Association of Body Composition, Blood Pressure, Physical Activity and Fitness with Cardiovascular and Metabolic Health in Children

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List of Abbreviations

AGEs	Advanced glycation end products
au	arbitrary units
AVR	arteriolar-to-venular diameter ratio
BMI	Body mass index
CRAE	Central retinal arteriolar equivalents
CRVE	Central retinal venular equivalents
CVD	Cardiovascular disease
NO	Nitric oxide
PWV	Pulse wave velocity
RAGE	Receptor for advanced glycation end products
SAF	skin autofluorescence

Summary

Background

Cardiovascular disease (CVD) has its origin early in life and is the number one cause of death worldwide. Obesity is a main predictor of the pathophysiological development of hypertension and cardiometabolic disease. Insufficient physical activity and fitness lead to overweight and obesity from childhood until adulthood. Advanced glycation end products (AGEs) accumulate in adults with micro- and macrovascular complications. Therefore, the association of cardiovascular risk factors such as childhood obesity, high blood pressure and physical inactivity with cardiometabolic health need to be investigated in a systems physiology approach.

Aims

We aimed to investigate the association of obesity, high blood pressure, physical activity and fitness with micro- and macrovascular health in young children. Furthermore, we aimed to examine whether AGEs are related to cardiovascular risk factors early in life.

Methods

First, we conducted a systematical review and meta-analysis in over 5000 children to investigate the association of body mass index (BMI), blood pressure and physical fitness with retinal vessel diameters. An electronic literature search was performed throughout the databases of PubMed, EMBASE, Ovid, Web of Science and the Cochrane Register of Controlled Trials.

In a cross-sectional approach, over 1000 children (aged 7.2±0.4 years) were screened for BMI, blood pressure, retinal arteriolar (CRAE) and venular diameters (CRVE), pulse wave velocity (PWV) and subcutaneous AGEs. A shuttle run and a 20-m sprint test were performed to assess physical fitness parameters in children. Physical activity was reported by questionnaires. Based on data from the population-based German KiGGS Study and according to the American Academy of Pediatrics guidelines, blood pressure was categorised in children with normal, high-normal blood pressure and hypertension.

Results

Our results showed that CRAE and PWV were associated with obesity and high blood pressure. Low physical fitness and physical inactivity (screen time) in childhood were determinants for unfavourable micro- and macrovascular health, but not independent of BMI and blood pressure. Moreover, physical fitness and screen time were independently associated with a higher accumulation of subcutaneous AGEs.

Conclusions

Our study showed that obesity and high blood pressure are associated with vascular alterations already in young children. We found a beneficial association of physical fitness with vascular health and AGEs. Future primary prevention programs will have to address the improvement of physical activity and fitness to promote cardiometabolic health in children. Cardiovascular risk stratification using different vascular screening tools may be important to help recognize subclinical changes in children at risk. Long-term follow-up studies are needed to clarify whether early cardiovascular changes are predictive for the development of cardiometabolic disease later in life.

Chapter 1 Introduction

1.1 Cardiovascular Disease and Mortality in Adulthood

Cardiovascular disease (CVD) is a chronic inflammatory disease of the circulatory system with long duration and slow progression. Globally, over 17.5 million of all deaths every year are caused by CVD, representing one third of all deaths worldwide[1]. Coronary heart disease and stroke are the first and second leading causes of global death and are responsible for an enormous economic burden and public health concern[2,3]. Obesity and high blood pressure are two of modern day's largest risk factors for cardiovascular morbidity and mortality.

Over the past three decades, obesity rates have been gradually increased and the number of persons with obesity has nearly doubled in Western countries[4]. In the year 2014, almost 70% of all adults aged 18 years and older were classified as overweight or obese[1]. The rise of obesity and related diseases is not only an emerging health concern in developed nations but this pandemic is arising in developing countries as well[5]. Epidemiological evidence suggests that obesity in early adulthood is linked to a three-fold higher risk of hypertension at older age[6]. High blood pressure, defined as blood pressure over 140/90mmHg, has been found in over one third of the Swiss population and in only 40% blood pressure was controlled[7]. The prevalence of hypertension in the year 2025 has been predicted to reach 29% of the global population[8]. About 54% of stroke and 47% of coronary heart disease have been burden attributed to high blood pressure[9]. The spread of the obesity pandemic and hypertension are main underlying causes for the high prevalence of CVD worldwide. Primary prevention of obesity and hypertension early in life is of highest clinical and socioeconomic relevance to curtail the rise of CVD in adulthood.

1.2 Childhood Obesity, High Blood Pressure and Cardiovascular Health

CVD has its origin in early life due to classical and lifestyle-associated risk factors such as physical inactivity, abnormal dietary intake and consequently, weight gain and an increased prevalence of childhood obesity. One in four children in Europe is overweight or obese[10]. Children with a body mass index (BMI) over the 90th percentile are categorised as overweight and over the 95th percentile as obese. Over the last several decades, the prevalence of overweight and obese children has risen by 47%[10], even in children below the age of 5 years[11].

In Switzerland, studies reported that the prevalence of overweight and obese children stabilised during the period between 2002 and 2012[12,13]. However, an epidemiological survey of the population and sample studies demonstrated that 17% of Swiss children are overweight and 3.9 % are affected by obesity[14]. The number of overweight and obese children in Switzerland has to be considered as high. Childhood obesity is an important contribution to the early onset of type 2 diabetes[15], hypertension[16] and long-term vascular complications in adulthood[17]. Recent longitudinal data showed in a populations of 2.3 million adolescents that higher BMI is strongly associated with increased cardiovascular morbidity and mortality during 40 years of follow-up[18]. In the years from 2000 to 2020, adolescent overweight has been predicted to increase the prevalence of obesity from 30 to 37% in men and 34 to 44% in women, leading to an estimated increase incidence of coronary heart disease by a range of 5 to 16% in the year 2035[19]. Furthermore, several paediatric epidemiology studies over the past decades demonstrated that more than 60% of overweight children will be overweight or obese in adulthood and suffer from adult CVD[20–23]. A previous study found that young overweight children aged 2- to- 5-year old are at a four-fold greater risk of becoming obese adults compared to normal weight peers[23]. The probability of an overweight child becoming obese in adulthood increased with the time at which the data were collected.

