somewhat larger in boys than in girls, although there was overlap of the 95% confidence intervals for most cardiometabolic markers (Table 4). When we additionally adjusted for maternal prepregnancy BMI, associations remained statistically significant (supplemental Table 1). Children in the gradually developing and persistent overweight patterns had advanced puberty compared with those in the never overweight pattern, but this mediated the associations with TC, HDLC, systolic and diastolic BP only slightly (supplemental Table 2).

Table 2. Differences[1] in levels of cardiometabolic markers of three overweight patterns compared with a never overweight pattern

	Never overweight pattern	Early transient overweight pattern		Gradually developing overweight pattern		Persistent overweight pattern	
	Absolute level (reference)	difference[3]	*P*	difference[3]	*P*	difference[3]	*P*
Total cholesterol (mmol/L)	4.4	0.01 (-0.13; 0.16)	.86	**0.21 (0.03; 0.39)**	**.02**	0.06 (-0.15; 0.27)	.56
HDL cholesterol (mmol/L)	1.5	0.05 (-0.02; 0.12)	.14	**-0.21 (-0.30; -0.13)**	**<.0001**	**-0.17 (-0.26; -0.08)**	**<.001**
TC/HDLC ratio	3.0	-0.12 (-0.29; 0.06)	.20	**0.75 (0.54; 0.96)**	**<.0001**	**0.55 (0.30; 0.79)**	**<.0001**
Systolic blood pressure (mmHg)[2]	118.9	-0.11 (-2.11; 1.88)	.91	**4.90 (2.45; 7.36)**	**<.0001**	**4.30 (1.51; 7.09)**	**<.01**
Diastolic blood pressure (mmHg)[2]	69.3	-0.30 (-1.69; 1.09)	.67	**1.78 (0.07; 3.49)**	**.04**	3.41 (1.46; 5.35)	<.01
HbA1c (mmol/mol)	33.1	-0.07 (-0.66; 0.52)	.82	-0.21 (-0.93; 0.50)	.56	-0.11 (-0.93; 0.72)	.80

[1]Adjusted for sex, ethnicity, maternal education, and paternal education.[2]Additionally adjusted for cuff size. [3]Regression coefficients (95% confidence intervals) of the mean level of the cardiometabolic marker in each of the overweight patterns versus the mean level in the never overweight pattern

Table 3. Relative risks[1] of having high[3] (>90th centile) cardiometabolic markers for three overweight patterns compared with a never overweight pattern

	Never overweight pattern	Early transient overweight pattern	Gradually developing overweight pattern	Persistent overweight pattern
		RR (95%CI)	RR (95%CI)	RR (95%CI)
High total cholesterol	Ref.	1.15 (0.54-2.44)	**2.04 (1.02-4.09)**	0.93 (0.32-2.70)
Low HDL cholesterol (<10th centile)	Ref.	0.88 (0.42-1.86)	**2.58 (1.53-4.35)**	1.59 (0.71-3.56)
High Total/HDL cholesterol ratio	Ref.	0.49 (0.17-1.35)	**3.32 (2.01-5.48)**	**2.85 (1.53-5.33)**
High systolic BP[2]	Ref.	0.80 (0.38-1.71)	**3.16 (1.47-6.79)**	2.18 (0.85-5.62)
High diastolic BP[2]	Ref.	0.58 (0.24-1.43)	1.99 (0.87-4.57)	**2.97 (1.42-6.21)**
High HbA1c	Ref.	1.05 (0.56-1.97)	1.11 (0.53-2.35)	0.87 (0.34-2.23)
Clustering of two or more cardio-metabolic markers with high levels[4]	Ref.	0.66 (0.24-1.85)	**2.34 (1.16-4.73)**	1.96 (0.82-4.66)

[1]Adjusted for sex, ethnicity, maternal education, and paternal education.

[2]Additionally adjusted for cuff size.

[3]Cut-offs for >90th percentile (for HDLC <10th percentile) were: TC >4.9 mmol/L; HDLC <1.0 mmol/L; TC/HDLC ratio >4.2;
systolic BP >127 mmHg; diastolic BP >75 mmHg; and HbA1c >35.5 mmol/mol.

[4]Having two or more of HDLC <10th centile or TC >90th centile or
TC/HDLC ratio >90th centile; systolic or diastolic BP >90th centile; and HbA1c >90th centile.

Associations with a significance level of P<0.05 are boldfaced.

Table 4. Differences[1] in levels of cardiometabolic markers of three overweight patterns compared with a never overweight pattern, by sex

	Never overweight pattern	Early transient overweight pattern		Gradually developing overweight pattern		Persistent overweight pattern	
	Absolute level (reference)	difference[3]	*P*	difference[3]	*P*	difference[3]	*P*
Girls							
Total cholesterol (mmol/L)	4.0	0.03 (-0.19; 0.24)	.80	-0.008 (-0.26; 0.25)[†]	.95	-0.04 (-0.32; 0.24)[†]	.76
HDL cholesterol (mmol/L)	1.3	0.001 (-0.10; 0.10)	.98	**-0.22 (-0.33; -0.09)**	**<.001**	**-0.18 (-0.30; -0.05)**	**.01**
TC/HDLC ratio	3.2	-0.002 (-0.25; 0.25)	.99	**0.54 (0.24; 0.83)[†]**	**<.001**	**0.56 (0.24; 0.88)**	**<.01**
Systolic blood pressure (mmHg)[2]	122.6	-0.26 (-3.25; 2.72)	.86	**4.67 (0.84; 8.50)**	**.02**	**4.11 (0.06; 8.15)**	**.05**
Diastolic blood pressure (mmHg)[2]	70.0	-0.65 (-0.02; 4.84)	.52	2.31 (-0.21; 4.84)	.07	2.44 (-0.23; 5.10)	.07
HbA1c (mmol/mol)	32.7	0.22 (-0.70; 1.14)	.65	-0.10 (-1.23; 1.04)	.87	0.24 (-0.97; 1.44)	0.70
Boys							
Total cholesterol (mmol/L)	4.0	0.03 (-0.17; 0.24)	.75	**0.40 (0.15; 0.65)[†]**	**<.01**	0.23 (-0.08; 0.55)[†]	.14
HDL cholesterol (mmol/L)	1.4	0.09 (0.001; 0.19)	.05	**-0.20 (-0.32; -0.09)**	**<.001**	**-0.16 (-0.30; -0.01)**	**.03**
TC/HDLC ratio	3.1	-0.18 (-0.43; 0.07)	.15	**0.92 (0.62; 1.22)[†]**	<.0001	0.56 (0.19; 0.94)	<.01
Systolic blood pressure (mmHg)[2]	122.0	0.12 (-2.56; 2.80)	.93	**5.07 (1.89; 8.24)**	**<.01**	**4.57 (0.68; 8.46)**	**.02**
Diastolic blood pressure (mmHg)[2]	70.6	-0.06 (-2.05; 1.93)	.95	1.59 (-0.77; 3.94)	.19	**4.47 (1.59; 7.36)**	**<.01**
HbA1c (mmol/mol)	32.7	-0.29 (-1.04; 0.46)	.44	-0.31 (-1.21; 0.60)	0.51	-0.45 (-1.59; 0.69)	.44

[1]Adjusted for ethnicity, maternal education, and paternal education.
[2]Additionally adjusted for cuff size
[3]Regression coefficients (95% confidence intervals) of the mean level of the cardiometabolic marker in each of the overweight patterns versus the mean level in the never overweight pattern
[†]Statistically significant (p<.15) interaction with sex.

Discussion

In our prospective study of 1500 children, we observed that already in early adolescence cardiometabolic profiles are unfavourable in children who develop overweight across childhood and in children with persistent overweight, compared to never-overweight children. These children had a 2-3 fold higher risk of being in the upper 10th percentile of TC/HDLC ratio and of systolic or diastolic BP. In children whose overweight gradually diminished in their preschool years, cardiometabolic profiles at age 12 years were comparable to those in children who never had overweight.

Our findings extend evidence from retrospective studies showing that adults with diabetes and CVD more often have accelerated weight gain during childhood and suggest that an unfavourable cardiometabolic profile already builds up during childhood. The pattern of gradually developing overweight reflects accelerated weight gain after being relatively thin in infancy. It is remarkable that children with this pattern had levels of cardiometabolic markers at age 12 as high as for children with persistent overweight. As a potential mechanism to explain the increased cardiometabolic risk in individuals with early accelerated weight gain Barker suggested that relative thinness at birth is associated with persistent reductions in muscle mass, and that subsequent accelerated weight gain may lead to a disproportionately high fat mass in relation to muscle mass[1]. It has also been suggested that growth acceleration in childhood programs abnormal vascularization and endothelial dysfunction, signs of early atherosclerosis[26]. Singhal and Lucas called this the 'growth acceleration hypothesis' and stated that the adverse effects may not be exclusive to the infancy period or any specific time window[26].

Three previous studies used a data-driven approach such as LLCA, to identify distinct patterns of (over)weight development in the paediatric population and related these to cardiometabolic markers. Their findings were similar to ours, in that, in addition to persistent overweight, a pattern of gradually developing overweight was associated with higher blood pressure at age 18[27], insulin resistance at age 14[22] and multiple cardiometabolic markers in girls at age 15[28]. Extending previous findings, our study showed that unfavourable cardiometabolic markers were already present at the age of 12 years, and that there was clustering of cardiometabolic markers especially in children who gradually developed overweight. Our results also show that the cardiometabolic profile for those with early transient overweight is similar to those who were never overweight, as was previously observed for systolic blood pressure[27] and insulin resistance[22] in adolescents.

This is important because it may point towards potential benefits of interventions aimed at limiting excessive weight gain in children who start life with a relatively high BMI.

High birth weight is a strong risk factor for overweight later in childhood, but heavy infants are not necessarily predestined to become overweight children. In a substantial proportion (around 70%) of the heavier infants in our study population, overweight gradually diminished before the age of 7 years. These children, following the early transient overweight pattern, had levels of cardiometabolic markers at age 12 years that were similar to the levels of children who were never overweight. At the same time, low overweight risk in infancy is no guarantee that overweight risk will remain low throughout childhood. In a subgroup of children without overweight in infancy, overweight risk started to rise already in the first year of life and continued to rise throughout childhood. These children, following the gradually developing overweight pattern, had similarly unfavourable cardiometabolic profiles at age 12 years as children with persistent overweight throughout childhood. Remarkably, the direction in which overweight risk will develop (i.e. decreasing or increasing) seems to be established already in the first years of life, making this a particularly sensitive period for strategies aiming to prevent excessive weight gain and related unfavourable cardiometabolic markers.

Strengths of the study are the longitudinal design with 10 repeated assessments of height and weight, the large sample size, and the availability of measurements of multiple cardiometabolic markers. We investigated the potential role of accelerated pubertal development by conducting a mediation analysis. A potential limitation may be that we cannot extend our results throughout young adulthood yet. Our study was restricted to children who had attended a clinical assessment at age 12. The prevalence of overweight in these children (12.3%) was similar to the Dutch countrywide prevalence among 13-year-olds in 2009 (boys 12.0%, girls 12.5%)[29]. Children with a lower socio-economic position (as reflected by lower parental education) were underrepresented in the current study population, compared with the total study population. This implies that the gradually developing and persistent overweight patterns, which were more prevalent among children with parents of lower socio-economic position, are likely to be even more prevalent in the general population than in our study. However, although socioeconomic disadvantage is strongly associated with adult cardiovascular disease, associations of overweight trajectories with cardiometabolic markers may be assumed to be in the same direction for children of lower or higher socio-economic position.

