Early life determinants of cardiovascular disease

Effects of cardiovascular risk factors in childhood and adolescence on the carotid intima media thickness in healthy, young adolescents

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Abbreviations

ACE	Adverse childhood events
AEPC	Association for European Paediatric Cardiology
BMI	Body mass index
BP	Blood pressure
cIMT	Carotid intima media thickness
CVD	Cardiovascular disease
LGA	Large for gestational age
PAT	Peripheral arterial tonometry
PWV	Pulse wave velocity
SGA	Small for gestational age
WHISTLER	Wheezing Illnesses Study Leidsche Rijn
WHR	Waist-hip-ratio

Abstract

Purpose: The purposes of this study were to primarily investigate whether determinants measured in adolescence could predict carotid intima-media thickness in adolescents aged 12-16 years. And secondly to determine whether predictors measured in childhood could predict cIMT in adolescents in addition to these measurements in adolescence.

Methods: For this study, data was used from a Dutch birth cohort initiated for the Wheezing Illnesses study Leidsche Rijn, in which healthy children were included at birth and follow-up was obtained until the age of 12-16 years for 232 children. Data from physical measurements combined with questionnaires at the age of 5 and 12-16 was included. With this, univariable and multivariable linear regression analyses were performed for the determinants and predictors separately.

Results: As for the determinants, no statistically significant associations were found with carotid intima-media thickness in adolescence, neither in univariable, nor multivariable linear regression analyses. Regarding the predictors, carotid intima-media thickness in childhood was found to be associated with carotid intima-media thickness in adolescence in univariable linear regression analysis (unstandardized beta 0.337 [95% CI 0.032-0.642]) and multivariable linear regression analysis (unstandardized beta 0.412 [95% CI 0.094-0.729]). The other predictors showed no statistically significant association with cIMT in adolescence.

Conclusion: Carotid intima-media thickness in childhood is found to be a significant predictor for carotid intima-media thickness in adolescence. Apart from this, none of the investigated determinants or predictors showed a significant association with carotid intima-media thickness in adolescence.

Introduction

The mortality rates of cardiovascular disease (CVD) have drastically decreased in most high-income countries since the middle of the 20th century,[1] partly due to the identification and treatment of risk factors.[2] In the Netherlands, mortality due to CVD decreased with 46% in men and 33% in women from 1980 to 2018.[3] Nevertheless, CVD persists to be the most common cause of premature adult mortality in Europe, costing more than 60 million potential years of life annually.[4]

The pathophysiology of CVD is essentially based on the development of atherosclerotic plaques, starting with fatty streaks, gradually developing into atheromatous plaques, with the risk of rupturing and causing local thrombosis.[1,5] The major clinical manifestations of atherosclerosis include ischemic heart disease, ischemic stroke and peripheral arterial disease.[1]

Even though the burden of these diseases is mostly carried out in adulthood, atherosclerosis emerges in the early years of life, as was found by the Bogalusa Heart study in 1998.[6] In this study, autopsies were performed on 204 persons aged 2 to 39 years, who died mostly of accidental causes. They found that fatty streaks and fibrous plaques were present in these young subjects and the extent increased with age.[6] Although the pathologic effects mostly occur in adult life, atherosclerosis can therefore not be viewed as a purely adult disease.

Adult risk factors, identifying the patients who are at risk for CVD, have been established over the last decades, the most relevant factors being: hypertension, high cholesterol, overweight, male sex and smoking.[2] Identifying children and adolescents who are affected by early atherosclerosis remains challenging.[7] This is unfortunate, because research showed that implementing lifestyle interventions in late youth and beginning adolescence could contribute to prevention of progression of early atherosclerosis, with additional effect to adult prevention programs.[8,9]

A promising method in identifying adolescent risk groups is the measurement of the carotid intima-media thickness (cIMT), as it is associated with cardiovascular risk factors and cardiovascular outcomes.[10] In this ultrasound examination, the span from the lumen-intima interface to the media-adventitia interface of the carotid wall is measured.[10] An increased cIMT of the common carotid wall indicates atherosclerosis in the intima layer.[10] cIMT measurement is easy, fast and reproducible.[11] It could be used as a surrogate marker for cardiovascular risk, and therefore have an important impact in implementing preventive interventions.[11] Further research into the long-term predictive value of cIMT is necessary, as its value in cardiovascular prevention, over and above traditional risk factors, is a matter of ongoing debate.[10,11] But, despite that, the Association for European Paediatric Cardiology (AEPC) Working Group on Cardiovascular Prevention strongly recommends the use of cIMT in screening patients with elevated cardiovascular risk.[11] In conclusion, cIMT could be viewed as a reliable surrogate marker for cardiovascular risk,[10,11] and could possibly be used to identify the children and adolescents who would benefit from lifestyle interventions.

But, how to select the subjects who are at risk and thus need screening through cIMT for cardiovascular risk? Risk factors that significantly predict an elevated cIMT in children and young adults are yet to be established.