The recent obesity epidemic among children and adolescents is related to a concomitant increase in absolute blood pressure levels in childhood[24]. The prevalence of childhood hypertension has increased almost four-fold since the year 1970 and is estimated to be between 2.2 to 4.9% in Europe and about 3.5% in US children[25]. Over the past 20 years, epidemiological surveys demonstrated that high blood pressure coincides with obesity, generating a vicious circle that potentially fosters the development of subclinical atherosclerosis in children and adolescents[26,27]. The longitudinal Cardiovascular Risk in Young Finns Study showed that elevated blood pressure can be observed early in life, it persists into adulthood and is associated with increased intima-media thickness in adulthood[28]. The clinical categorisation of blood pressure in children is defined as systolic and diastolic blood pressure over 90 percentile as high-normal blood pressure and over 95 percentile for hypertension based on reference percentiles of healthy children. An analysis of data from the National Childhood Blood Pressure database in USA indicated that the rate of progression from high-normal blood pressure to hypertension is 7% per year after a 2 year follow-up evaluation.

High-normal blood pressure persisted in 50% of boys and in 24% of girls who were initially categorised as having high-normal blood pressure[29]. Given that children and adolescents presenting high-normal blood pressure and overweight have major risk of hypertension and obesity in adulthood, it seems to be necessary to focus on early screening and treatment strategies to counteract the increasing burden of CVD in adulthood.

1.3 Childhood Physical Inactivity as a Cardiovascular Risk Factor

Obesity and hypertension have become a public health concern with an increasing global prevalence of physical inactivity and unhealthy lifestyle. A global analysis of major non-communicable diseases reported an increased economic burden due to physical inactivity as a pathophysiological risk factor for obesity-related hypertension and CVD[30]. In 2012, physical inactivity and low physical activity were responsible for 3.2 million global deaths as the fourth leading cause for mortality worldwide[31]. About 20% of men and 27% of women do not reach the global recommendations on physical activity for health of at least 150 minutes moderate-to-vigorous physical activity throughout the week[32]. Physical inactive individuals have a 30 % higher risk for all-cause mortality compared to individuals reaching the recommendations on physical activity[33]. In children, physical activity is essential for a healthy development from childhood into adulthood and provides a number of wide-ranging health benefits such as prevention of early development of endothelial dysfunction and pre-atherosclerosis[34,35]. Physical activity recommendations and guidelines for children aged 5- to 17-years suggest at least 60 minutes of moderate-to-vigorous daily physical activity and additional amounts of physical activity provides further health benefits[32]. Nevertheless, over 80% of adolescents aged 13- to- 15 years are less than 60 minutes moderate-to-vigorous physical active per day[36]. A previous observational study has shown that only 16.8% of the boys and 4.6% of the girls reach the recommended physical activity levels for children in Europe[37]. The highest amounts of physical activity were recorded in Switzerland with 27.8% of the boys and 12.5% of the girls fulfil the current physical activity recommendations. The percentage of physical inactive children has to be regarded as high, since The European Youth Heart Study found that physical activity levels should be at least 90 minutes of moderate intensity to prevent clustering of CVD risk factors[38].

In addition, several studies demonstrated that sedentary behaviour and time spent in front of a screen have been associated with higher cardiovascular risk, independent of physical activity levels[39–41]. A previous systematic review of sedentary behaviour showed that the risk for obesity and high blood pressure increases in a dose response manner with increasing screen time in school-aged children and youth[40]. More than two hours per day spending time in front of a screen was associated with decreased VO_2max and lower cardiorespiratory fitness. A recent study found that obese children aged 2- to- 5 years are more than twice as likely to spend more than 4 hours in front of a screen per day[42]. Moreover, there is growing evidence that childhood physical inactivity tracks through youth into adulthood[43,44] and seems to be responsible for hypertension, insulin-resistance and CVD later in life[45,46]. Therefore, it seems necessary to include not only physical activity but also sedentary behaviour into study design and future recommendations.

Besides physical activity and sedentary behaviour, objectively measured physical fitness has been shown to be a more powerful predictor of mortality than established cardiovascular risk factors[47]. A recent study found that childhood physical activity, sedentary behaviour patterns and obesity are mediated by cardiorespiratory fitness[48]. Physical fitness, specifically cardiorespiratory fitness, during childhood plays an important role in the development of obesity in adulthood[49]. Further studies analysing the influence of physical activity, sedentary behaviour and physical fitness on cardiovascular health in children are warranted.