Conclusion

Children with gradually developing risks of overweight during childhood, and those with persistent overweight had unfavourable levels of cholesterol and blood pressure already at the age of 12 years, whereas children with early transient overweight avoided these unfavourable outcomes. Together with findings from earlier studies that adults with diabetes and CVD more often had accelerated weight gain after birth, our results support the hypothesis that specific overweight patterns predispose to an adverse cardiometabolic profile, which is already apparent in early adolescence before progressing to adult cardiometabolic disease.

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Supplemental text S1. Description of the overweight patterns

The patterns we used in this paper were described previously[1]. For determining the distinct patterns of overweight development from age 3 months to 11 years latent growth analysis was used (SAS proc traj), a statistical technique that can be used to estimate the effects of growth in different periods of the life course on health outcomes at a later point in time[2]. The latent growth model allowed individuals to have incomplete BMI data over the course of follow-up so that they could be retained in the analytic sample: a minimum of 4 observations of BMI was required. The starting point for the procedure was a one-pattern model, which describes the probability of being overweight (i.e. ≥90th percentile) for the population at each age. We entered a linear, quadratic, and cubic term for age in the model. Non-significant terms were removed from the model, until the highest order term was significant. The resulting model was the best model for the one-pattern model, and the log-likelihood of this model was used for comparison with the log-likelihood of the model with two patterns. The same procedure was followed for the models with consecutively more patterns, until allowing more patterns did not further improve model fit (e.g. the log-likelihood of the model with more patterns was not significantly more towards 0). Statistically, the data was best described with 6 patterns. However, since one pattern had wide confidence intervals and therefore showed overlap with other patterns, we decided to remove this pattern. Furthermore, we merged two patterns since they were parallel and very similar; one with a persistent very low probability of overweight and one with a persistent low probability of overweight. In addition, the mean posterior probabilities for the five- and six-pattern model were below 0.8. Thus, we identified four plausible patterns. Each individual was assigned a probability of belonging to each of the four patterns and we used these probabilities in the analyses with the cardiometabolic markers. The four patterns were characterized as: never overweight (77.6%), persistent overweight (4.0%), reducing overweight (10.0%), and late onset overweight (8.4%) (Figure 1). In the current study, we use the terms 'early transient overweight' instead of 'overweight reduction' for pattern 3, and 'gradually developing overweight' instead of 'late onset' for pattern 1. The membership probabilities for the four overweight patterns, for the current study population (a), for children in that pattern (b), and the corresponding percentages (b/a) were, respectively; never overweight (1167, 1147, 98%); early transient overweight (159, 110, 69%); gradually developing overweight (117, 81, 69%); and persistent overweight (57, 49, 86%).

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Table S1. Differences[1] in levels of cardiometabolic markers of three overweight patterns compared with a never overweight pattern, additionally adjusted for maternal pre-pregnancy BMI

	Never overweight pattern	Early transient overweight pattern		Gradually developing overweight pattern		Persistent overweight pattern	
	Absolute level (reference)	difference[3]	*P*	difference[3]	*P*	difference[3]	*P*
Total cholesterol (mmol/L)	3.9	0.02 (-0.14; 0.17)	.81	**0.23 (0.04; 0.42)**	**.02**	0.05 (-0.18; 0.28)	.66
HDL cholesterol (mmol/L)	1.5	0.05 (-0.02; 0.12)	.16	**-0.19 (-0.27; -0.11)**	**<.0001**	**-0.15 (-0.25; -0.05)**	**<.01**
TC/HDLC ratio	2.7	-0.10 (-0.28; 0.07)	.25	**0.68 (0.47; 0.90)**	**<.0001**	**0.51 (0.24; 0.77)**	**<.001**
Systolic blood pressure (mmHg)[2]	119.2	-0.06 (-2.13; 2.01)	.95	**4.05 (1.49; 6.62)**	<.01	4.41 (1.42; 7.39)	<.01
Diastolic blood pressure (mmHg)[2]	69.6	-0.04 (-1.47; 1.40)	.96	1.57 (-0.20; 3.35)	.08	**3.51 (1.44; 5.58)**	**<.01**
HbA1c (mmol/mol)	31.7	-0.02 (-0.63; 0.60)	.96	-0.34 (1.09; 0.41)	.37	-0.33 (-1.23; 0.58)	.48

[1]Adjusted for sex, ethnicity, maternal education, paternal education, and maternal pre-pregnancy BMI.
[2]Additionally adjusted for cuff size
[3]Regression coefficients (95% confidence intervals) of the mean level of the cardiometabolic marker in each of the overweight patterns versus the mean level in the never overweight pattern

Table S2. Accounting for mediation by puberty development in associations[1] between two overweight patterns and levels of cholesterol and blood pressure

	Never overweight pattern	Gradually developing overweight pattern			Persistent overweight pattern		
	Absolute level (reference)	difference[3]	s.e.	*P*	difference[3]	s.e.	*P*
Total cholesterol (mmol/L)	4.4	**0.28**	**0.09**	**<.01**	0.12	0.11	.26
HDL cholesterol (mmol/L)	1.5	**-0.18**	**0.04**	**<.0001**	**-0.16**	**0.05**	**<.01**
Systolic blood pressure (mmHg)[2]	112.0	**3.87**	**1.20**	**<.01**	4.06	1.42	<.01
Diastolic blood pressure (mmHg)[2]	65.6	1.25	0.84	.14	**3.38**	**0.99**	**<.01**

[1]Adjusted for sex, ethnicity, maternal education, and paternal education.
[2]Additionally adjusted for cuff size
[3]Regression coefficients (95% confidence intervals) of the mean level of the cardiometabolic marker in each of the overweight patterns versus the mean level in the never overweight pattern

Chapter 6

Early or late pubertal timing and cardiometabolic markers at age 16 in boys and girls from a contemporary cohort

Nina E. Berentzen
Alet H. Wijga
Lenie van Rossem
Dirkje S. Postma
Ulrike Gehring
Henriëtte A. Smit

Submitted

Abstract

Background

Women with early or late menarche have a higher risk of overweight and cardiovascular disease than those with intermediate menarche. The role of childhood adiposity in these associations is unclear, and studies in men and boys are scarce.

Methods and Results

We analysed data of 799 boys and girls participating in a Dutch birth cohort, who self-reported pubertal development at ages 11, 14 and 16, and who had information on cardiometabolic markers (waist circumference, cholesterol, blood pressure (BP), glycated haemoglobin (HbA1c)) at age 16. We classified participants as early (84 girls, 88 boys), intermediate (240 girls, 211 boys), or late pubertal timing (89 girls, 85 boys) and estimated differences in cardiometabolic markers using linear regression analysis. At age 16, girls with early pubertal timing had 1.54 cm larger waist circumference (95% CI 0.05; 3.03) and 3.98 mmHg higher systolic BP (95% CI 1.69; 6.27) than girls with intermediate pubertal timing. The association with systolic BP remained after adjusting for childhood BMI (age 8), but attenuated after adjusting for BMI in adolescence (age 16). Boys with early pubertal timing had 0.79 mmol/mol lower HbA1c (95% CI -1.38; -0.20) than boys with intermediate pubertal timing. Girls and boys with late pubertal timing had a tendency for lower waist circumference, but no differences in other cardiometabolic markers.

Conclusions

Girls with early pubertal timing have unfavourable blood pressure levels at age 16, independently of BMI in childhood. Late pubertal timing does not appear a risk factor for unfavourable cardiometabolic markers in adolescence.

Introduction

Women who had early menarche (<12 y) have an increased risk of overweight and cardiometabolic disorders such as hypertension, hypercholesterolemia, and coronary heart disease[1-4]. Childhood overweight is associated with earlier menarche[5,6] and may therefore explain at least part of the association with later cardiometabolic disorders. However, studies in adults lack data on childhood adiposity and therefore have been unable to investigate the role of this potential confounder in the association between pubertal timing and adult cardiometabolic outcomes[1]. Prospective studies in younger populations have the advantage of assessments of childhood adiposity preceding puberty. The Bogalusa Heart Study and the Fels Longitudinal Study have shown that girls with early menarche develop an unfavourable cardiometabolic profile independent of childhood adiposity, which appears to persist into early adulthood[7,8]. In contrast, others have observed that early menarche follows increased childhood adiposity, and stated that a strong independent effect of early menarche on adult cardiometabolic risk is unlikely[9].

Within this discussion, two issues have remained largely unaddressed: consequences of early pubertal timing for boys and men, and consequences of late puberty. The limited available evidence in men suggests associations with cardiometabolic outcomes in directions similar to those in women[1,4]. In this prospective study, we examined levels of cardiometabolic markers at age 16 in relation to pubertal timing in Dutch boys and girls. We investigated the potential confounding role of adiposity in childhood and the potential mediating role of adiposity in adolescence. Since recent studies suggested a U-shaped association between menarcheal age and cardiovascular disease (CVD) risk[2,4,10], we separately considered early and late pubertal timing in relation to cardiometabolic markers.

Methods

Study participants

We used data from a population-based contemporary Dutch birth cohort: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, with prenatal inclusion of 3963 children in 1996/1997. A detailed description of the study design was published previously[11]. At age 16 years, 3263 adolescents (82%) were still in the study. A random

subsample of the participants still in the study was invited to a clinical assessment (n=2159), excluding the Rotterdam area due to budget restraints, and 802 participated (response rate 37%). The study population for the current study consisted of 799 adolescents (415 girls and 384 boys) of whom data were available for any of the cardiometabolic markers at age 16, and at least one of the puberty variables. The 799 participants included in the current study had parents with a higher education and less frequently had a non-Western ethnicity than those not included in the current study population, but no differences were found in levels of puberty variables at age 11, 14 and 16 between those included and excluded in the current study. The study protocol was approved by the medical ethics committees of the participating institutes. All parents gave written informed consent for the general study and separately for the clinical assessment; additionally, participants themselves gave written informed consent for the clinical assessment.

Assessment of pubertal timing at ages 11, 14 and 16

Pubertal stage was assessed with the Pubertal Development Scale (PDS)[12]. Participants rated their pubertal development on a 4-point scale for five items: growth spurt, pubic hair, skin changes, and additionally menarche and breast development (girls) and voice change and facial hair (boys). The total PDS scores for the five items were averaged to maintain the original metric (range 1 to 4). The PDS was reported during three waves of follow-up, around age 11, 14, and 16. Correction for the influence of variation in age at each wave of follow-up on the PDS was achieved by standardizing the PDS values, using the residuals obtained when regressing the PDS on the participant's age.