To determine these risk factors, known adult risk factors could be researched in adolescence in cross-sectional context, such as gender, age, body mass index (BMI), blood pressure (BP), presence of CVD in the family history and smoking.[5,8,9,12] Besides, factors that

are thought to be childhood/adolescence risk factors for CVD could be included, such as physical activity and fruit intake.[5,8] Adverse childhood events (ACE's) are recently found to be an additional risk factor for developing CVD.[13] ACE's are divided into three categories: neglect, abuse and household dysfunction.[14] On top of that, some new ACE's have recently been added, such as bullying, not having good friends, not having good grades in school, exposure to community violence, exposure to nature disasters, severe overweight, severe unintentional injury, disease or death of someone close, parental unemployment and homelessness. [13,15]

Furthermore, measurements performed in childhood have been seen to be associated with progression of cIMT.[8] For example, an elevated BMI in childhood is associated with an increased cIMT in young adulthood.[16] Another factor that might predict the cIMT, is birth weight, being born small for gestational age (SGA) or large for gestational age (LGA) is associated with a higher cardiovascular risk later in life.[17] Also, Sjöholm et al. found that children born SGA have a significantly lower cIMT at the age of 18-19 years, compared to children born LGA.[18] Apart from BMI and birth weight, a large gain in weight throughout childhood has been found to be associated with a higher blood pressure and other cardiovascular markers, among which cIMT.[19] Systolic BP has been seen to increase the progression of cIMT in early adolescence in the young Finns study.[8] Also, an elevated blood pressure in childhood is associated with an elevated cIMT in early adulthood.[16] Lastly, even though it has not been extensively described in current literature, we presumed cIMT measured in early childhood to be a possible predictor for cIMT in adolescence.

Therefore, this study primarily aims to investigate which determinants in cross-sectional context, are associated with an elevated cIMT in healthy adolescents aged 12 to 16 years old. More specifically, the following determinants will be taken into account: gender, overweight, hypertension, physical activity, smoking, CVD in family history, fruit intake and ACE's.
Secondarily, this study aims to investigate whether longitudinal predictors measured in early childhood, are able to predict an elevated cIMT in healthy adolescents, in addition to these measurements in adolescence. Herein, gender, age, BMI, systolic and diastolic BP, birth weight and cIMT will be considered.

Methods

Study design and population

Data were obtained from a Dutch birth cohort: the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), which was originally set up to investigate determinants of wheezing illnesses.[20] Children born between 2001 and 2012 were included in Leidsche Rijn, a newly built district in Utrecht, the Netherlands. Besides determinants of wheezing illnesses, many other topics have been researched, from birth up to the age of 12-16 years old at the last follow-up rounds, also including cIMT.

In this study, 232 adolescents aged 12 to 16 years from the WHISTLER population were enrolled, as they completed the measurements and questionnaires performed between January 2019 and October 2020, as well as previous measurements and questionnaires, among which a set performed at the age of 5. Informed consent was obtained from all participants or their parent/guardian.

In this study, all variables measured at the same age as the outcome variable are referred to as determinants, more specifically, these are measured at the age of 12 to 16 years. All variables measured earlier in life are referred to as predictors, these are measured before the age of 12 years, mostly done at 5 years of age.

Definition of outcome

cIMT was measured on the right common carotid artery, approximately 0.5cm proximal to the bifurcation. Three measurements were performed, of which a mean was calculated.

Determinants

The exposure variables of interest were obtained by physical measurements or questionnaires, based on the HBSC 2017 questionnaires.

Blood pressure

BP was measured on the right arm, using an automatic device. At least two measurements were done, and if a difference of 10mmHg or more was found in any of the measurements (diastolic BP, systolic BP, mean arterial pressure or heart rate), a third measurement was performed. At the age of 5, BP was measured while seated, in adolescence, BP was measured laying down. For both the childhood and adolescence measurements, the least of these two or three was used to calculate a percentile, based on sex, height and age, using the 2017 AAP Guidelines for Screening and Management of High Blood Pressure.[21]

Anthropometrics

Height was measured in centimeters without shoes, rounded to one decimal. Weight was measured in kilograms without clothes and shoes, rounded to 0.5kg (e.g. 63.3kg > 63.5kg). Height, weight, age and sex were used to calculate a Z-score for BMI, using the Dutch 'JGZ-richtlijn lengtegroei.'[22]

Birth weight was requested at inclusion in the WHISTLER cohort, shortly after birth. A Z-score for birth weight was calculated, also using the Dutch 'JGZ-richtlijn lengtegroei,'[22] in which gestational age and sex were inserted.

The increase in BMI Z-score from childhood to early adolescence was calculated by subtracting the Z-score at 5 years old from the Z-score in adolescence. Because BMI in

adolescence was now represented in the Z-score increase, the BMI Z-score in adolescence was removed from the longitudinal analysis, to prevent multicollinearity.

Including the waist-hip-ratio (WHR) has been considered, since there is evidence regarding that, not in particular BMI, but moreover body composition and fat distribution provides for an elevated cardiovascular risk.[23,24] However, a strong relationship between gender and WHR and between age and WHR exists, whereas WHR is higher in men and decreases with increasing age.[25,26] On top of that, the extent of this decrease with age, differs between men and women.[25] A statistically significant correlation was also found in this cohort, for age and WHR (Pearson's R -0.184 (p-value 0.005)) and for gender and WHR (Pearson's R 0.497 (p-value 0.000)), as was expected regarding the earlier mentioned literature. Subsequently, due to the high risk of multicollinearity between gender and/or age and WHR, WHR was not included in analyses.