1.4 Retinal Vessel Diameters and Cardiovascular Health in Children

Childhood obesity promotes chronic pro-inflammatory processes in adipose tissue, leading to the initiation and progression of atherosclerosis during lifespan[50]. The mechanisms of early subclinical vascular complications from childhood until adulthood are still poorly understood. Over the last decades, retinal vessel analysis has been used for the non-invasive investigation of microvascular health[51]. Retinal vessels are regulators of local cerebrovascular blood flow and can serve as representative biomarkers for alterations in coronary microvessels[52]. It has previously been shown that narrowing of retinal arterioles and widening of retinal venules are predictors for cardiovascular outcome during lifespan[53]. Smaller central arteriolar (CRAE) and larger central venular equivalents (CRVE) are associated with increased risk of obesity[54,55],

hypertension[56,57], stroke[58] and a higher cardiovascular mortality and morbidity rate[59] in adulthood. Alterations of CRAE and CRVE seem to occur before common cardiovascular risk factors become evident. The Blue Mountains Eye Study found that retinal arteriolar narrowing is associated with a subsequent 5-year incident of severe hypertension in older adults, independent of baseline blood pressure[60]. In children, obesity and high blood pressure have been predominantly associated with smaller CRAE[61,62]. A previous study found that arteriolar narrowing due to elevated systolic blood pressure can already be detected in preschool-age children[63]. There is evidence that higher BMI is responsible for retinal venular widening in 8-year old children[62,64]. In contrast, a large cross-sectional study found no association of BMI with CRVE in young children[65]. Gishti et al. found an inverse association between systolic blood pressure and CRVE, whereas Li et al. found a positive association between the two[63]. An overview on the association of childhood BMI and blood pressure with retinal vessel diameters is needed to optimise cardiovascular risk stratification and improve primary prevention of CVD.

Few studies have investigated the association of physical activity and sedentary behaviour with retinal microvascular health. A previous study showed that low physical activity and higher screen time are associated with wider retinal venules in adults[66]. Three studies investigated physical activity and retinal vessel diameters in children[67–69]. A school-based German study found an association between arteriolar-to-venular ratio (AVR) and physical inactivity[69]. An Australian study demonstrated that time spent in front of a screen was negatively correlated with CRAE and outdoor physical activity resulted in wider retinal arteriolar diameters[68]. Imhof et al. showed that cardiorespiratory fitness was associated with smaller CRVE[67]. There is an urgent need for studies investigating physical activity and fitness and retinal vessel diameters in children to improve early stage cardiovascular risk stratification and treatment strategies.

1.5 Arterial Stiffness and Cardiovascular Risk

With regard to the macrovascular bed, several studies have investigated large artery stiffness, commonly measured by aortic pulse wave velocity (PWV) as a surrogate end point for cardiovascular risk stratification[70–72]. The European Society of Cardiology guidelines for the

management of hypertension included aortic PWV measurement for the assessment of target organ damage and CVD in clinical practise[73]. A systematic review and meta-analysis of longitudinal studies over 7.7 years demonstrated an increase of carotid-femoral PWV by 1m/s represents a risk increase of about 15% in total cardiovascular and all-cause mortality[71]. Higher PWV was associated with a twofold increased hazard for major cardiovascular events and mortality compared to low arterial stiffness in adults[71]. A large longitudinal study showed that the development of obesity predicts increased age-related progression of arterial stiffness in later midlife[74]. In addition, elevated blood pressure seems to play a key role for the development of high arterial stiffness[75]. In the systemic circulation increased blood pressure and its related higher PWV contributes to expose small vessels such as the cerebral microcirculation to high pulsatile pressure and is thereby leading to microvascular impairments[76]. Several studies demonstrated that aerobic exercise training has the potential to reduce arterial stiffness in children with obesity and hypertension[77–79].

Data on arterial stiffness in children and associations with cardiovascular risk are scarce and the mechanisms of CVD development in childhood are poorly understood. Studies that examined childhood obesity and arterial stiffness found that children with hypercholesterolemia[80] and severe obesity[81] showed lower arterial compliance compared to healthy children. The Bogalusa Heart Study demonstrated that childhood blood pressure is a potential predictor of arterial stiffness in young adults[82]. A previous 27-year follow-up study concluded that elevated blood pressure is tracked from childhood until adulthood and is responsible for subsequent atherosclerotic processes[83]. Few studies examined the association of physical activity and fitness with arterial stiffness in children. Schack-Nielsen et al. reported that patterns of physical inactivity, high fat intake and breast-feeding in infancy are related to large artery stiffness during childhood[84]. In an Australian population, low cardiorespiratory fitness seems to be associated with higher PWV in 10-year old schoolchildren[85]. Subclinical micro- and macrovascular changes seem to exist long before vascular complications can be diagnosed[60,86]. The association and interrelation of body composition, blood pressure and physical activity/fitness with micro- and macrovascular health has never been investigated in young children.

1.6 Association of Body Composition, Blood Pressure and Physical Activity with Advanced Glycation End Products

Advanced Glycation end products (AGEs) are formed by the interaction of reduced sugars, such as aldose with proteins or lipids and subsequent non-enzymatic molecular transformation, resulting in a group of fluorescent compounds. The formation of AGEs undergo complex multistep reactions to form reversible stable pentosidine and irreversible AGEs[87]. AGEs can be detected in biological fluids and in skin (fluorescence), accumulate during ageing and its formation accelerated under hyperglycaemic, inflammatory and oxidative stress conditions[87,88]. It is evident that AGEs interact with cell surface receptors for AGEs (RAGE) and play a pivotal role in the development of diabetes mellitus and chronic CVD[89–91]. Accumulation of AGEs in blood serum and RAGE correlate with subcutaneous AGEs formation[92]. Concentrations of AGEs in tissue and the vessel wall induce distinct and maladaptive collagen cross-linking. They seem to be associated with higher arterial stiffness in adults[93–95]. Subcutaneous AGEs, measured by skin autofluorescence (SAF), are strongly related to cumulative metabolic diseases[90] and microvascular complications[89] in diabetic patients.