Definition of early and late pubertal timing

Based on the age-standardized PDS at age 11 and 16 (girls) or age 14 and 16 (boys) we classified participants as early, intermediate or late pubertal timing. Early pubertal timing was defined as a PDS >75th percentile at age 11 (girls), or 14 (boys) and late pubertal timing was defined as a PDS <25th percentile at age 16. Boys and girls not classified as either early or late, were classified as intermediate pubertal timing. Corresponding cut-off values and *n* were; early pubertal timing (girls: PDS>2.3 n=84, boys PDS>3.4 n=88); late pubertal timing (girls: PDS≤3.2 n=89, boys: PDS<2.8 n=85); intermediate pubertal timing (all others, girls: n=240, boys: n=211). According to this classification, 13 girls and no boys had both early and late pubertal timing. We classified these girls as intermediate pubertal timing. For 49 participants (28 girls and 21 boys), the PDS was available at one

age only. We retained these participants in the analyses and classified them according to their single PDS value. Power calculations showed that with the current group sizes for early, intermediate and late pubertal timing, and a fixed 80% power (0.8) at a significance level (alpha) of 0.05, a clinically relevant difference[13] of 2.4 cm waist circumference could be detected in boys and girls.

Definition of early and late menarche

Additionally to groups of pubertal timing, we assessed early, intermediate and late menarche for comparison with studies in the adult population. During three waves of follow-up, around the age of 11, 14 and 16, girls reported whether menstrual periods had begun and, if so, the age at initiation, in years and months. Regarding reliability of self-reported age at menarche, the reported menarcheal age at the first (11 y) and second wave (14 y) or first (11 y) and third (16 y) wave differed by ≤1 y among 86% of the girls, and among 82%, respectively. Mean difference in recalled menarcheal age between any two waves (of 11 y, 14 y, and 16 y) was <0.3 y (SD: 0.7). Bland-Altman plots[14] did not provide evidence for systematic variation of differences with age. We used the menarcheal age reported at the first available wave to reduce potential misclassification, since errors in recalling age at menarche are likely to increase with time. If a participant reported years of age at menarche, but not months, the month was imputed as 6 months later than the reported integer age at menarche (n=3). We classified girls as early (≤11 y, n=43), intermediate (12-14 y, n=319) or late (≥15 y, n=49) menarche. Those who had not yet reached menarche by the age of 16 were thus included in the late menarche group.

Assessment of cardiometabolic markers at age 16

Clinical assessments at age 16 (range 15.9–17.5 y) were performed by trained staff at visits to medical centres, according to standardized protocols. Systolic and diastolic blood pressure (BP) were measured according to the recommendations of the American Heart Association Council on High Blood Pressure Research[15]. BP readings were obtained from the non-dominant upper arm using an Omron M6 monitor while the child was seated. The first measurement was taken after≥5 minutes of rest, without talking. Depending on arm circumference, 17 to 22 cm or 22 to 42 cm cuffs were used. BP was measured at least twice with 5 minutes intervals. If two consecutive measures differed by >5 mm Hg, a third measurement was taken. The means of (2 or 3) systolic and diastolic measurements were used in analyses. Blood was drawn for measurement of cholesterol and glycated

haemoglobin (HbA1c). Serum total and high-density lipoprotein (HDL) cholesterol were determined enzymatically using Roche automated clinical chemistry analysers (Roche Diagnostics, Indianapolis). The ratio between total and HDL cholesterol was calculated (TC/HDLC ratio). For analysis of HbA1c, erythrocytes from blood samples were stored, a 5 µl cell mass was lysated and HbA1c was measured by ion-exchange chromatography using the Adams A1c, HA-8160 HPLC Auto analyser (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial (DCCT) standards.

Assessment of anthropometry

Weight, height and waist circumference were measured in cm during clinical assessments at ages 8, 12 and 16. BMI (kg/m^2) was used in the analysis as age- and sex-specific standard deviation scores (z-scores), calculated using the reference growth curves of the Dutch Fourth Nation-wide Growth Study carried out in 1997[16]. Waist-to-height ratio was calculated. Overweight (including obesity) and thinness were defined based on international cut-off values[17].

Assessment of other characteristics

Characteristics that were used to describe the study population besides pubertal development were child's ethnicity, age (months) and maternal and paternal education. Ethnicity was based on country of birth of the child's parents, and was categorized as Dutch, Non-Dutch western or nonwestern. Mother's and father's educational level were categorized as low (primary school, lower vocational or lower secondary education), intermediate (intermediate vocational education or intermediate/higher secondary education) or high (higher vocational education and university).

Statistical analyses

Mean levels of cardiometabolic markers and other characteristics were compared between groups with early and late pubertal timing, stratified by sex. Differences in cardiometabolic markers between adolescents with early or late vs intermediate pubertal timing, or menarche, were assessed by multiple linear regression analyses. The regression models were run separately for boys and girls, and included groups of pubertal timing or menarcheal age as independent variable and the cardiometabolic marker as dependent variable. Regression models were adjusted for age at the clinical assessment, ethnicity,

height, and maternal and paternal education in the first adjusted model. These potential confounders were selected based on prior knowledge and their associations with the outcome of interest. We considered the potentially confounding role of smoking, since it is an important risk factor for adult CVD, but the prevalence of smoking (at least once a day) was low in our study population of 16-year-olds (5%) and was not associated with pubertal timing in an exploratory analysis. Therefore, we did not include smoking in the regression models.

There is evidence that increased childhood adiposity is associated with earlier pubertal timing in girls[5], whereas in boys the relationship is less clear[6]. As childhood adiposity is also associated with unfavourable cardiometabolic outcomes, it may act as a confounder in the relationship between pubertal timing and cardiometabolic markers; we investigated this by separately including and excluding childhood (age 8) BMI z-score or waist circumference z-score as a covariate in the linear regression models and comparing the regression coefficients. Furthermore, increased adiposity at the time of cardiometabolic marker assessment may explain part of the association with pubertal timing; therefore, we subsequently included BMI or waist circumference at the time of clinical assessment (age 16) in the linear regression models. All of the data analysis was performed by using SAS software (version 9.3; SAS Institute Inc, Cary, NC).

Results

The mean PDS increased with age from 3.4 (age 11) to 4.0 (age 16) in girls with early pubertal timing, and from 1.1 (age 11) to 2.8 (age 16) in girls with late pubertal timing (Table 1). The mean PDS increased from 1.5 (age 11) to 3.8 (age 16) in boys with early pubertal timing, and from 1.1 (age 11) to 2.0 (age 16) in boys with late pubertal timing. Girls with early pubertal timing were consistently more often overweight/obese from age 8 (24% vs 10%), and age 12 (20% vs 8%) to age 16 (18% vs 8%) than girls with intermediate pubertal timing. Boys with early pubertal timing were more often overweight than boys with intermediate pubertal timing at age 8 (18% vs 12%), but no longer at age 12 (8% vs 13%) and age 16 (7% vs 10%).

After adjustment for confounders, girls with early pubertal timing had 1.54 cm larger waist circumference (95% CI 0.05; 3.03) and had 3.98 mmHg higher systolic BP (95% CI 1.69; 6.27) at age 16, than those with intermediate pubertal timing (Table 2). Levels of

other cardiometabolic markers did not differ between girls with early vs intermediate pubertal timing. When we additionally adjusted for childhood BMI z-score (age 8), the association with systolic BP remained, at a difference of 3.69 mmHg (95% CI 1.18; 6.21). When we adjusted for BMI z-score at the time of cardiometabolic assessment (age 16), the association with systolic BP attenuated to a difference of 2.67 mmHg (95% CI 0.43; 4.92) (Table 2). Because waist circumference may be a better proxy for adiposity than BMI, we repeated analyses adjusting for waist circumference z-score instead of BMI. This resulted in similar effect sizes and did not change interpretations (data not shown).

Girls with early (≤11 y) menarche had 3.24 cm higher waist circumference (95% CI 1.32; 5.17) than those with intermediate (12-14 y) menarche, and a tendency for higher systolic BP, although this effect estimate attenuated after adjustment for confounders (2.10 mmHg; 95% CI -0.92; 5.13) (Table 3). In boys, those with early pubertal timing had 0.79 mmol/mol lower HbA1c (95% CI -1.38; -0.20) than those with intermediate pubertal timing (Table 2). Levels of other cardiometabolic markers did not differ between boys with early vs intermediate pubertal timing.

Girls and boys with late pubertal timing had a tendency for lower waist circumference, although the effect estimates were not statistically significant after adjustment for confounders (Table 2). Levels of other cardiometabolic markers did not differ for girls and boys with late vs intermediate pubertal timing. Girls with late (≥15 y) menarche had a borderline significantly lower waist circumference of 1.83 cm (95% CI -3.63; -0.03, p=.05) than those with intermediate menarche, but no differences in levels of other cardiometabolic markers (Table 3).

Table 1. Characteristics of the study population within categories of pubertal timing, based on the puberty development scale[1]