Carotid intima-media thickness in childhood

At the age of 5, the cIMT was measured on the right common carotid artery, approximately 0.5cm proximal to the bifurcation. Three to four measurements were performed, of which a mean was calculated.

Physical activity and life style

All determinants regarding physical activity and life style were determined in the adolescence follow-up round, at the age of 12-16 years.

The level of physical activity was established, through 3 questions from the HBSC 2017 questionnaire, which was found to be a valid measurement of physical activity.[27] The questions were regarding the frequency of physical activity, the time spent playing/sporting per week and how much time is spent at a sports club. A scoring system was created, following the method used by the Young Finns study.[28] For every question a score of 1-3 points could be achieved, summing up to a total score, with a minimum of 3 to a maximum of 9 points.

For fruit intake, the following options were included in the questionnaire, scored as the number given: never (0), less than once a week (1), once a week (2), 2-4 times a week (3), 5-6 times a week (4), once a day (5) and more than once a day (6).

Smoking data were collected through anonymous questionnaires, so that participants could answer confidentially. The questions used have been proven to be valid, as there is no significant difference found when test-retest study is performed.[29] Subjects who reported smoking at least once a week or more were considered smokers.

Family history was assessed through a questionnaire for the parents/guardians, asking for the presence of cardiovascular disease in parents or grandparents before the age of 60 years.

To find whether an association between ACE's and cIMT could be established in the WHISTLER population, household composition, bullying, chronical illnesses in the family and life satisfaction were included in this analysis, being assessed through the questionnaire. Household composition was investigated using questions about who lives in the same house as the subject, the groups were made based on whether someone lives with both their parents in the same house or not. Bullying was measured using questions on real-life bullying and bullying through the internet, subjects who reported being bullied at least two to three times a month, regardless of the setting, were considered as being bullied. Chronical illness was defined as someone in the family being chronically ill, including the subject themselves. Lastly,

life satisfaction was measured using the Cantril ladder, which is a validated and reliable measurement of life satisfaction in adolescents.[30]

Statistical analysis

To investigate the effect of risk variables on cIMT, for the physical measured variables, Z-scores and percentiles were calculated for birth weight, BMI and BP, to correct for possible biases caused by age, sex and height. Outliers of determinants were removed if they were 3 interquartile ranges higher than the third quartile, or lower than the first quartile. For cIMT, outliers were removed if the difference between the 3 individual measurements was higher than 2 standard deviations. In this data, no outliers were present. Firstly, for each determinant univariable linear regression analysis was performed, and after that a multivariable linear regression analysis was performed combining all determinants. Secondly, for all predictors also univariable linear regression analyses were performed, and after that, a multivariable linear regression analysis was done, including the predictors and physical measured determinants combined. All using IBM SPSS Statistics 26. Statistical significance was defined by 95% confidence intervals.

Results

Study population

In this section, the standard deviation is given between brackets, following the mean.

In total, 232 adolescents were enrolled in statistical analyses, among which 121 were female (52.2%) with a mean age of 14.77 years (1.28) and a mean cIMT of 442.35um (73.15). As for the physical measurements, the mean systolic and diastolic blood pressure were 112mmHg (10.4) and 57mmHg (6.6) respectively and the mean BMI was 19.8kg/m^2 (3.1). Overall a mean physical activity index of 7.1 (1.8) was found, with the maximum possible score being 9. The mean fruit index was 3.9 (1.4), whereas an index of 4 means fruit is eaten four to six times a week. 87.9% of included children lived with both their parents in the same household, and the children reported a mean Cantril ladder score of 7.6 (1.2). In 25 cases (11.2%), there was someone close with a chronical illness and in 33.5% a known family history of cardiovascular disease was reported. The mean birth weight was 3530gram (505). The mean BMI at the age of 5 years old was 15.1 kg/m^2 (1.2) and the mean increase in BMI Z-score from the age of 5 to adolescence was 0.36 (0.99). The mean systolic and diastolic blood pressure at 5 years old were 104mmHg (7.1) and 54mmHg (7.1) respectively. The mean cIMT at 5 years old was 380.26um (34.69). For baseline characteristics, see Table 1.

In merely 2 cases smoking was reported, which provides for 0.9%, and only 3 children reported being bullied (1.4%), so these determinants were excluded from statistical analyses due to low numbers.

Characteristics adolescence	*N =*	*Frequencies or mean*
Gender (m/f)	232	111/121 (47.8%/52.2%)
Age (years)	232	14,77 (1.28)
Systolic blood pressure (mmHg)	231	112 (10.4)
Diastolic blood pressure (mmHg)	231	57 (6.6)
BMI (kg/m^2)	232	19.8 (3.1)
Physical activity score	223	7.1 (1.8)
Fruit intake score	223	3.9 (1.4)
Smoking (yes/no)	223	2/221 (0.9%/99.1%)
Family history (+/-)	227	76/151 (33.5%/66.5%)
Both parents in household (no/yes)	223	27/196 (12.1%/87.9%)
Being bullied (yes/no)	221	3/218 (1.4%/98.6%)
Life satisfaction (Cantril ladder)	222	7.6 (1.2)
Chronical illness (yes/no)	223	25/198 (11.2%/88.8%)
cIMT (um)	232	442.35 (73.15)
Characteristics 5 years old	**N =**	**Frequencies or mean**
Birth weight (gram)	225	3530 (506)
BMI (kg/m^2)	199	15.1 (1.2)
BMI Z-score increase	199	0.36 (0.99)
Systolic blood pressure (mmHg)	197	104 (7.1)
Diastolic blood pressure (mmHg)	197	54 (7.1)
cIMT (um)	179	380.26 (34.69)

Table 1: Baseline characteristics. For continuous variables the mean (standard deviation) and for nominal variables, the frequencies (percentages) are presented.