Results on the association of obesity and AGEs formation are scarce and do not seem to be consistent. A recent study has shown that an accumulation of subcutaneous AGEs is associated with central obesity in patients with the metabolic syndrome, but not in healthy obese participants[96]. A previous cross-sectional study in Western Europe found no association of higher waist circumference and SAF[97]. There is strong evidence that AGEs mediate as a predictor for vascular impairments such as endothelial dysfunction and arterial stiffness[98,99]. Few studies examined the association of AGEs with blood pressure. Systolic and diastolic blood pressure seem to be associated with subcutaneous AGEs accumulation in men[100]. McNulty et al. found that plasma AGEs were higher in hypertensive than in normotensive participants and related to central/aortic arterial stiffness[99]. There is evidence that, physical inactivity and abnormal dietary behaviour, for example hyperglycaemic diets and dietary AGEs intake trigger accumulation of AGEs in tissue[101]. Physical exercise combined with dietary restriction have the potential to reduce AGEs accumulation in adults[102]. A previous study indicated that life-long regular endurance exercise reduces the age-related AGE accumulation

in skin[103]. In addition, physical activity and compliance with physical activity guidelines are associated with lower AGEs-levels in a population over 65-year-old[104].

There are very few studies on AGEs metabolism in children. Recent evidence describes the role of AGEs in the pathogenesis of obesity and β-cell damage by inducing oxidative cell stress[105]. AGEs seem to be involved in the development of diabetes mellitus type 2 during childhood[106,107]. A previous study found that childhood high body fat is related to soluble RAGE in blood plasma[108]. These above findings suggest that changes in AGEs metabolism are present at an early stage of the development of diabetes and CVD. Future studies are needed to clarify the clinical relevance of AGEs metabolism early in life.

Chapter 2 Aims and Hypotheses

2.1 Aims

In a first phase, this dissertation aimed to identify, critically evaluate and summarise the association of childhood obesity, high blood pressure and physical inactivity with retinal vessel diameters. Within the cross-sectional **EX**ercise and **A**rterial **M**odulation **IN YOUTH** (EXAMIN YOUTH) study (Appendix A), this PhD-Project aimed to focus on the cross-talk between large arterial stiffness and retinal microvascular health in 6- to 8- year old children. We investigated whether body composition, blood pressure, physical activity and fitness differently affect large and small arteries in young children. Further objectives included the analysis of non-invasive, subcutaneous AGEs as an indicator of the glucose metabolism. The main aims of this PhD project were:

Aim 1: to systematically review and meta-analyse associations of BMI, blood pressure and physical activity with retinal vessel diameters in children and adolescents (Chapter 3)

Aim 2: to determine the association of BMI, blood pressure, physical activity and fitness with retinal vessel diameters and arterial stiffness in 6- to 8- year-old children (Chapter 4)

Aim3: to determine the association of BMI, blood pressure, physical activity and fitness with AGEs in 6- to 8- year-old children (Chapter 5)

2.2 Hypotheses

The main hypotheses to be evaluated for this PhD project were:

Hypothesis 1: Childhood obesity, high BP and low physical activity are associated with retinal arteriolar narrowing and retinal venular widening.

Hypothesis 2: High BMI and blood pressure and low physical activity/fitness are associated with retinal arteriolar narrowing, retinal venular widening and higher arterial stiffness in 6- to 8- year-old children.

Hypothesis 3: High BMI and blood pressure and low physical activity/fitness are associated with higher levels of subcutaneous AGEs in young children.

Chapter 3 Publication 1: Obesity, Blood Pressure, and Retinal Vessels: A Meta-analysis

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Chapter 4 Publication 2: Obesity, High Blood Pressure, and Physical Activity Determine Vascular Phenotype in Young Children: The EXAMIN YOUTH Study

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Chapter 5 Publication 3: Association of physical fitness with advanced glycation end products in children

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Abstract

Purpose: Advanced glycation end products (AGEs) accumulate with age and development of cardiovascular disease. Higher AGEs have been shown in children with diabetes but little is known about their association with obesity, hypertension and physical fitness in childhood. We aimed to investigate whether body composition, blood pressure and physical fitness affect AGEs formation in young children.

Methods: In this cross-sectional study, 1075 primary school children (aged 7.2±0.4years) were screened for subcutaneous AGEs (skin autofluorescence; SAF in arbitrary units (au)), body mass index (BMI), blood pressure (BP), and cardiorespiratory fitness (CRF) as estimated by shuttle run stages using standardized procedures for children. Group comparisons were performed in clinical BP and BMI categories and tertiles of CRF. BP was categorized according to the reference values of the population-based German KiGGS study.

Results: Children with higher physical fitness showed lower SAF (0.99 (1.03;1.05) au) compared to children with low CRF (1.09 (1.03;1.05) au, p<0.001). An increase of one shuttle run stage was associated with a mean reduction in SAF of -0,032(CI: -0,042;-0,024) au, independent of BMI and BP (p<0.001). Girls showed lower AGEs and lower fitness levels compared to boys. BMI and BP were not independently associated with AGEs in this large cohort of primary school children.

Conclusion: Low physical fitness but not BMI and BP were associated with higher levels of AGEs. Primary prevention programs in young children may need to focus on improving physical fitness in game settings in order to reduce the growing prevalence of metabolic disease during childhood and later in life.