	Girls (n=413)			Boys (n=384)		
	Early pubertal timing (n=84)	Intermediate pubertal timing (n=240)	Late pubertal timing (n=89)	Early pubertal timing (n=88)	Intermediate pubertal timing (n=211)	Late pubertal timing (n=85)
Socio-demographic characteristics (%)						
Ethnicity						
Dutch	91.6	91.1	92.9	93.0	95.2	91.5
Non-Dutch western	2.4	3.8	4.7	2.3	3.4	1.2
Nonwestern	6.0	5.1	2.4	4.7	1.4	7.3
Maternal education						
Low	17.9	10.8	15.7	20.5	15.2	16.5
Intermediate	36.9	42.9	40.5	35.2	39.3	35.3
High	45.2	46.3	43.8	44.3	45.5	48.2
Paternal education						
Low	16.9	18.1	16.9	21.6	12.0	15.3
Intermediate	37.4	29.5	30.3	34.1	33.7	36.5
High	45.8	52.3	52.8	44.3	54.3	48.2
Pubertal assessment (mean, std)						
Puberty development scale at age 11 (1-4)	3.4 (0.8)	1.5 (0.7)	1.1 (0.6)	1.5 (0.9)	1.2 (0.6)	1.1 (0.5)
Puberty development scale at age 14 (1-4)	3.9 (0.5)	3.4 (0.8)	2.5 (1.3)	4.1 (0.4)	2.6 (0.7)	1.6 (1.0)
Puberty development scale at age 16 (1-4)	4.0 (0.3)	3.8 (0.4)	2.8 (0.6)	3.8 (0.5)	3.4 (0.5)	2.0 (0.6)
Pubertal assessment at age 16 (mean, std)						
Growth spurt (1-4)	4.0 (0.0)	4.0 (0.2)	3.4 (1.1)	3.8 (0.5)	3.7 (0.5)	3.0 (0.8)
Skin change (1-4)	3.5 (0.5)	3.4 (0.5)	2.7 (0.9)	3.4 (0.5)	3.2 (0.4)	2.5 (0.7)
Pubic hair (1-4)	3.9 (0.4)	3.8 (0.4)	3.1 (0.4)	3.6 (0.5)	3.3 (0.5)	3.0 (0.4)
Breast development (1-4)	3.7 (0.5)	3.4 (0.5)	2.9 (0.4)	-	-	-
Age at menarche (years)	11.9 (1.0)	13.2 (1.0)	13.9 (1.2)	-	-	-
Facial hair (1-4)	-	-	-	3.0 (0.5)	2.6 (0.6)	2.0 (0.6)
Voice change (1-4)	-	-	-	3.8 (0.4)	3.6 (0.5)	2.6 (0.7)
Cardiometabolic assessment						
At age 8 (mean, std)						
BMI (z-score)	0.4 (0.9)	-0.0 (0.9)	-0.2 (1.0)	0.2 (1.0)	0.1 (0.9)	-0.1 (0.9)
Overweight/obese (%)	24.0	9.8	10.4	17.6	12.1	4.2
Thin or very thin (%)	1.3	6.8	11.7	5.4	5.5	7.0
At age 12 (mean, std)						
BMI (z-score)	0.5 (1.0)	-0.0 (1.0)	-0.2 (1.0)	0.2 (0.9)	0.2 (1.1)	-0.1 (1.0)
Overweight/obese (%)	20.0	7.6	6.5	7.8	13.1	7.7
Thin or very thin (%)	2.7	8.1	15.6	6.5	9.1	9.0
At age 16 (mean, std)						
BMI (z-score)	0.4 (1.0)	0.1 (0.9)	-0.3 (1.0)	0.1 (0.9)	0.1 (1.0)	-0.3 (1.0)
Overweight/obese (%)	18.3	8.3	5.6	6.8	10.4	5.9
Thin or very thin (%)	6.1	8.8	15.7	3.4	9.0	17.7
Age at clinical assessment (years)	16.4 (0.2)	16.4 (0.2)	16.3 (0.2)	16.4 (0.2)	16.3 (0.2)	16.3 (0.2)
Height (cm)	170.1 (5.8)	170.4 (6.2)	168.9 (6.1)	183.6 (6.3)	182.0 (6.1)	179.6 (7.7)
Height-for-age (z-score)	0.2 (0.9)	0.2 (1.0)	0.0 (0.9)	0.5 (0.8)	0.3 (0.8)	-0.0 (1.0)
Waist circumference (cm)	71.9 (7.2)	70.4 (6.2)	68.9 (5.1)	74.8 (5.9)	74.6 (6.7)	73.0 (6.5)
Waist-to-height ratio	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)
Total cholesterol (mmol/L)	4.1 (0.7)	4.2 (0.7)	4.1 (0.8)	3.6 (0.8)	3.7 (0.6)	3.6 (0.6)
HDL cholesterol (mmol/L)	1.4 (0.3)	1.5 (0.4)	1.5 (0.3)	1.2 (0.3)	1.3 (0.3)	1.3 (0.3)
Total/HDL cholesterol ratio	3.0 (0.8)	3.0 (0.8)	3.0 (1.1)	3.0 (0.8)	3.0 (0.7)	2.8 (0.7)
Systolic blood pressure (mmHg)	116.5 (10.2)	112.4 (8.6)	111.9 (9.5)	121.6 (8.4)	119.3 (9.6)	118.2 (10.2)
Diastolic blood pressure (mmHg)	68.7 (6.5)	67.2 (6.8)	66.5 (6.6)	65.9 (6.0)	65.0 (6.5)	64.7 (7.2)
HbA1c (mmol/mol)	33.1 (4.2)	33.6 (4.2)	33.1 (2.3)	32.7 (2.0)	33.4 (2.2)	33.4 (2.5)

[1]Early puberty was defined as a puberty development scale (PDS) >P75 at age 11 for girls, and age 14 for boys.
Late puberty was defined as a PDS <P25 at age 16 for girls and boys.

Table 2. Differences[1] in levels of cardiometabolic markers at age 16 among girls and boys with early or late pubertal timing (based on the puberty development scale), compared to those with intermediate pubertal timing

	Waist circumference (cm)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	TC/HDLC ratio	Systolic blood pressure (mmHg)[3]	Diastolic blood pressure (mmHg)[3]	HbA1c (mmol/mol)
Girls with intermediate pubertal timing - absolute level (reference)	70.4	4.2	1.5	3.0	112.4	67.2	33.6
Girls with early pubertal timing[4]							
Crude	1.44 (-0.11; 2.99)	-0.07 (-0.26; 0.12)	-0.01 (-0.10; 0.08)	-0.04 (-0.26; 0.18)	**4.13 (1.84; 6.42)***	1.50 (-0.18; 3.17)	-0.51 (-1.51; 0.50)
Adjusted[2]	**1.54 (0.05; 3.03)**	-0.09 (-0.28; 0.10)	-0.01 (-0.10; 0.09)	-0.08 (-0.30; 0.15)	**3.98 (1.69; 6.27)***	1.43 (-0.27; 3.14)	-0.52 (-1.54; 0.51)
+ BMI (z-score) age 8	-	-0.09 (-0.30; 0.13)	-0.01 (-0.11; 0.08)	-0.07 (-0.31; 0.18)	**3.69 (1.18; 6.21)***	0.84 (-0.99; 2.68)	-0.76 (-1.91; 0.39)
+ BMI (z-score) age 16	-	-0.15 (-0.34; 0.04)	0.01 (-0.08; 0.10)	-0.15 (-0.38; 0.07)	**2.67 (0.43; 4.92)***	0.73 (-0.98; 2.43)	-0.47 (-1.51; 0.57)
Grls with late pubertal timing[4]							
Crude	-1.52 (-3.03; -0.01)	-0.10 (-0.29; 0.09)	0.01 (-0.08; 0.10)	-0.05 (-0.27; 0.17)	-0.70 (-2.95; 1.55)	-0.87 (-2.51; 0.78)	-0.48 (-1.48; 0.52)
Adjusted[2]	-0.98 (-2.45; 0.49)	-0.09 (-0.29; 0.10)	0.001 (-0.09; 0.09)	-0.03 (-0.26; 0.19)	-0.55 (-2.83; 1.73)	-0.82 (-2.52; 0.87)	-0.66 (-1.69; 0.37)
+ BMI (z-score) age 8	-	-0.08 (-0.29; 0.13)	-0.002 (-0.10; 0.09)	-0.02 (-0.26; 0.23)	-0.57 (-3.04; 1.91)	-0.48 (-2.28; 1.33)	-0.79 (-1.92; 0.34)
+ BMI (z-score) age 16	-	-0.04 (-0.23; 0.15)	-0.01 (-0.10; 0.08)	0.03 (-0.19; 0.25)	-0.17 (-2.37; 2.03)	-0.67 (-2.34; 1.01)	-0.67 (-1.70; 0.36)
Boys with intermediate pubertal timing - absolute level (reference)	74.6	3.7	1.3	3.0	119.3	65.0	33.4
Boys with early pubertal timing[4]							
Crude	0.26 (-1.36; 1.87)	-0.07 (-0.23; 0.09)	-0.03 (-0.10; 0.04)	0.003 (-0.18; 0.19)	2.34 (-0.03; 4.71)	0.86 (-0.77; 2.49)	**-0.75 (-1.33; -0.18)***
Adjusted[2]	-0.05 (-1.71; 1.61)	-0.02 (-0.18; 0.14)	-0.04 (-0.11; 0.03)	0.05 (-0.13; 0.24)	1.78 (-0.64; 4.20)	0.60 (-1.06; 2.25)	**-0.79 (-1.38; -0.20)***
+ BMI (z-score) age 8	-	-0.08 (-0.25; 0.09)	-0.04 (-0.11; 0.04)	0.01 (-0.19; 0.21)	1.47 (-1.00; 3.95)	-0.09 (-1.86; 1.69)	-0.61 (-1.23; 0.02)
+ BMI (z-score) age 16	-	-0.05 (-0.21; 0.11)	-0.04 (-0.11; 0.03)	0.03 (-0.15; 0.22)	2.00 (-0.31; 4.32)	0.80 (-0.86; 2.45)	**-0.80 (-1.38; -0.21)***
Boys with late pubertal timing[4]							
Crude	-1.57 (-3.22; 0.07)	-0.07 (-0.22; 0.09)	0.05 (-0.02; 0.11)	-0.18 (-0.37; 0.0002)	-1.28 (-3.69; 1.13)	-0.45 (-2.11; 1.21)	0.02 (-0.55; 0.59)
Adjusted[2]	-1.18 (-2.88; 0.51)	-0.09 (-0.25; 0.07)	0.03 (-0.04; 0.10)	-0.17 (-0.36; 0.02)	-1.22 (-3.68; 1.25)	-0.44 (-2.13; 1.25)	0.08 (-0.51; 0.67)
+ BMI (z-score) age 8	-	-0.09 (-0.27; 0.09)	0.05 (-0.03; 0.12)	-0.18 (-0.39; 0.02)	-2.49 (-5.02; 0.04)	-1.50 (-3.31; 0.32)	0.41 (-0.23; 1.05)
+ BMI (z-score) age 16	-	-0.02 (-0.18; 0.14)	0.02 (-0.05; 0.09)	-0.08 (-0.27; 0.10)	-0.48 (-2.86; 1.90)	-0.37 (-2.07; 1.33)	0.12 (-0.48; 0.71)

[1]Regression coefficient (95% confidence interval) of the mean level of the cardiometabolic marker among adolescents with early or late pubertal timing compared to the mean level among those with intermediate pubertal timing.

[2]Adjusted for age at clinical assessment, height, ethnicity, maternal education, and paternal education. Subsequently, models additionally adjusted for BMI (z-score) either at age 8, or at age 16 (excluding height).

[3]Adjusted for cuff size in all models.

[4]Early puberty was defined as a puberty development scale (PDS) >P75 at age 11 for girls, and age 14 for boys. Late puberty was defined as a PDS <P25 at age 16 for girls and boys.

*p<.05

Table 3. Differences[1] in levels of cardiometabolic markers at age 16 among girls with early or late menarche, compared to girls with intermediate menarche

	Intermediate menarche (12-14 years)	Early menarche (≤11 years)	Late menarche (≥15 years)
	Absolute level (reference)	difference	difference
Waist circumference (cm)	70.5	**3.24 (1.32; 5.17)***	-1.83 (-3.63; -0.03)
Total cholesterol (mmol/L)	4.1	-0.01 (-0.26; 0.24)	-0.03 (-0.27; 0.20)
HDL cholesterol (mmol/L)	1.4	0.01 (-0.11; 0.13)	0.01 (-0.10; 0.12)
TC/HDLC ratio	3.0	0.01 (-0.28; 0.30)	0.04 (-0.23; 0.30)
Systolic blood pressure (mmHg)[2]	113.3	2.10 (-0.92; 5.13)	-1.92 (-4.81; 0.97)
Diastolic blood pressure (mmHg)[2]	67.6	0.34 (-1.89; 2.57)	-1.82 (-3.95; 0.31)
HbA1c (mmol/mol)	33.4	-0.15 (-1.48; 1.19)	-0.30 (-1.54; 0.93)

[1]Regression coefficients (95% confidence intervals), adjusted for age at clinical assessment, height, ethnicity, maternal education, and paternal education.
[2]Additionally adjusted for cuff size.
*p<.05

Discussion

Main findings

Girls with early pubertal timing had a higher systolic BP at age 16 than girls with intermediate pubertal timing, independently of BMI in childhood. Boys with early pubertal timing had lower HbA1c levels than boys with intermediate pubertal timing, but no differences in other cardiometabolic markers. Girls and boys with late pubertal timing had a tendency for lower waist circumference and no differences in other cardiometabolic markers.