Univariable analyses of determinants

Firstly, univariable linear regression analyses were performed for all determinants independently, as related to the cIMT. For results see Table 2. The unstandardized beta in predicting cIMT for gender was -4.870 [95% CI -23.843-12.104], for age 1.029 [95% CI -6.382-8.439], for systolic BP 0.083 [95% CI -0.310-0,475], for diastolic BP -0.012 [95% CI –0.610-0.586], for BMI 2.541 [95% CI -5.784-10.865], for physical activity -0.637 [95% CI -6.054-4.780], for fruit intake -2.600 [95% CI -9.753-4.554], for family history of CVD -0.090 [95% CI -20.439-20.260], for household composition 8.166 [95% CI -21.561-37.893], for life satisfaction 2.710 [95% CI -5.390-10.811] and for chronical illness 10.963 [95% CI -19.760-41.685]. Overall, there was no statistically significant effect found for any determinant in predicting cIMT.

		95% CI		
Determinant	B	lower bound	upper bound	p-value
Gender	-4.870	-23.843	14.104	0.614
Age	1.029	-6.382	8.439	0.785
Systolic BP percentile	0.083	-0.310	0.475	0.678
Diastolic BP percentile	-0.012	-0.610	0.586	0.968
BMI Z-score	2.541	-5.784	10.865	0.548
Physical activity score	-0.637	-6.054	4.780	0.817
Fruit intake score	-2.600	-9.753	4.554	0.475
Family history	-0.090	-20.439	20.260	0.993
Household composition	8.166	-21.561	37.893	0.589
Life satisfaction	2.710	-5.390	10.811	0.510
Chronical illness	10.963	-19.760	41.685	0.483

Table 2: Results of univariable linear regression analyses from cross-sectional analyses including the presented determinants measured in adolescence related to the mean cIMT at 12-16 years old. B = unstandardized beta.

Multivariable analyses of determinants

Secondly, a multivariable linear regression analysis was performed. The model provided for an R^2 of 0.010. For results, see Table 3. The unstandardized beta in predicting cIMT for gender was -5.951 [95% CI -28.032-16.130], for age -0.149 [95% CI -8.570-8.272], for systolic BP 0.081 [-0.406-0.568], for diastolic BP -0.045 [95% CI -0.792-0.702], for BMI 3.851 [95% CI -5.992-13.694], for physical activity -1.008 [95% CI -6.906-4.891], for fruit intake -1.252 [95% CI -8.989-6.486], for family history 1.587 [95% CI -19.927-23.101], for household composition 4.826 [95% CI -27.446-37.097], for life satisfaction 2.169 [95% CI -7.105-11.442] and for chronical illness 9.379 [95% CI -24.043-42.800]. So, as in the univariable analyses, there were no statistically significant effects found in predicting cIMT in any of the determinants tested.

Determinant	B	95% CI		p-value
		lower bound	upper bound	
Gender	-5.951	-28.032	16.130	0.596
Age	-0.149	-8.570	8.272	0.972
Systolic BP percentile	0.081	-0.406	0.568	0.743
Diastolic BP percentile	-0.045	-0.792	0.702	0.905
BMI Z-score	3.851	-5.992	13.694	0.441
Physical activity score	-1.008	-6.906	4.891	0.737
Fruit intake score	-1.252	-8.989	6.486	0.750
Family history	1.587	-19.927	23.101	0.885
Household composition	4.826	-27.446	37.097	0.768
Life satisfaction	2.169	-7.105	11.442	0.645
Chronical illness	9.379	-24.043	42.800	0.581

Table 3: Summary of multivariable linear regression model from cross-sectional analysis, including the presented determinants measured in adolescence related to the mean cIMT. B = unstandardized beta.

Univariable analyses of predictors

For the predictors, measured at the age of 5 years, also univariable linear regression analyses were performed. For results see Table 4. The unstandardized beta value in predicting cIMT in adolescence for birth weight was -6.164 [95% CI -16.411-4.083], for BMI 5.519 [95% CI -4.200-15.239], for the increase in BMI Z-score -2.305 [95% CI -12.811-8.200], for systolic BP 0.371 [95% CI -0.146-0.888], for diastolic BP 0.022 [95% CI-0.434-0.477] and for cIMT 0.337 [95% CI 0.032-0.642]. In these univariable analyses, a statistically significant effect was found for cIMT measured at 5 years old in predicting the cIMT in adolescence. The other predictors were found to not be statistically significant related to cIMT in adolescence.