Keywords: Childhood obesity, blood pressure, cardiorespiratory fitness, cardiometabolic risk; primary prevention

Introduction

Advanced glycation end products (AGEs) form when proteins or lipids interact with reduced sugars for an extended period of time, subsequently undergoing progressive irreversible molecular transformation. Growing evidence suggested that AGEs interact with cell surface receptors for AGEs (RAGE) under hyperglycemic conditions, leading to increased oxidative stress and inflammation.(1–3) Interactions of AGEs with RAGE impart distinct and maladaptive remodeling of cross-linked collagen in the vascular wall.(4,5)

Concentrations of serum AGEs correlate with AGEs accumulation in the skin.(6) Subcutaneous AGEs seem to be related to long-term diabetic risk factors and glycemic control.(6) Data on the relationship between obesity and AGEs formation are scarce and inconsistent. Den Engelsen et al.(7) found no association of central obesity and subcutaneous AGEs. In contrast, a more recent study demonstrated an association of skin AGEs with incidence metabolic syndrome, higher waist circumference and elevated blood pressure (BP) in adults. (8) However, no association of subcutaneous AGEs with obesity was found in participants without the metabolic syndrome(8) In a recent meta-analysis of patients with high CV risk, skin AGEs has been shown to be predictive of CV and all-cause mortality.(9) Sedentary lifestyle and unbalanced diet have been associated with an accumulation of AGEs.(10) The combination of exercise and diet seems to be an effective means to reduce AGEs accumulation.(11) A recent study has shown that life-long exercise can counteract the age-related accumulation of AGEs.(12) In older adults it was recently shown that higher physical activity was associated with lower AGEs levels(13). Hypertension and arterial stiffness have both been associated with increased plasma concentrations of AGEs in adults.(14)

There are very few studies on AGEs in children. A previous review on the role of dietary AGEs in childhood suggested that AGEs are involved in the pathogenesis of adiposity and β-cell failure.(15) Jaisson and colleagues found that serum AGEs were elevated on first diagnosis of diabetes mellitus and may play a role in developing long-term complications.(16) Children with five years exposure to diabetes have been reported to have higher accumulation of skin AGEs, comparable to levels of healthy adults and equivalent to about 25 years of chronologic aging.(17) Our study, for the first time, aimed to examine the association of body composition, BP and cardiorespiratory fitness (CRF) with subcutaneous accumulation of AGEs in an

unselected population of young children. We hypothesize that cardiovascular risk factors such as obesity, high blood pressure and low physical fitness are associated with higher concentration of skin AGEs early in life.

Methods

Study design and Participants

This cross-sectional study was embedded in the large scale, cross-sectional EXAMIN YOUTH study in Switzerland. The study protocol was approved by the Ethics Committee of the University of Basel (EKBB: 258/12). The study was performed in accordance with the Helsinki Declaration of Guideline For Good Clinical Practice(18) and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.(19) In a predominantly Caucasian population, children aged 6- to 8-years were screened for body composition, BP, CRF and subcutaneous AGEs accumulation. An informed parental written informed consent was obtained from all participants. The study was registered a priori in a clinical trials registry (ClinicalTrials.gov: NCT02853747).

Measurements

Advanced glycation end products

AGEs were assessed by subcutaneous skin autofluroescence (SAF). Measurements of SAF were performed using the validated AGE Reader® device (DiagnOptics Technologies BV, Groningen, Netherlands).(6,20) The AGE Reader® involves an integrated spectrometer to analyze reflected excitation light. The ratio between the emission light and reflected light multiplied by 100 was used to calculate SAF, expressed in arbitrary units (au). The emission light ranges between 420 to 600nm, whereas the reflected excitation light ranges between 300 to 420nm. SAF allows the non-invasive, validated method to analyze AGEs in connective tissue and it is strongly correlated to AGEs accumulation in the blood serum.(6) For further analysis, the arithmetic mean of three measurements at different areas at the right ventral side of the forearm was used.

Anthropometrics

Body height was measured with a wall-mounted stadiometer (Seca 206, Basel, Switzerland), weight and percentage body fat (BF) were measured with the InBody device (InBody 170 Biospace device; InBody Co., Seoul, Korea). According to cut off points for BMI (kg/m^2) incorporating age and sex, children were classified in body composition groups.(21) Children with a BMI over the 85th percentile in their sex and age group were categorized as being overweight and over the 95th percentile as children with obesity. BP parameters were assessed using an automated oszillograph (Oscillomate, CAS Medical Systems, Branford, CT, USA). All children were measured in a sitting position after a five minute rest based on the recommendations of the American Heart Association. BP was measured five times in series and the mean of the three measurements with the smallest variation were taken for the further analysis. According of the population-based German KiGGS study, children over the 90th percentile were categorized as having high-normal BP and over the 95th percentile as children with hypertension.(22)

Physical fitness

The CRF assessment took place during the physical education lessons with the same equipment used for every school. After a 5-min warm-up, a 20m shuttle run was performed. This is a well-established and validated test to measure physical fitness.(23,24) A previous meta-analysis has demonstrated the feasibility and validity of the 20 m shuttle run as a surrogate marker for cardiorespiratory fitness in children.(25) It is concluded that, although spiroergometry remains to be the gold standard, the shuttle run is an established alternative if a laboratory-based test is not feasible. In this progressive endurance test, the children had to run back and forth between two lines of 20m with an initial running speed of 8.0 km/h and an increase of 0.5km/h every minute, paced by beeps from an audio device programmed for the timing of the shuttle run test. The individual maximum was reached if the child did not cross the line for two consecutive 20m trials within the given time, defined by the audio beeps. A 2m range for crossing the line was allowed. The score was assessed by the numbers of stages (1 stage = 1min) reached with a precision of 0.5 stages.

Statistical analysis

Variance homogeneity was assessed using Tukey-Anscombe Plots. To assess normality, we used normal QQ plots of the residuals. Mean SAF was analyzed across the clinical categories of BMI,BP and tertiles of CRF using univariate analysis of covariance (ANCOVA). Bivariate analysis was performed to compare clinical relevant BMI categories, physical fitness and SAF. Pearson's correlation was used to compare CRF with AGEs. Multiple linear regression analysis was applied to compare changes in SAF with changes in BMI, BF, BP and CRF. Four different models were fitted to adjust for age and sex as well as BMI, BP and CRF. 95% confidence intervals were presented for measures of effect to indicate the amount of uncertainty and a 2-sided level of significance of 0.05 denotes statistical significance. For analyses and graphics, an up-to-date version of Stata 15 (StataCorp LP, College Station, TX, USA) was used. The sample size of the cross-sectional study was given by the number of children and parents giving their consent.