Previous studies in adolescents

Many studies have described cardiometabolic markers during stages of normal pubertal development, but only four studies have investigated differences in cardiometabolic markers between adolescents with early or late pubertal timing[7-9,18]. Three studies assessed menarcheal age in girls[7-9], and one assessed Tanner stages (a puberty scale similar to the PDS based on physical exam of external primary and secondary sex characteristics) in boys and girls[18]. As in our study, girls with self-reported early menarche or early pubertal timing developed increased blood pressure in late adolescence in two prospective studies[8,18] and this was independent of baseline BMI[18] or changes in body composition[8]. However, among girls in the Young Finns Study, early menarche was not associated with

increased blood pressure in adolescence (9–18 y)[9]. The wide age range in these girls and the fact that some of them had blood pressure measured before menarche may have prevented the detection of associations that arise after menarche. Early menarche was not associated with higher blood pressure in girls from the Bogalusa Heart Study, but this study was conducted more than two decades ago and the authors noted that girls included in the non-early group (menarche ≥12 y) were not lean, thus their adiposity could obscure subtle differences in BP[7]. Our results in boys are consistent with one prospective study in African-American boys, showing that early vs non-early maturing boys did not develop unfavourable BMI, waist circumference and BP[18].

We found no evidence of unfavourable cardiometabolic markers in boys and girls with late pubertal timing or menarche. Only two previous studies investigated late menarche in girls and our results are similar to the findings of these studies in that age at menarche was inversely associated with BMI or waist circumference[8,9]. To our knowledge, the current study is one of few to investigate pubertal timing in relation to cardiometabolic markers in boys, and the first to investigate the association of specifically late pubertal timing (in addition to late menarche) with cardiometabolic markers in girls and boys.

Interpretation

Mild transient insulin resistance occurs during normal puberty, with higher circulating insulin levels near the end of puberty (Tanner stage 5)[19]. Those with advanced puberty may have higher insulin levels and lower glucose levels earlier than those with later puberty, possibly explaining the lower HbA1c levels at age 16 in boys with early compared to intermediate pubertal timing. Although this is an interesting finding, it is difficult to interpret. It may be that these differences will diminish towards adulthood.

Although levels of cholesterol change with age during adolescence, we found no association of early or late pubertal timing with cholesterol levels (HDLC, TC, or TC/HDLC ratio) which is consistent with previous research in adolescent populations[1,7-9].

Our study showed that girls with early puberty have higher systolic blood pressure, which suggests that the increased cardiometabolic risk observed in adult women with early menarche may be present already in adolescence. However, the increase in blood pressure may also be temporary, as part of a normal transition to adulthood. Longer follow-up of these girls should provide more insight into the longitudinal development of cardiometabolic risk following early puberty. Differences in waist circumference and blood pressure for menarcheal timing and pubertal timing were comparable in terms of magnitude

and direction of effect. As an explanation for the increased risk of later overweight and CVD in women with early menarche, it has been suggested that this association reflects earlier timing of puberty in girls who were already overweight in childhood[1,9]. In our study, adjusting for childhood (age 8) BMI or waist circumference did not explain the association of early puberty with higher blood pressure in girls. However, these measures may not be optimal markers of adiposity, and we cannot exclude the possibility that specific aspects of childhood adiposity (e.g. the proportion or distribution of body fat, or the proportion of visceral vs subcutaneous fat) may exert effects on blood pressure beyond BMI or waist circumference. Adiposity and puberty may be associated via hormonal mechanisms. It may be that early pubertal timing has an additional influence on cardiometabolic risk, by altering the timing of exposure to reproductive hormones, or the lifetime cumulative dose[20].

Intriguingly, two recent large studies from the UK (the Million Women Study and the Biobank Study) found that adult women with late menarche (≥15 y) had increased risk of coronary heart disease (CHD)[2,4], and in two other studies menarche at 16-18 y was associated with a slightly increased risks of all-cause and CHD mortality[21,22]. Remarkably, women with late menarche had a lower BMI in these individual studies and in a previous meta-analysis (0.24 kg/m^2 lower BMI than women with menarche <15 y)[1]. Inflammatory diseases such as inflammatory bowel disease[23], and childhood undernutrition and underweight[24,25] are associated with delayed puberty and may partly explain these findings. These associations might also be due to exposures after adolescence. Our results show that the increased cardiometabolic risk in women with late pubertal timing or menarche is not yet apparent in adolescence, suggesting that these associations might arise in adulthood.

Strengths and limitations

Key strengths of this study are the prospectively collected, repeatedly assessed data on childhood adiposity and puberty in both boys and girls from a population-based contemporary cohort, and the objectively measured cardiometabolic markers. Where previous studies used a binary classification for pubertal timing (e.g. early vs non-early), we included a third category of intermediate pubertal timing that allowed us to better differentiate adolescents with early or late pubertal timing. We assessed a multidimensional puberty measure (i.e. the PDS) in addition to the single indicator of menarcheal age. Assessment of the PDS in boys and girls makes the interpretation of sex differences in associations with cardiometabolic markers more straightforward than with sex-specific measures such as menarche or voice breaking.

Some limitations should be addressed. Puberty was self-reported and not validated with physical examinations. Although puberty is reported fairly accurately[12,26] adolescents may under- or overestimate pubertal maturation relative to their peers[26]. Since it is unlikely that pubertal maturation was more frequently misreported in adolescents with unfavourable levels of cardiometabolic markers or in those with a higher BMI[26], this may have resulted in underestimation of the true association. The number of adolescents that could be included in our study was relatively small due to the small sample size for the clinical assessment. However, power calculations showed that the sample size was sufficient to detect a clinically relevant difference in waist circumference. We acknowledge that our study population consisted of only 20% (799/3963) of the original cohort, which is a potential weakness. Adolescents with a lower socio-economic position (as reflected by parental education) and of non-Western ethnicity were underrepresented in the current study population, compared with the total study population. The mean age at menarche in our study population (13.1 y, SD 1.2) was comparable to the 50th centile age at menarche in 6,270 girls in the Netherlands in 2009 (13.1 y, 95% CI 12.9–13.2)[27], but our results may not be generalizable to other ethnic groups. Further, although socioeconomic disadvantage is strongly associated with adult CVD, associations of pubertal timing with cardiometabolic markers may be assumed to be in the same direction for adolescents of lower or higher socio-economic position.

Implications

Early puberty may have negative consequences for adult cardiometabolic health, and has been associated with several other disorders, e.g. polycystic ovarian syndrome, and breast-, ovarian-, and endometrial cancers[20,28]. Overweight is a major risk factor for these disorders, which, combined with our finding that girls with early pubertal timing have a higher BMI already during childhood, emphasizes the role of adiposity in associations with pubertal timing. The variety and impact of diseases related to pubertal timing underscores the importance of overweight prevention as a public health priority. In boys however the limited number of studies prevent from drawing such conclusions. Further studies in boys need to investigate whether early or late pubertal timing is a risk factor for later cardiometabolic disease. Since repeated assessments of pubertal development allow studying associations with cardiometabolic risk factors more thoroughly, the potential value of monitoring pubertal development for research purposes should be evaluated.

Conclusion

Girls with early pubertal timing had higher blood pressure levels, independently of BMI in childhood. Early pubertal timing may be important for girls' cardiometabolic risk already in adolescence, particularly for blood pressure. Boys with early pubertal timing had lower HbA1c than boys with intermediate pubertal timing, which may reflect earlier transient insulin resistance. Both girls and boys with late pubertal timing had a tendency for lower waist circumference and no differences in levels of other cardiometabolic markers. Contrary to findings in women, late pubertal timing does not appear to be a risk factor for unfavourable cardiometabolic markers in adolescence.

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Chapter 7

General discussion

The aim of this thesis was to identify and characterize determinants of an unfavourable cardiometabolic profile in childhood and adolescence. These determinants were familial factors (family history of cardiovascular disease or diabetes), lifestyle factors (sleep, sedentary behaviour, physical activity, and snack consumption), and biological factors (patterns of overweight development and pubertal timing). The child's cardiometabolic profile was characterized by adiposity, total cholesterol (TC), HDL cholesterol (HDLC), total/HDL cholesterol ratio (TC/HDLC ratio), blood pressure and glycated haemoglobin (HbA1c).

Summary and interpretation of main findings

This thesis shows that already in adolescence (ages 12 and 16 years) there was variation in levels of cardiometabolic markers that could be explained partly by determinants in childhood which are well-known risk factors for adult cardiovascular disease (Table 1).

Familial factors

Children with a strong family history of either cardiovascular disease or diabetes had higher levels of TC and TC/HDLC ratio than those without a family history of these diseases. The type of disease (cardiovascular disease or diabetes) determined which type of cardiometabolic marker was elevated in these children. Family history of cardiovascular disease was important for children's cholesterol levels, a typical risk factor for cardiovascular disease, whereas family history of diabetes was important for children's waist circumference and HbA1c, typical risk factors for diabetes (Chapter 2).

Lifestyle factors

Specific aspects of sleep were associated with unfavourable levels of cardiometabolic markers in girls, but not in boys. In girls, long sleep duration was associated with lower adiposity, whereas going to bed late/rise early, night-time awakenings and feeling tired were associated with different measures of cholesterol (TC, HDLC and TC/HDLC ratio). Other aspects of sleep were not associated with cardiometabolic markers. The relative importance of sleep duration versus sleep quality for cardiometabolic markers deserves further study (Chapter 3).

Table 1. Associations of familial factors, lifestyle factors and biological factors with cardiometabolic markers described in this thesis

		Adiposity (BMI, waist circumference or waist-to-height ratio)	Total cholesterol	HDL cholesterol	TC/HDLC ratio	Systolic BP	Diastolic BP	HbA1c
Familial factors								
Strong family history of myocardial infarction	Chapter 2	0	+ (girls and boys)	- (girls and boys)	+ (girls and boys)	0	0	0
Strong family history of stroke	Chapter 2	0	0	0	0	0	0	0
Strong family history of diabetes	Chapter 2	+ (girls and boys)	+ (girls and boys)	0	+ (girls and boys)	0	0	+ (girls and boys)
Lifestyle factors								
Sleep duration	Chapter 4	- Long sleep duration (girls)	0	0	0	0	0	0
Sleep quality	Chapter 4	0	+ Late to bed/ Early rise (girls) + Night-time awakenings (girls)	+ Late to bed/ Early rise (girls)[a] - Feeling tired (girls)	+ Feeling tired (girls)[a]	0	0	0
Screen time	Chapter 5	+ (girls and boys)[b]	NI	NI	0	- (girls and boys)	0	0
Moderate physical activity	Chapter 5	- (girls and boys)	NI	NI	- (girls and boys)[b]	0	0	0
Snack consumption	Chapter 5	0[c]	NI	NI	0	0	0	0
Biological factors								
Early transient overweight pattern	Chapter 3	NI	0	0	0	0	0	0
Gradually developing overweight pattern	Chapter 3	NI	+ (girls and boys)[b]	- (girls and boys)	+ (girls and boys)	+ (girls and boys)	0	0
Persistent overweight pattern	Chapter 3	NI	0	- (girls and boys)	+ (girls and boys)	+ (girls and boys)	+ (girls and boys)[b]	0
Early pubertal timing	Chapter 6	+ (girls)	0	0	0	+ (girls)[a]	0	- (boys)
Late pubertal timing	Chapter 6	0	0	0	0	0	0	0

Abbreviations; BMI, body mass index, BP, blood pressure, HbA1c, glycated haemoglobin, HDL, high-density-lipoprotein, TC/HDLC, total-to high-density-lipoprotein.
Plus signs indicate a positive association of the risk factor with the cardiometabolic marker, and minus signs a negative association. Zeros indicate no difference in the cardiometabolic marker. NI, not investigated.
[a]Association attenuated after adjusting for BMI or waist circumference.
[b]Only in boys in stratified analyses.
[c]Negative association in boys in stratified analyses.