Predictor	B	95% CI		p-value
		lower bound	upper bound	
Birth weight	-6.164	-16.411	4.083	0.237
BMI Z-score 5yr old	5.519	-4.200	15.239	0.264
BMI Z-score increase	-2.305	-12.811	8.200	0.666
Systolic BP 5yr old	0.371	-0.146	0.888	0.158
Diastolic BP 5yr old	0.022	-0.434	0.477	0.925
cIMT 5yr old	0.337	0.032	0.642	0.031

Table 4: Results of univariable linear regression analyses from longitudinal analyses including the presented predictors related to the mean cIMT at 12-16 years old. B = unstandardized beta.

Multivariable analysis of determinants and predictors

Lastly, a multivariable linear regression analysis was performed using the data measured at 5 years old, combined with the data measured in adolescence in relation to the cIMT in adolescence. For results, see Table 5. The total model provided an R^2 of 0.079. The unstandardized beta in predicting cIMT for gender was 1.918 [95% CI -21.062-224.899], for age 1.895 [95% CI -6.787-10.578], for systolic BP in adolescence 0.048 [95% CI -0.484-0.579],

for diastolic BP in adolescence -0.024 [95% CI -0.799-0.750], for birth weight -9.062 [95% CI-21.590-3.466], BMI at 5 years old 8.952 [95% CI -2.918-20.822], for the increase in BMI Z-score -5.120 [95% CI -18.429-8.189], for systolic BP at 5 years old 0.384 [95% CI -0.244-1.013], for diastolic BP at 5 years old 0.075 [95% CI -0.453-0.603] and for cIMT at 5 years old 0.412 [95% CI 0.094-0.729]. In the multivariable analysis, complementary results were found to the univariable analysis, that is, a statistically significant predicting effect of cIMT in childhood in predicting cIMT in adolescence. Besides that no statistically significant relation of other determinants or predictors to cIMT in adolescence.

		95% CI		
Determinant/predictor	B	lower bound	upper bound	p-value
Gender	1.918	-21.062	24.899	0.869
Age adolescence	1.895	-6.787	10.578	0.667
Systolic BP adolescence	0.048	-0.484	0.579	0.860
Diastolic BP adolescence	-0.024	-0.799	0.750	0.951
Birth weight	-9.062	-21.590	3.466	0.155
BMI Z-score 5yr old	8.952	-2.918	20.822	0.138
BMI Z-score increase	-5.120	-18.429	8.189	0.449
Systolic BP 5yr old	0.384	-0.244	1.013	0.229
Diastolic BP 5yr old	0.075	-0.453	0.603	0.780
cIMT 5yr old	0.412	0.094	0.729	0.011

Table 5: Summary of multivariable linear regression model including longitudinal predictors and cross-sectional determinants, as related to cIMT in adolescence. B = unstandardized beta.

Analysis on blood pressure

Since no association between the risk factors and cIMT was found, and systolic blood pressure is also regarded as a possible cardiovascular risk marker in adolescence,[31] analysis were repeated with systolic BP as outcome. For these results, see Supplement 1.

Discussion

Main results

The primary aim of this study was to investigate whether cIMT in healthy adolescents could be predicted by determinants measured in adolescence. Herein, no association was found between any of the determinants and the cIMT in the univariable nor the multivariable analyses. Secondarily, we aimed to investigate whether predictors measured in childhood were associated with an elevated cIMT in adolescence, additionally to these measurements in adolescence. In this, cIMT measured in childhood was the only predictor that was found to be of predictive value for cIMT in adolescence, both in univariable and multivariable linear regression analyses. Besides this, no effect was found in any of the predictors both in univariable and multivariable regression analyses.

Results in the context of previous literature

In line with the current study, Baroncini et al. found no relation with cIMT for gender, age and BMI, when looking at sixty children, who were 11 to 15 years old.[32] Another study with similar results is the EVA Tyrol study, which observed the effect of birth weight on cIMT at the age of 16, and, no significant relation was found.[33]

But, on the other hand, there is literature reporting contradictory results, in which a relation between cIMT and risk factors is described. For example, the Cardiovascular Risk in Young Finns study did find an association between several risk factors (systolic BP, BMI, physical activity and fruit consumption) and cIMT, when 1809 adolescents were studied.[8] However, in this study the cIMT is measured later in life, at 20-35 years of age, which is in contrast to the early adolescent cIMT measurements at 12-16 years in the current study. Noteworthy, is that in most other studies that did find associations between risk factors and cIMT, cIMT measurements were performed in a later stage of adolescence. This is seen in Eikendal et al. who performed cIMT measurements at 28 years of age,[16] Sjöholm et al. where measurements were performed at the age of 18[18] and Sadasivam et al. who included cIMT measurements from the age of 18 through 25.[34]

In the earlier mentioned studies by Baroncini et al. and the EVA Tyrol study, a similar age group as WHISTLER's is examined, 11-15 year old's and 16 year old's in the Baroncini and EVA Tyrol study respectively, and complementing this study's findings, no relation between risk factors and cIMT is found in these age groups.[32,33] This complements the idea that age is of great relevance in this matter.