Results

Participants

From the 3068 children that were invited in the study, 1690 (55%) had a written consent from their parents to participate. 221 children dropped out because of illness or were otherwise absent. Due to skin pigmentation or a temporary technical default of the device (AGE Reader®), 394 children had to be excluded from the data analysis, leaving 1075 children with complete measurements (Figure 1). Age, body weight and height, BF, BMI and shuttle run data of the 615 excluded children are presented in supplement Table S1. Excluded children had slightly higher fitness levels compared to children included in the study. Population characteristics are shown in Table 1. Based on a modified questionnaire survey(26), 95% of children were Caucasian. In our cohort, 87% of children presented with normal weight (n=934), 10% with overweight (n=103) and 3% (n=38) with obesity. Based on systolic BP, 77% were categorized as children with normal BP (n=827), 9% as having high-normal BP (n=99) and 14% as children with hypertension (n=149). Boys were fitter (CRF: 4.0±1.6 stages) but

showed higher subcutaneous AGEs (SAF: 1.07±0.2au) compared to girls (CRF: 3.4±1.3 stages; SAF: 1.03±0.2 au, p<0.001).

Figure 1. Flow diagram

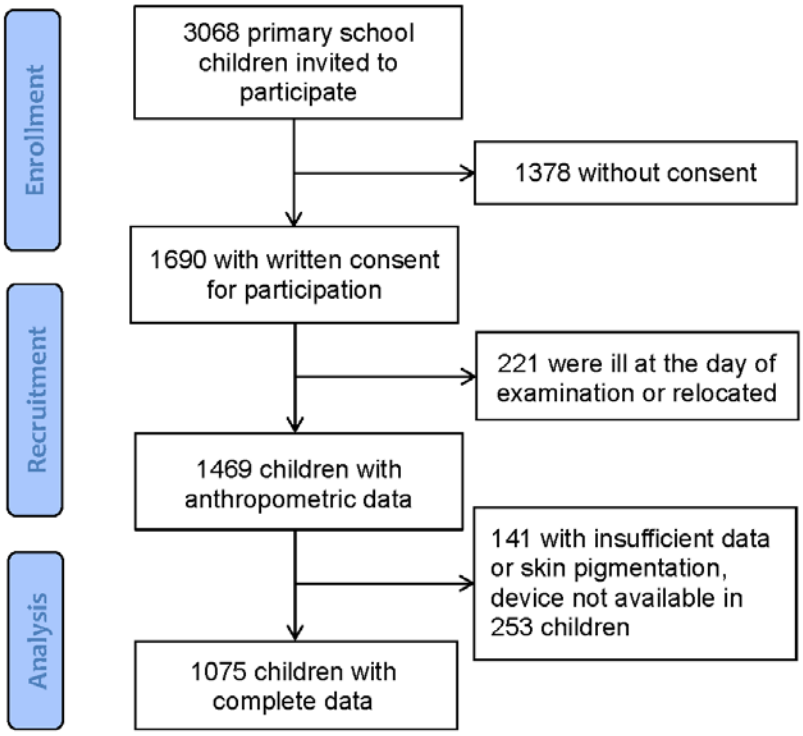


Table S1. Population characteristics of the main population as compared to children excluded.

Parameter	**Main population** **Mean±SD**	**n**	**Excluded children** **Mean±SD**	**n**	**p**
Age (years)	7.2±0.4	1075	7.2±0.4	615	0.722
Height (cm)	124.4±5.5	1075	124.4±5.4	615	0.670
Weight (kg)	24.7±4.7	1075	24.8±5.1	615	0.558
BMI (kg/m^2)	15.9±2.2	1075	15.9±2.2	615	0.828
Percentage body fat (%)	15.6±7.7	1075	15.8±7.9	615	0.587
20-m Shuttle Run (stages)	3.7±1.5	1075	3.8±1.5	615	0.040

Abbreviations: BMI, body mass index; SD, standard deviation

Table 1. Population characteristics of the study.

Parameter	**Total** **Mean±SD**	**n**	**Boys** **Mean±SD**	**n**	**Girls** **Mean±SD**	**n**	**p**
Age (years)	7.2±0.4	1075	7.2±0.4	509	7.2±0.4	566	0.073
Height (cm)	124.4±5.5	1075	124.8±5.4	509	124.0±5.5	566	0.025
Weight (kg)	24.7±4.7	1075	25.0±4.7	509	24.4±4.6	566	0.068
BMI (kg/m^2)	15.9±2.2	1075	15.9±2.2	509	15.8±2.2	566	0.295
Percentage body fat (%)	15.6±7.7	1075	14.1±7.3	509	16.9±7.8	566	<0.001
Heart rate (bpm)	85.8±10.3	1075	85.5±10.2	509	86.2±10.5	566	0.278
Systolic BP (mmHg)	103.7±7.7	1075	103.6±7.6	509	103.9±7.8	566	0.547
Diastolic BP (mmHg)	64.2±6.8	1075	64.1±6.8	509	64.3±6.8	566	0.569
SAF (au)	1.05±0.20	1075	1.07±0.20	509	1.03±0.20	566	<0.001
20-m Shuttle Run (stages)	3.7±1.5	1075	4.0±1.6	509	3.4±1.3	566	<0.001

Abbreviations: BMI, body mass index; BP, blood pressure; SAF, skin autofluorescence; au, arbitrary units; SD, standard deviation; n, number

Group differences

The results for between group differences are shown in Table 2. Clinical BMI, systolic and diastolic BP categories were not associated with SAF in our cohort of children. Children with higher physical fitness showed lower SAF (0.99 (1.03;1.05) au) compared to children with low CRF (1.09 (1.03;1.05) au, p<0.001).The bivariate analysis illustrates the interrelation between fitness, body composition and SAF levels. Children with low fitness and overweight or obesity had the highest subcutaneous AGEs levels (Figure 2).