The higher adiposity in children with high levels of screen time (as indicator for sedentary time), was partly explained by less physical activity, but not by higher snack consumption. The higher TC/HDLC ratio in children with more screen time was almost completely explained by higher adiposity and to a minor extent by less physical activity (Chapter 4).

Biological factors

The presence of overweight from the age of 3 months to 11 years, either gradually developing during childhood or persistently present from early infancy onwards, was strongly associated with unfavourable levels of cardiometabolic markers, and with clustering of two or more of these markers. Children with gradually developing overweight and those with persistent overweight over this period had a 2-3 fold higher risk of being in the upper percentile of TC/HDLC ratio, systolic and diastolic blood pressure by the age of 12 years. In contrast, children with early transient overweight had no unfavourable levels of cardiometabolic markers at age 12 years (Chapter 5).

Girls with early (compared with intermediate) pubertal timing had unfavourable blood pressure levels at age 16 years, suggesting one pathway by which early menarche may contribute to a higher risk for cardiovascular disease in adult women. Boys with early pubertal timing did not have unfavourable levels of cardiometabolic markers. Boys and girls with late (compared with intermediate) pubertal timing did not have unfavourable levels of cardiometabolic markers in adolescence (Chapter 6).

Which determinants in this thesis are most important for cardiometabolic risk?

This thesis shows that several well-known risk factors for cardiovascular disease in adults were also determinants of the cardiometabolic profile in children and adolescents. In general, children with a strong family history of cardiovascular disease or diabetes, those with less physical activity, those with gradually developing or persistent overweight in childhood, girls with lower sleep quality, and girls with early pubertal timing had unfavourable levels of cardiometabolic markers in childhood and adolescence. Of all these determinants, developing overweight in childhood and a strong family history of diabetes may be most unfavourable for future cardiovascular disease risk because these factors were associated with a wider range of cardiometabolic markers. The magnitude of the effect of developing overweight and having a strong family history of diabetes on the levels of cardiometabolic markers was substantial. For example, TC was 0.21 mmol/l higher in children who followed a pattern of gradually increasing overweight and 0.19 mmol/l higher in children with a

strong family history of diabetes, independent of child's body mass index (BMI). Since the prevalence of both factors was relatively high (of the 12-year olds in the PIAMA population, 12% were overweight and 10% had a strong family history of diabetes) this may have a meaningful effect on the future disease burden in the population.

How can the null findings be interpreted?

For some of the determinants investigated in this thesis, no associations were observed, for example between short sleep duration or late pubertal timing and cardiometabolic markers. It may be that in reality, the associations exist but these were not detected by studies in this thesis, or it may be that the associations truly do not exist.

Inaccurate assessment of lifestyle factors such as diet, physical activity and sedentary behaviour may be one explanation. Lifestyle behaviours are difficult to assess accurately; they can be measured by self-report or objective measures, but each method has inherent strengths and limitations. Often, no gold standard for measurement is available. Thus, imprecise or inaccurate assessment may have resulted in some misclassification of the lifestyle behaviours, and may explain why some factors considered as risk factors for cardiovascular disease (such as snacking or short sleep duration), were not found to be a risk factor for the child's cardiometabolic profile in this thesis.

Reverse causation may be another possible explanation for the difference between the null findings in this thesis and associations observed in adults. For example, short sleep duration may increase adiposity, but the reverse could also be true: high adiposity may increase the risk for obstructive sleep apnoea, which in turn may negatively affect sleep duration. Another example includes late pubertal timing, which may follow undernutrition or subclinical inflammatory disease in childhood and subsequently increase the risk for cardiovascular disease. Thus, reverse causation may be present in adult studies and may explain the null findings in this thesis. It is important for studies in children and adults to consider risk factors for cardiovascular disease that may precede the determinants of interest, and therefore adult studies need to take in account the potential influence of childhood exposures on the development of cardiovascular risk.

Another possibility may be that for some determinants, associations with cardiometabolic risk are not readily noticeable in childhood or adolescence, and arise in adulthood only after longer cumulative exposure. Therefore, it is important to continue to follow the adolescents in the PIAMA study in order to evaluate the associations with cardiometabolic risk at a later age.

To what extent is cardiometabolic risk dependent on adiposity?

Adiposity contributed importantly to unfavourable levels of cardiometabolic markers. Children who developed overweight that persisted after the age of 5 years had unfavourable levels of cholesterol measures and blood pressure at age 12 years (Chapter 5). In addition, these children more often had clustering of two or more of these markers, which may indicate a higher degree of atherosclerosis[1]. Lifestyle factors (sleep, screen time and physical activity) were associated with higher levels of cardiometabolic markers mainly through higher adiposity. This suggests that adiposity is an important mediator in the pathway of unfavourable lifestyle behaviours to cardiovascular outcomes, and is consistent with the observation that elevated levels of cardiometabolic markers are often preceded by overweight development[2-4]. Although overweight is a major risk factor for later cardiovascular disease, some determinants were associated with an unfavourable cardiometabolic profile independent of BMI. Children with a strong family history of myocardial infarction or diabetes had unfavourable levels of cardiometabolic markers even if they had a healthy BMI (Chapter 2). Girls with lower sleep quality, such as nighttime awakenings, and girls who felt tired during the day, had unfavourable levels of TC and HDLC, independent of waist circumference and BMI (Chapter 3). Children with more physical activity had a lower TC/HDLC ratio, which was only partly explained by a lower adiposity (Chapter 4). These findings imply that cardiometabolic risk is not determined by adiposity only and that children with a healthy weight may also develop a higher risk for later cardiovascular disease depending on other determinants, such as family history and lack of physical activity.

Methodological considerations

Generalizability of the observed associations

Participants in cohort studies generally have a higher socioeconomic position than non-participants, and may have healthier behaviours, especially when follow-up is long. An important question in many of those studies is to what extent the observed associations are valid for the general population. Studies in this thesis have been restricted to participants of the PIAMA study who attended a clinical assessment at age 12 or 16 years, respectively 38% and 20% of the baseline PIAMA population of 3963 children. In these children, parental education was higher and the prevalence of overweight was

lower (only in the 16 years study sample) than in the original PIAMA population. However, selective loss to follow up of those with a lower parental education does not necessarily imply bias in the observed associations[5]. The studies in this thesis were adjusted for level of education of the parents and this did not have a major impact on the observed associations. Furthermore, the associations may be assumed to be in the same direction for children of parents with low or high education, implying that the findings of this thesis may be generalizable to children with lower educated parents as well.

HbA1c as a marker for cardiometabolic risk

Blood pressure and cholesterol measures (TC, HDLC, TC/HDLC ratio) are established risk factors for cardiovascular disease, but there is ongoing debate on HbA1c as a marker for insulin resistance and diabetes, particularly in children[6-8]. This thesis shows that levels of HbA1c are higher in children with a strong history of diabetes in parents and grandparents, independent of child's BMI. Previous studies showed that HbA1c levels are also elevated in children born to mothers with gestational diabetes[9] and in children with parental diabetes[10]. HbA1c was not associated with adiposity and lifestyle factors in this thesis, which is in agreement with other studies[6,9]. HbA1c reflects blood glucose levels over the preceding 2 to 3 months and has the practical advantage over glucose that it does not require fasting samples. HbA1c is stable over time[11,12] and high levels of HbA1c are predictive for cardiovascular disease morbidity and mortality[8,13]. Although this suggests that HbA1c is a relevant marker for assessment of cardiometabolic risk early in life, further studies need to investigate whether early life measurements of HbA1c contribute to later life risk for diabetes and cardiovascular disease.

Implications

Reversibility of adverse levels of cardiometabolic markers in childhood

An important question is whether an unfavourable cardiometabolic profile in childhood and adolescence is reversible in later life. It can be argued that adverse cardiometabolic effects of unfavourable lifestyle behaviours in childhood such as insufficient physical activity or an unhealthy diet can be reversed with favourable lifestyle changes or medication. For example, reduction of blood cholesterol levels by diet or drugs reverses the risk for cardiovascular disease over a relatively short period[14]. Further, adverse cardiometabolic

effects of childhood adiposity are reversible by weight loss[15]. On the other hand it can be argued that childhood exposures increase the risk for cardiovascular disease by increasing the cumulative lifetime exposure[14]. For example, the effect of sodium restriction during the first 6 months of life on blood pressure levels increases with age[16]. Mortality from heart disease across countries strongly correlates with dietary fat consumption and cholesterol levels in the past (>20-30 years preceding disease occurrence) but only weakly to recent levels[17]. Furthermore, greater cumulative exposure to higher blood pressure levels is associated with increased risks for cardiovascular disease, despite long-term antihypertensive treatment.[18]. Thus, adverse effects of childhood exposures may be reversed if people alter their behaviour in adulthood, but for some exposures, the adverse effects accumulate over the life course and result in a higher risk for cardiovascular disease in adulthood.

Relevance for health of populations and individuals

Although the observed effect sizes in this thesis were modest, these may be important for the future burden of cardiovascular disease under the assumption that these effects are causally related to the occurrence of cardiovascular disease. Firstly, because children with elevated levels of cardiometabolic markers are likely to continue with high levels into adulthood and will subsequently have an increased risk of cardiovascular disease. Secondly, small elevations in levels of cardiometabolic markers and associated small relative risks may be relevant if they occur in a large proportion of the population. For example, a small increase in blood pressure levels in a large proportion of the population may result in a large increase in the prevalence of hypertension and associated CVD risk in the total population[19,20]. Thirdly, the prevalence of some of the determinants was substantial in the PIAMA population. This implies that a large part of the future adult population may be at risk for an unfavourable cardiometabolic profile, and may subsequently develop cardiovascular disease. For instance, one third of the PIAMA children had a strong family history of either cardiovascular disease or diabetes. Furthermore, overweight and the metabolic syndrome are more prevalent in the generation of the PIAMA cohort, than in previous generations[21,22]. Since these conditions strongly increase the risk for cardiovascular disease, such trends may contribute to a higher future burden of cardiovascular disease.