These findings suggests that age is of paramount interest in this matter, whereas a relation between risk factors and cIMT seems to be found only in a later stadium of adolescence. This is strengthened by the findings of Doyon et al. that sex differences in cIMT start from the age of 15 years.[35] On top of that, the varying pubertal status within the included population might explain these findings, since Zanini et al. established that an increase in cIMT takes place during puberty.[36]

One study that included a very similar population to WHISTLER, also including a similar age group with a mean age of 14.5 years, but actually did find an association between risk factors and cIMT is the study by Koskinen et al. that combined three birth cohorts to a total of 2893 patients.[12] The major difference between the current study and theirs' is the way cIMT is analyzed, namely in an ordinal scale, using a cut-off point at the 90th percentile, while in this study a continuous scale was used. When using the normative data provided by a large systematic review,[37] only 8 children in the WHISTLER cohort would have a cIMT above the 90th

percentile, which indicates that this study's cIMT measurements were generally lower than in Koskinen et al. Another study performed on 11-13 year old children found significant associations between risk factors and cIMT, yet the mean cIMT in their population was also slightly higher; 490um, while in the current study the mean cIMT was 442um.[38]

Remarkable, is the resemblance in study design between the current study and a study performed in Germany, Turkey, Poland and Sweden, by Doyon et al., since the populations are very much alike, regarding age (subgroup of 12-14.99 year old's in their population, compared to 12-16 year old's in this study), BMI (mean 20.0kg/m^2 (SD 2.6) and 19.8kg/m^2 (SD 3.1) for Doyon et al. and the current study respectively) and BP (mean systolic BP 110.2mmHg (SD 9.7) and 112mmHg (SD 10.4) for Doyon et al. and the current study respectively).[35] The mean cIMT in their population was even lower than in the current study, since their mean cIMT was 383-396um for boys and 380-385um for girls aged 12-14.99 years, and the mean in WHISTLER was 442um. However, Doyon et al. found a significant predicting effect of age, BMI and BP on cIMT when performing univariable and multivariable analyses, in contrast to the findings in the current population. In contrast to this study's set-up, the analyses were performed in a combined group of 6-18 year old's, yet the contrasting outcomes remain interesting. Information regarding ethnicity and socio-economic status is not provided by Doyon et al., differences in this matter might add to explanation of variation in findings.

Interpretation

The fact that cIMT in childhood is associated with cIMT in adolescence, strengthens the hypothesis that the development of atherosclerosis is determined at a very young age, since an elevated cIMT in early childhood is associated with this measurement later in life. Apart from cIMT in childhood, no association between risk factors and cIMT in adolescence is found. In our opinion, this could be explained by either one of two explanations.

First of all, there could be no effect of the investigated risk factors in the development of cardiovascular disease (CVD), since cIMT was a marker for the cardiovascular risk status, and no association is seen here. However, this seems unlikely, since the risk factors are of great importance in predicting cardiovascular risk in adults. As is confirmed, when looking at the Framingham Risk score, based on age, gender, smoking, cholesterol and BP, which is commonly used and has been validated to properly predict cardiovascular risk in several ethnic groups in differing settings.[39] On top of that, Jacobs et al. performed a large study including 38589 participants, in which the significance of BP, BMI, smoking and cholesterol in childhood is established, using a mean follow-up of 35 years to see whether cardiovascular events occur.[9] These findings make it very unlikely that these risk factors are of no effect in predicting cardiovascular disease.

An alternative conclusion is that cIMT is no representative marker for cardiovascular risk status in the current population of healthy children aged 12-16 years. As seen previously, age seems to play a critical role, since most studies that do present significant relations between risk factors and cIMT have included slightly older subjects. This is actually not surprising, since Berenson et al. described the formation of atherosclerosis, which initiates in the iliac arteries and abdominal aorta, so the carotid artery follows in a later stadium.[6]

Besides that, during childhood physiological changes in the media layer occur as a consequence of increasing body size, as opposed to atherosclerosis in the intima.[23] Yet, these two factors are combined in the measurement of cIMT.[10] This may provide for false positives when searching for elevated cIMT, since it could be elevated due to these physiological changes, even when no atherosclerosis is present.[23] These factors may particularly be relevant

in young adolescents, since the prevalence of risk factors is low and growth is of significant interest.[23] These two explanations, along with the results of the current study, indicate that cIMT is less suited as a reliable marker for subclinical atherosclerosis and thus cardiovascular risk.

In opposition, as discussed earlier, there is evidence on the existence of associations between cIMT and risk factors. Even though, this is mostly in different study set-ups or different populations, it does suggest the presence of such relations. Possibly, the current study might be underpowered for subgroups such as obese children and children with an elevated BP, which could provide for an underestimation of the effects of these risk factors in predicting cIMT. On top of that, experts in the field are predominantly positive regarding cIMT as a cardiovascular marker, since the Association for European Paediatric Cardiology (AEPC) Working Group on Cardiovascular Prevention strongly recommends the use of cIMT in screening patients with elevated cardiovascular risk, despite the necessity for further research into the topic.[11] Lastly, the fact that an association between cIMT in childhood and cIMT adolescence was found, confirms that changes in the vascular walls may start at a very early age and strengthen the idea that cIMT could be a consistent marker.

The participants in the WHISTLER cohort were recruited from Leidsche Rijn, a new and upcoming district in Utrecht, with a predominantly rich, white and highly educated population, which, on average, shows more health improving behavior than other populations. The baseline characteristics confirm that this is a predominantly healthy group, since the vast majority is member of a sports club, has a high fruit intake and smoking numbers are extremely low. As a consequence, results of this study may not be generalizable to populations with lower income, different ethnicity or lower education.