Table 2. Advanced glycation end products in relation to clinical categories of body mass index, blood pressure and tertiles of shuttle run.

Parameter	n	SAF (au) Mean (95% CI)	p
BMI[a]			0.685
Normal weight	934	1.05 (1.04;1.06)	
Overweight	103	1.03 (0.99;1.07)	
Obese	38	1.04 (0.98;1.11)	
BF[a]			0.975
First (lowest)	347	1.04 (1.02;1.07)	
Second	354	1.05 (1.02;1.07)	
Third	339	1.05 (1.03;1.07)	
Systolic BP[b]			0.799
Normotensive	827	1.05 (1.03;1.06)	
High-normal BP	99	1.04 (1.01;1.08)	
Hypertensive	149	1.04 (1.00;1.07)	
Diastolic BP[b]			0.181
Normotensive	840	1.04 (1.03;1.05)	
High-normal BP	84	1.07 (1.03;1.11)	
Hypertensive	151	1.06 (1.03;1.10)	
Shuttle run[c]			<0.001
First	476	1.09 (1.03;1.05)	
Second	275	1.03 (1.03;1.11)	
Third (fittest)	324	0.99 (1.03;1.10)	

Data adjusted for age and sex

P value across lowest and highest category (univariate analysis of covariance)

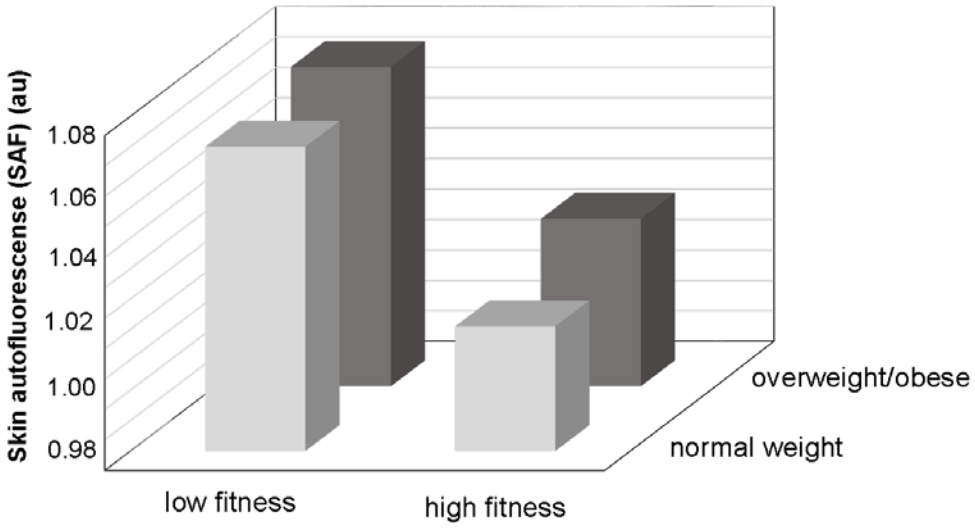
[a] Additionally adjusted for shuttle run, systolic and diastolic blood pressure

[b] Additionally adjusted for shuttle run and BMI

[c] Additionally adjusted for BMI, systolic and diastolic blood pressure

Abbreviations: BMI, body mass index; BF, body fat; BP, blood pressure; SAF, skin autofluorescence; au, arbitrary units; CI, confidence interval

Figure 2. Skin autofluorescence by body mass index categories and median of physical fitness.



Regression analysis

In the regression analysis, BMI was associated with SAF (0.006 (0.2E-3;0.011) au, p=0.042) but not independently of age and sex (Table 3). Percentage BF was associated with increased SAF (p=0.001). This association disappeared after adjustment for BP and CRF. No association of systolic and diastolic BP with SAF was found. One stage increase in shuttle run was significantly associated with decreased SAF,independent of BMI and BP (Figure 3). CRF alone explained 5% of the SAF variance. As expected, children with higher CRF had lower BMI (p<0.001) and lower systolic (p<0.001) and diastolic BP (p=0.035).

Table 3. Regression analysis for the association of body composition, peripheral blood pressure, physical fitness and activity with advanced glycation end products

Parameter	**Model**	**SAF (au change per unit)**	
		B (95% CI)	p
BMI (kg/m^2)	1	0.005 (-0.001;0.010)	0.085
	2	-0.002 (-0.008;0.004)	0.439
Percentage body fat (%)	1	0.003 (0.001;0.004)	0.001
	2	0.2E-2 (-0.002;0.002)	0.861
Systolic BP (mmHg)	1	0.001 (-0.001;0.002)	0.575
	3	-0.6E-4 (-0.001;0.002)	0.936
Diastolic BP (mmHg)	1	0.001 (-0.001;0.002)	0.542
	3	0.2E-3 (-0.002;0.002)	0.815
20-m Shuttle Run (stages)	1	-0.032 (-0.040;-0.024)	<0.001
	4	-0.033 (-0.042;-0.024)	<0.001