Some effect sizes observed in this thesis may be relevant on the individual level since these were clinically relevant in adult populations. For example, children with a strong family history of diabetes had 2.25 cm larger waist circumference, which is approximately 3% of

their mean waist circumference (Chapter 2). A reduction in waist circumference of >3% from initial waist circumference may be considered clinically relevant for overweight adults in the long term[23]. Furthermore, children who followed a pattern of gradually increasing overweight had 0.75 higher TC/HDLC ratio (Chapter 5). In adult men, a 1 unit increase in TC/HDLC ratio is associated with a 53% higher risk of myocardial infarction[24]. Although the long-term clinical importance of the observed differences in this thesis still needs to be established, the current body of evidence suggests that also these small differences may translate into a higher future risk for cardiovascular disease at the individual level.

Implications for prevention of cardiovascular disease

In a Policy Statement, the American Heart Association noted, "with primary prevention, cardiovascular disease is largely preventable"[25]. Primary prevention strategies may encourage individuals to change their behaviour, e.g. by campaigns to promote healthy eating; or they may facilitate or force behavioural change in the total population, e.g. by infrastructure that encourages cycling and other physical activities, smoking bans, or policies for eliminating artificial trans-fats from the diet.

This thesis shows that lifestyle factors (sleep quality and duration, physical activity, and sedentary behaviour) are determinants of the cardiometabolic profile at age 12 years. Assuming a causal relationship, this suggests that preventing unfavourable lifestyle behaviours in children may have beneficial effects on the cardiometabolic profile from childhood into adulthood, and may ultimately lower the population burden of cardiovascular disease. In addition, childhood and adolescence are important periods for establishing healthy lifestyle habits and for preventing overweight, emphasizing the importance of starting prevention of cardiovascular disease already in childhood.

The finding that children with more screen time have less physical activity and higher adiposity (Chapter 4) highlights the importance of encouraging physical activity as well as limiting sedentary screen based behaviours for overweight prevention. However, only those who are highly motivated will maintain such healthy behaviours in the long term, particularly in an 'obesogenic' environment that promotes sedentary behaviour. Efforts for increasing physical activity while limiting sedentary time during school hours were effective both for decreasing adiposity and improving cognitive function[26]. Thus, creating an activity-friendly environment by infrastructural measures and regulations is important for children and adolescents to achieve a healthy lifestyle and may thereby improve cardiovascular health of the population.

Conclusions

The findings from this thesis provide insight into determinants of the cardiometabolic profile in childhood and adolescence, and support evidence that development of cardiometabolic risk starts early in life. Already at age 12 years, there was variation in levels of cardiometabolic markers that was associated with family history, lifestyle factors, and overweight development earlier in childhood. In general, children and adolescents with a strong family history of cardiovascular disease or diabetes, persistent overweight or gradually developing overweight, less physical activity, girls with lower sleep quality, and girls with early pubertal timing had unfavourable levels of cardiometabolic markers. Lifestyle factors were associated with the cardiometabolic profile partly through adiposity. Family history of diabetes and developing overweight in childhood may be most important for future cardiovascular disease because these factors were associated with a wide range of cardiometabolic markers. The findings in this thesis highlight the importance of adiposity, as a risk factor in itself, and as a pathway in between determinants and cardiometabolic risk. Although the observed effect sizes of determinants investigated in this thesis were modest, these determinants are important for the future cardiometabolic burden at a population level. With population strategies for prevention of overweight and unfavourable lifestyle behaviours starting early in life, the long-term cardiovascular health of the entire population may be improved.

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Summary

Chapter 1 provides the background of this thesis. The atherosclerotic process leading to cardiovascular disease begins early in life and is influenced over time by several risk factors. Investigating determinants that contribute to an unfavourable cardiometabolic profile in childhood and adolescence is important for specifying time windows suitable for prevention or intervention early in the process of cardiovascular disease development, and ultimately for improving cardiovascular health of the population. The aim of this thesis was to identify and characterize determinants of an unfavourable cardiometabolic profile in childhood and adolescence. The cardiometabolic profile was characterized by indicators of adiposity, levels of total cholesterol (TC), HDL cholesterol (HDLC), total/HDL cholesterol ratio, blood pressure and glycated haemoglobin (HbA1c). As indicators of adiposity, body mass index (BMI), waist circumference and waist-to-height ratio were used. The individual studies presented in this thesis were embedded in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study; an ongoing population based prospective study in the Netherlands that has followed children from before birth until young adulthood.

Already in adolescence (ages 12 and 16 years), there was variation in levels of cardiometabolic markers that could partly be explained by determinants in childhood, which are well-known risk factors for adult cardiovascular disease. These determinants were familial factors (family history of cardiovascular disease or diabetes), lifestyle factors (sleep, sedentary behaviour, physical activity, and snack consumption), and biological factors (patterns of overweight development and pubertal timing).

Chapter 2 describes how the disease burden of cardiovascular disease and/or diabetes that runs within families is associated with the child's cardiometabolic profile, and examines disease specific associations with cardiometabolic markers. The study population consisted of 1374 children who had parental reports on family history of CVD (myocardial infarction (MI) and stroke) and diabetes in two generations (e.g. biological parents and grandparents), and for whom adiposity and cardiometabolic markers at age 12 years were available. Children were classified either as having 'no'; 'moderate'; or 'strong' family history, based on early/late disease onset in parents and grandparents. One third of the children had a strong family history of CVD and/or diabetes. These children had higher levels of TC and TC/HDLC ratio at age 12 years than those without a family history of these diseases. The type of disease (cardiovascular disease or diabetes)

determined which type of cardiometabolic marker was elevated in these children. Family history of cardiovascular disease was important for children's cholesterol levels, a typical risk factor for cardiovascular disease, whereas family history of diabetes was important for children's waist circumference and HbA1c, typical risk factors for diabetes.

Chapter 3 describes how several aspects of sleep contribute to the child's cardiometabolic profile at age 11-12 years: sleep duration, sleep-wake pattern and sleep quality (night-time awakenings and daytime outcomes). The study population consisted of 1481 children who reported sleep items by questionnaire at age 11 years and for whom adiposity and cardiometabolic markers at age 12 years were available. Specific aspects of sleep were associated with unfavourable levels of cardiometabolic markers in girls, but not in boys. In girls, long sleep duration was associated with lower adiposity, whereas going to bed late/rise early, night-time awakenings and feeling tired were unfavourably associated with different measures of cholesterol (TC, HDLC and TC/HDLC ratio). Other aspects of sleep were not associated with cardiometabolic markers. The relative importance of sleep duration versus sleep quality for the cardiometabolic profile is difficult to determine and deserves further study.

Chapter 4 describes pathways from screen time to higher adiposity (via more snacking or less physical activity) and to the cardiometabolic profile. The study population consisted of 1447 children who reported screen time and other lifestyle factors by questionnaire at age 11 years, and for whom adiposity and cardiometabolic markers at age 12 years were available. A mediation analysis was conducted to study the different pathways from screen time to adiposity and cardiometabolic profile. The higher adiposity in children with high levels of screen time (as indicator for total sedentary time), was partly explained by less physical activity, but not by higher snack consumption. The higher TC/HDLC ratio in children with more screen time was almost completely explained by higher adiposity and to a minor extent by less physical activity.

Chapter 5 describes the association between patterns of overweight development from 3 months to 11 years and the child's cardiometabolic profile at age 12 years. Parents reported height and weight (thus BMI) during 10 waves of follow-up over the course of 11 years. Taking in account all of these repeated BMI reports, four distinct overweight development patterns were derived using longitudinal latent class analysis; 'never'; 'early transient'; 'gradually developing' and 'persistent' overweight. The study population consisted of 1500 children who had cardiometabolic markers measured at age 12 years. The presence of overweight from the age of 3 months to 11 years,

either gradually developing during childhood or persistently present from early infancy onwards, was strongly associated with unfavourable levels of cardiometabolic markers, and with clustering of two or more of these markers. Children with gradually developing overweight and those with persistent overweight over this period had a 2-3 fold higher risk of being in the upper percentile of TC/HDLC ratio, systolic and diastolic blood pressure by the age of 12 years. In contrast, children with early transient overweight had no unfavourable levels of cardiometabolic markers at age 12 years.

Chapter 6 describes whether boys and girls with early or late pubertal timing have different levels of cardiometabolic markers than those with intermediate pubertal timing. The study population consisted of 799 boys and girls who self-reported the Pubertal Development Scale (PDS) throughout adolescence (at age 11, 14 and 16 years), and for whom adiposity and cardiometabolic markers at age 16 years were available. Using percentile cut-off values for the age-standardized PDS, adolescents were classified as early, intermediate or late pubertal timing. Girls with early (compared with intermediate) pubertal timing had higher blood pressure levels at age 16 years, suggesting one pathway by which early menarche may contribute to a higher risk for cardiovascular disease in adult women. Boys with early pubertal timing did not have more unfavourable levels of cardiometabolic markers than those with intermediate pubertal timing. Boys and girls with late (compared with intermediate) pubertal timing had no differences in levels of cardiometabolic markers in adolescence.

Chapter 7 provides a general discussion of the main findings of this thesis, the methodological considerations and implications for individual and population cardiovascular health. This thesis shows that several well-known risk factors for cardiovascular disease in adults were also determinants of the cardiometabolic profile in children and adolescents and supports evidence that the development of cardiovascular risk starts early in life. In general, children with a strong family history of cardiovascular disease or diabetes, those with less physical activity, those with gradually developing or persistent overweight in childhood, girls with lower sleep quality, and girls with early pubertal timing had unfavourable levels of cardiometabolic markers in childhood and adolescence. Of all these determinants, developing overweight in childhood and a strong family history of diabetes may be most unfavourable for future cardiovascular disease risk because these factors were associated with a wider range of cardiometabolic markers. Lifestyle factors (sleep, screen time and physical activity) were associated with the cardiometabolic profile mainly through higher adiposity, suggesting that adiposity is an important mediator in

the pathway of unfavourable lifestyle behaviours to cardiovascular outcomes. Although the observed effect sizes of determinants investigated in this thesis were modest, the high prevalence of these determinants in the general populations may lead to a relevant contribution to the future burden of cardiovascular disease at a population level. With population strategies for prevention of overweight and unfavourable lifestyle behaviours starting early in life, the long-term cardiovascular health of the entire population may be improved.

Nederlandse samenvatting

Hoofdstuk 1 beschrijft de achtergrond van dit proefschrift. Het atherosclerotische proces dat ten grondslag ligt aan hart- en vaatziekten begint vroeg in het leven en wordt beïnvloed door verschillende risicofactoren. Het is van belang om te onderzoeken welke determinanten bijdragen aan een ongunstig cardiometabool profiel in de kindertijd en adolescentie. Dit geeft inzicht in de perioden die het meest geschikt zijn voor vroege preventie van hart- en vaatziekten, en daarmee kan de cardiovasculaire gezondheid van de hele bevolking verbeterd worden. Het doel van dit proefschrift was om determinanten van een ongunstig cardiometabool profiel in de kindertijd en de adolescentie te identificeren en te beschrijven. Met het cardiometabool profiel wordt in dit proefschrift bedoeld: het profiel van meetbare cardiometabole markers zoals middelomtrek, cholesterol en bloeddruk, die bij volwassenen gerelateerd zijn aan het risico op hart- en vaatziekten. De deelstudies in dit proefschrift zijn uitgevoerd binnen de Preventie en Incidentie van Astma en Mijt Allergie (PIAMA) geboortecohort studie; een lopende Nederlandse prospectieve studie waarin kinderen vanaf de geboorte tot in de vroege volwassenheid gevolgd zijn.