Recommendation for further research

If cIMT is found not to be a good marker for cardiovascular risk in this population, further research into alternative markers is necessary. A review by Magnussen et al. in 2016 established that childhood BP may have a direct effect on adult cardiovascular health.[31] Given this promising association, an additional analysis was performed in this population, to see whether risk factors were associated with BP, for results, see Supplement 1. In conclusion, a statistically significant association was found between BMI and systolic BP in cross-sectional univariable and multivariable analyses. In longitudinal analyses, BMI increase and systolic BP at 5 years old were associated with systolic BP in adolescence. Further research on this topic might add to the reliability and feasibility of BP as a marker for cardiovascular risk.

Another promising marker is pulse wave velocity (PWV), which is a measure of arterial stiffness.[40] Kulsum-Mecci et al. found a strong relationship between PWV and obesity and hypertension, when looking at healthy children aged 4 to 18 years.[40] Another study on the association between PWV and cIMT on one hand and traditional cardiovascular risk factors (such as smoking, BMI, BP, glucose and cholesterol) on the other hand, showed that PWV is associated with BP and BMI, whereas for cIMT only an association with cholesterol was found.[41] This is line with the results of the current study, where no relations with cIMT were found and it suggests PWV to be a promising marker, even in a population where cIMT appears not to be.

Lastly, a possible marker of preclinical atherosclerosis is endothelial dysfunction, which can be determined through peripheral arterial tonometry (PAT).[42] In adults, these measurements are established to predict adverse cardiovascular events, such as cardiac death, myocardial infarction and cardiac hospitalization.[43] Further research for validation of PAT in

pediatrics is necessary, yet it is considered a promising addition to the markers for preclinical atherosclerosis.[42]

When assuming the hypothesis that no relation between risk factors and cIMT is found here, because the alterations in the vascular wall are still too small to detect, due to the young and fairly healthy population, it might be interesting to repeat these experiments in a population where more extensive alterations are expected. For example, a population of healthy children with more variation regarding social factors such as education level, income and ethnicity, but also more diversity in physical health, including more subjects with an increased BMI, higher BP, more smokers and subjects with lower frequency of physical activity and fruit consumption.

Besides that, in children suffering from chronic disease, the deviations in cIMT are probably bigger, since inflammation plays a significant role in the formation of atherosclerosis.[44] So, development of atherosclerosis might be accelerated in chronically ill children, as is already seen in children suffering from type 1 diabetes, Kawasaki disease and chronic kidney disease.[5] Therefore, it would be interesting to repeat this study in a population of children with chronic disease.

In this, it would be interesting to include analyses of blood samples, such as glucose levels, cholesterol measurements and inflammation markers, since those are associated with cIMT changes.[8,12]

Limitations and strengths

The strength of this study lies in its extensive collection of data in a large cohort over a lengthy follow-up time. Secondly, a specific age group is included (12-16 years), especially when comparing to earlier performed studies, in which large age groups (e.g. 6-18 years or 20-35 years) are included. Since cIMT changes with age, this provides for more specific outcomes and conclusions. On top of that, consistent measurements were performed in childhood as well as in adolescence, using similar protocols, therefore the data is comparable in several subjects.

This study also has its limitations. Firstly, the BP is measured while lying down in adolescence, which would have preferably been measured sitting up, as is done in childhood. Secondly, cIMT was measured by different persons, which presents a risk of interobserver variation. Lastly, the scoring system for physical activity is not a validated score, but is set up by ourselves, using the available data. It is built up from 3 questions, which have overlapping elements and adds up to only 9 points, which might not be specific enough to determine different physical activity levels.

Conclusion

The primary aim of this study was to determine whether cross-sectional measurements in adolescence of gender, age, systolic and diastolic BP, BMI, physical activity, fruit intake, family history of CVD, household composition, life satisfaction and chronical illness in family members were associated with cIMT in adolescents. No association was found for any of these determinants in univariable as well as multivariable linear regression analyses.

The secondary aim of this study was to determine whether longitudinal measurements in childhood of systolic and diastolic BP, birth weight, BMI, BMI increase and cIMT were significant predictors for cIMT in adolescents. A statistically significant association was found for cIMT in childhood and cIMT in adolescence in the univariable analysis and multivariable analysis. Apart from that, no associations were found for the other predictors in the univariable nor the multivariable analysis.

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Supplement 1

Univariable analyses of determinants

For the included determinants measured in adolescence and cIMT in adolescence univariable linear regression analyses were performed. For results, see Table 1. The unstandardized beta value in the linear regression model in predicting cIMT in adolescence for gender was 0.477 [95% CI -5.805-6.758], for age 0.930 [95% CI -1.515-3.376], for BMI 5.296 [95% CI 2.637-7.955], for physical activity 0.981 [95% CI -0.791-2.753], for fruit intake -0.049 [95% CI -2.399-2.302], for family history 0.661 [95% CI -6.004-7.326], for household composition -3.475 [95% CI -13.191-6.241], for life satisfaction 1.072 [95% CI -1.589-3.733] and for chronic disease -7.601 [95% CI -17.607-2.406]. In this analysis, BMI was found to be statistically significantly associated with systolic BP, the other determinants were not associated with cIMT.

		95% CI		
Determinant	B	lower bound	upper bound	p-value
Gender	0.477	-5.805	6.758	0.881
Age	0.930	-1.515	3.376	0.454
BMI	5.296	2.637	7.955	<0.001
Physical activity	0.981	-0.791	2.753	0.276
Fruit intake	-0.049	-2.399	2.302	0.968
Family history	0.661	-6.004	7.326	0.845
Household composition	-3.475	-13.191	6.241	0.482
Life satisfaction	1.072	-1.589	3.733	0.428
Chronic disease	-7.601	-17.607	2.406	0.136

Table 1: Results of univariable linear regression analyses including the presented determinants related to the systolic BP at 12-16 years old. B = unstandardized beta.

Multivariable analysis of determinants

Secondly, a multivariable linear regression analysis was performed for the included determinants in relation to the cIMT, all measured at 12-16 years of age. For results, see Table 2. The total model provided an R^2 of 0.090. The unstandardized beta in predicting cIMT for gender was -0.566 [95% CI -8.623-7.492], for age -0.0119 [95% CI -2.798-2.560], for BMI 5.793 [95% CI 2.789-8.797], for physical activity 0.668 [95% CI -1.149-2.485], for fruit intake -0.357 [95% CI -2.051-2.766], for family history -0.876 [95% CI -7.552-5.801], for household composition -3.011 [95% CI -13.081-7.060], for life satisfaction 1.533 [95% CI -1.381-4.448] and for chronic disease -7.019 [95% CI -17.420-3.382]. In conclusion, only BMI showed a statistically significant association with systolic blood pressure.

Determinant	B	95% CI		p-value
		lower bound	upper bound	
Gender	-0.566	-8.623	7.492	0.890
Age	-0.119	-2.798	2.560	0.930
BMI	5.793	2.789	8.797	<0.001
Physical activity	0.668	-1.149	2.485	0.469
Fruit intake	0.357	-2.051	2.766	0.770
Family history	-0.876	-7.552	5.801	0.796
Household composition	-3.011	-13.081	7.060	0.556
Life satisfaction	1.533	-1.381	4.448	0.301
Chronic disease	-7.019	-17.420	3.382	0.185

Table 2: Results of multivariable linear regression analysis including the presented determinants related to the systolic BP at 12-16 years old. B = unstandardized beta.

Univariable analyses of predictors

For the longitudinal predictors, measured at the age of 5 years, also univariable linear regression analyses were performed with systolic BP as outcome. For results see Table 3. The unstandardized beta value in the linear regression model in predicting systolic BP in adolescence for birth weight was 2.118 [95% CI -1.251-5.487], for BMI 1.125 [95% CI -2.074-4.324], for BMI increase in Z-score 6.458 [95% CI 3.125-9.791], for systolic BP 0.443 [95% CI 0.285-0.602] and for diastolic BP 0.072 [95% CI -0.078-0.222]. In these univariable analyses, a statistically significant effect was found for the increase in BMI from 5 years old to 12-16 years old and systolic BP at 5 years old. The other predictors were found to not be statistically significant related to systolic BP in adolescence.

Predictor	B	95% CI		p-value
		lower bound	upper bound	
Birth weight	2.118	-1.251	5.487	0.217
BMI 5yr	1.125	-2.074	4.324	0.489
BMI increase	6.458	3.125	9.791	<0.001
Systolic BP 5yr	0.443	0.285	0.602	<0.001
Diastolic BP 5yr	0.072	-0.078	0.222	0.345

Table 3: Results of univariable linear regression analyses from longitudinal analyses including the presented predictors related to the systolic BP at 12-16 years old. B = unstandardized beta.

Multivariable analysis of determinants and predictors

Lastly, a multivariable linear regression analysis was performed using the data measured at 5 years old, combined with the data measured in adolescence in relation to the systolic BP in adolescence. For results, see Table 4. The total model provided an R^2 of 0.231. The unstandardized beta in predicting systolic BP for gender was -1.117 [95% CI -7.546-5.311], for age 0.260 [95% CI -2.227-2.746], for birth weight 1.476 [95% CI -2.165-5.116], BMI 2.950 [95% CI -0.367-6.267], for BMI increase 8.031 [95% CI 4.521-11.542], for systolic BP 0.460 [95% CI 0.297-0.622] and for diastolic BP -0.032 [95% CI -0.179-0.116]. In conclusion, just as in the univariable analyses, BMI increase and systolic BP at the age of 5 years old are established to

have a statistically significant association with systolic BP at 12-16 years old. The other determinants and predicators are found not to be statistically significantly associated to systolic BP in adolescence.

		95% CI		
Determinant/predictor	B	lower bound	upper bound	P-value
Gender	-1.117	-7.546	5.311	0.732
Age	0.260	-2.227	2.746	0.837
Birth weight	1.476	-2.165	5.116	0.425
BMI 5yr	2.950	-0.367	6.267	0.081
BMI increase	8.031	4.521	11.542	<0.001
Systolic BP 5yr	0.460	0.297	0.622	<0.001
Diastolic BP 5yr	-0.032	-0.179	0.116	0.671

Table 4: Summary of multivariable linear regression model including longitudinal predictors and cross-sectional determinants, as related to systolic BP in adolescence. B = unstandardized beta.