Al in de adolescentie (leeftijd 12 en 16 jaar) was er variatie in niveaus van cardiometabole markers die deels verklaard kon worden door determinanten in de kindertijd, welke tevens bekende risicofactoren voor hart- en vaatziekten zijn bij volwassenen. Deze determinanten waren familiaire factoren (familiegeschiedenis van hart- en vaatziekten of diabetes), leefstijlfactoren (slaap, sedentair gedrag, beweging en snacken) en biologische factoren (patronen van overgewicht ontwikkeling en timing van de puberteit).

Hoofdstuk 2 beschrijft hoe de ziektelast van hart- en vaatziekten en/of diabetes binnen de familie geassocieerd is met het cardiometabool profiel van het kind. De studiepopulatie bestond uit 1374 kinderen waarvan familiaire belasting van hart- en vaatziekten (hartinfarct en beroerte) en diabetes in twee generaties bekend was (ouders en grootouders), en waarbij cardiometabole markers waren gemeten op de leeftijd van 12 jaar. Kinderen werden geclassificeerd als 'geen', 'matige' of 'sterke' familiaire belasting, gebaseerd op vroege/late aanvang van de ziekte bij ouders en grootouders. Een derde van de kinderen had een sterke familiaire belasting van hart- en vaatziekten en/of diabetes. Deze kinderen hadden hogere niveaus van totaal cholesterol en totaal/HDL-cholesterol ratio dan kinderen zonder deze ziekten in de familie. Het soort ziekte (hart- en vaatziekten of diabetes) bepaalde welke cardiometabole marker verhoogd was bij deze kinderen. Familiaire belasting van hart- en vaatziekten was van belang voor cholesterolwaarden van

het kind – een typische risicofactor voor hart- en vaatziekten, terwijl familiaire belasting van diabetes van belang was voor de middelomtrek en het hemoglobine A1c – typische risicofactoren voor diabetes.

Hoofdstuk 3 beschrijft hoe verschillende aspecten van slaap bijdragen aan het cardiometabool profiel van het kind op de leeftijd van 11-12 jaar: slaapduur, slaap-ontwaak patroon en slaap kwaliteit ('s nachts wakker worden en moeheid overdag). De studiepopulatie bestond uit 1481 kinderen die een vragenlijst met vragen over slaap hadden ingevuld op de leeftijd van 11 jaar, en waarbij cardiometabole markers waren gemeten op de leeftijd van 12 jaar. Bepaalde aspecten van slaap waren geassocieerd met het cardiometabool profiel bij meisjes, maar niet bij jongens. Bij meisjes was een lange slaapduur geassocieerd met een lagere body mass index (BMI) en middelomtrek, terwijl laat naar bed gaan en vroeg opstaan, 's nachts wakker worden, en moeheid overdag ongunstig geassocieerd waren met verschillende maten van cholesterol (totaal cholesterol, HDL cholesterol en totaal/HDL-cholesterol ratio). Andere aspecten van slaap waren niet geassocieerd met cardiometabole markers. Het belang van slaapduur versus slaapkwaliteit voor het cardiometabool profiel is lastig te bepalen en zou verder onderzocht moeten worden.

Hoofdstuk 4 beschrijft hoe beeldscherm-tijd (TV kijken en computeren) bij zou kunnen dragen aan een hogere BMI en middelomtrek (via meer snacken of minder bewegen) en aan ongunstige niveaus van cardiometabole markers. De studiepopulatie bestond uit 1447 kinderen die beeldschermgebruik en andere leefstijlfactoren hadden gerapporteerd op de leeftijd van 11 jaar, en waarbij cardiometabole markers waren gemeten op de leeftijd van 12 jaar. De bijdrage van beeldschermgebruik aan een hogere BMI en middelomtrek en aan het cardiometabool profiel werd bestudeerd door middel van mediatie analyse. Dat kinderen met veel beeldscherm-tijd (als indicator voor de totale tijd die zittend wordt doorgebracht) een hogere BMI en middelomtrek hadden kon deels worden verklaard doordat ze minder actief waren, maar niet door een hogere snack consumptie. De hogere niveaus van totaal/HDL-cholesterol ratio bij kinderen met veel beeldscherm-tijd kon bijna volledig worden verklaard door een hogere BMI en middelomtrek en voor een klein deel door minder beweging.

Hoofdstuk 5 beschrijft de associatie tussen patronen van overgewicht ontwikkeling van de leeftijd 3 maanden tot 11 jaar en het cardiometabool profiel op de leeftijd van 12 jaar. Ouders rapporteerden lengte en gewicht (BMI) op 10 momenten gedurende 11 jaar. Door middel van longitudinale latente klasse analyse werden 4 verschillende patronen

van overgewicht ontwikkeling gevonden in de PIAMA populatie; 'nooit', 'voorbijgaand', 'geleidelijk ontwikkelend' en 'aanhoudend' overgewicht. De studiepopulatie bestond uit 1500 kinderen waarbij cardiometabole markers gemeten waren op de leeftijd van 12 jaar. Het ontwikkelen van overgewicht vanaf de leeftijd van 3 maanden tot 11 jaar, hetzij geleidelijk over deze periode, hetzij aanhoudend vanaf de vroege kindertijd, was sterk geassocieerd met ongunstige niveaus van cardiometabole markers en met het gelijktijdig optreden van twee of meer van deze ongunstige niveaus bij hetzelfde kind. Kinderen die geleidelijk overgewicht ontwikkelden, en kinderen met aanhoudend overgewicht van jongs af aan, hadden een twee- tot drievoudig verhoogd risico op ongunstige niveaus van totaal/HDL-cholesterol ratio, systolische en diastolische bloeddruk op de leeftijd van 12 jaar. Kinderen die op jonge leeftijd overgewicht hadden maar dat later kwijtraakten, hadden geen ongunstige waarden van cardiometabole markers.

Hoofdstuk 6 beschrijft of jongens en meisjes met vroege of late puberteit andere niveaus van cardiometabole markers hebben dan adolescenten met een gemiddelde timing van de puberteit. De studiepopulatie bestond uit 799 jongens en meisjes die tijdens de adolescentie (op leeftijd 11, 14 en 16 jaar) de ontwikkeling van secundaire geslachtskenmerken hadden gerapporteerd, en waarbij cardiometabole markers gemeten waren op de leeftijd van 16 jaar. Voor het navragen van de ontwikkeling van geslachtskenmerken is gebruik gemaakt van de Pubertal Development Scale (PDS). De adolescenten werden ingedeeld in 3 categorieën (vroege-, gemiddelde en late puberteit) aan de hand van afkapwaarden gebaseerd op percentielen van de PDS. Meisjes met vroege (vergeleken met gemiddelde) puberteit hadden hogere bloeddruk niveaus op leeftijd 16 jaar; dit suggereert dat vroege puberteit via een hogere bloeddruk zou kunnen bijdragen aan een verhoogd risico op hart- en vaatziekten bij volwassen vrouwen. Jongens met vroege (vergeleken met gemiddelde) puberteit hadden geen ongunstigere niveaus van cardiometabole markers. Jongens en meisjes met late (vergeleken met gemiddelde) puberteit hadden geen verschillende niveaus van cardiometabole markers in de adolescentie.

Hoofdstuk 7 geeft een algemene beschouwing van de belangrijkste resultaten van dit proefschrift, methodologische overwegingen en implicaties voor de cardiovasculaire gezondheid van individuen en van de totale bevolking. Dit proefschrift laat zien dat meerdere bekende risicofactoren voor hart- en vaatziekten bij volwassenen ook determinanten zijn van het cardiometabool profiel bij kinderen en adolescenten, en ondersteunt de hypothese dat de ontwikkeling van het cardiovasculaire risico vroeg in

het leven begint. Kinderen met een sterke familiaire belasting van hart- en vaatziekten of diabetes, kinderen die minder fysiek actief waren, kinderen die overgewicht ontwikkelden of die dat al vanaf jonge leeftijd hadden, meisjes met een lagere kwaliteit van slaap, en meisjes met vroege puberteit, hadden ongunstigere niveaus van cardiometabole markers in de kindertijd en de adolescentie. Van al deze determinanten leken het ontwikkelen van overgewicht en een sterke familiaire belasting van diabetes het meest ongunstig, omdat deze determinanten geassocieerd waren met meerdere, verschillende cardiometabole markers. Leefstijlfactoren (slaap, beeldschermgebruik en fysieke activiteit) waren voornamelijk via een hogere BMI en middelomtrek geassocieerd met het cardiometabool profiel, wat suggereert dat overgewicht een belangrijke mediator is op het pad van ongunstige leefstijlfactoren naar cardiovasculaire uitkomsten. Hoewel de gevonden effectschattingen bescheiden waren, kan de hoge prevalentie van deze determinanten van belang zijn voor de toekomstige ziektelast van cardiovasculaire ziekten in de totale bevolking. Met strategieën voor preventie van overgewicht en ongunstige leefstijlfactoren al vroeg in het leven, zou de cardiovasculaire gezondheid van de hele bevolking in de toekomst verbeterd kunnen worden.

Curriculum vitae

Nina Berentzen was born on September 21, 1983 in Amsterdam, the Netherlands. In 2004, she started with the study Nutrition and Dietetics at the Hogeschool van Amsterdam (Amsterdam University of Applied Sciences). She conducted her bachelor thesis in Delhi, India, where she worked on the DIVIDS trial (Delhi Infant Vitamin D Study) under supervision of Prof. Dr. Suzanne Filteau (London School of Hygiene & Tropical Medicine). In 2009 she obtained her BSc and continued studying Nutrition and Health at Wageningen University (specialization: Epidemiology and Public Health). The work for her master thesis was performed within the PIAMA study, a large birth cohort study in the Netherlands. She conducted a second research project at the Julius Center for Health Sciences and Primary Care, which resulted in a paper on the healthy diet indicator and overall cancer risk within the EPIC-NL study. After obtaining her MSc in 2011, she started her 4-years PhD project entitled 'Early origins of cardiovascular health' within the PIAMA study, under supervision of Prof. Dr. Jet Smit (Julius Center for Health Sciences and Primary Care, Utrecht University Medical Center) and Dr. Alet Wijga (National Institute of Public Health and the Environment - RIVM, Bilthoven). The results of this work are presented in this thesis. From February 2016 onwards she will be working as a postdoctoral researcher at the Netherlands Cancer Institute in Amsterdam.

List of publications

Manuscripts within this thesis

Berentzen NE, van Rossem L, Gehring U, Koppelman GH, Postma DS, de Jongste JC, Smit HA, Wijga AH. Overweight patterns throughout childhood and cardiometabolic markers in early adolescence. Int J Obes (Lond). 2015 Sep 23. [Epub ahead of print]

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