Model 1 = adjusted for age and sex

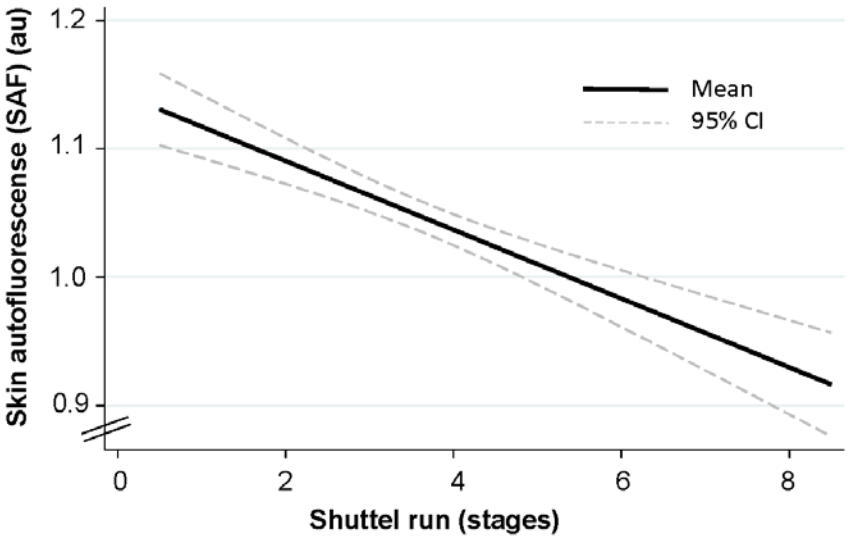
Model 2 = model 1 plus adjusted for systolic, diastolic blood pressure and shuttle run (stages)

Model 3 = model 1 plus adjusted for BMI and shuttle run (stages)

Model 4 = model 1 plus adjusted for BMI, systolic and diastolic blood pressure

Abbreviations: BMI, body mass index; BP, blood pressure; SAF, skin autofluorescence; au, arbitrary units; CI, confidence interval

Figure 3. The association of cardiorespiratory fitness with skin autofluorescence.



Discussion

This is the first study to examine the association of body composition, BP, and CRF with AGEs accumulation in children. Our findings demonstrate that low physical fitnessis associated with increased accumulation of subcutaneous AGEs in young children. In contrast to our hypothesis, we found no independent association of BMI and BP with subcutaneous AGEs.

Physical fitness and AGEs

Higher CRF wasassociated with reduced subcutaneous AGEs formation indicating a favorable glucose metabolism and a reduction of associated CV risk in these children. Data in a Slovak population suggested that regular self-reported physical activity is associated with lower SAF during lifespan.(27) Two studies have previously measured subcutaneous AGEs in a small number of healthy young children.(27,28) Our study is the first to assess AGEs in a large population-based unselected cohort of 6-8 year old children, offering reliable normal values for young Caucasian children (mean SAF 1.05±0.20au) and demonstrating the inverse association with objectively measured CRF.

CRF affects glycation processes in children, as at least 5% of the variance of SAF was explained by CRF in our cohort. One unit increase in CRF was associated with a 0.03au decrease in SAF and the difference in SAF between the lowest and the fittest tertile was 0.10au. In comparison, in adolescents with Type 1 Diabetes one unit increase in haemoglobin A1c (HbA1c) has been associated with a 0.06au increase in SAF.(29) Future studies will have to determine the long-term clinical and predictive value of subcutaneous AGEs for the development of cardiometabolic disease and the potential of physical fitnessto counteract accumulation of AGEs during childhood and later in life.

Body composition and AGEs

No independent association between BMI and SAF was found in our cohort of children. The association of BF with SAF was not independent of BP and CRF.

A previous study in adults also showed no association of subcutaneous AGEs with obesity in the absence of the metabolic syndrome.(8) A prior study in children suggested that BMI, BF and fat mass were associated with soluble RAGE in older children aged 12-14 years.(30) It is therefore possible, that obesity only affects AGEs formation after a longer-term exposure time to an increased BMI. In addition, sex seems to be a non-modifiable factor for the accumulation of AGEs in children. In our population of young children, boys showed higher AGE accumulation compared to girls independent of BMI and BP. In contrast to the gender differences in our children, it has been shown that plasma accumulation of AGEs is higher in women compared to men aged around 20 years.(31) Our children were examined in pre-puberty, whereas the aforementioned study investigated young adults. Sex-related differences in childhood development and puberty seems to be the most likely explanation for this conundrum.

Blood pressure and AGEs

In the regression analysis, no association of systolic and diastolic BP with SAF was found. However, there was a weak but significant association of diastolic BP and SAF in children

categorized as children with high-normal BP and hypertension. In adults, hypertension has been associated with increased accumulation of AGEs in plasma.(14) A recent study found an association of systolic and diastolic BP with subcutaneous AGEs in a general adult population.(32) In patients with the metabolic syndrome, high-normal BP was also associated with subcutaneous AGEs.(8) Childhood BP has been shown to predict development of CV disease in adulthood.(33) In children, higher BP does not seem to directly and independently affect AGEs accumulation and metabolic health. As argued before, exposure time to high BP may not be long enough to affect AGEs accumulation in young children. Based on our findings the clinical application of AGE`s in young children to differentiate cardiometabolic risk would appear premature. It remains to be determined if and to what extent BP, and indeed BMI, affect AGEs accumulation in older children and adolescents.

Potential mechanisms

Endothelial dysfunction and obesity-related inflammation are mediated trough oxidative stress conditions.(34) It is well known that oxidative stress is a main determinant for increased formation of AGEs(35–37). Sedentary behavior is characterized by reduced mitochondrial capacity and increased oxidative stress and exercise has the potential to reverse oxidative conditions.(38) Proteins are glycated to form AGEs through the so-called Maillard reaction. Early non-enzymatic glycation and formation of Schiff bases and Amadori products represent reversible cross-links between proteins and sugars.(39) We hypothesize that exercise can reverse formation of early glycation products preventing irreversible cross-links and tissue accumulation of AGEs forming fluorescent derivatives. In addition, exercise-induced formation of soluble RAGE may play an important role in reducing AGEs accumulation and associated oxidative stress. The circulating soluble RAGE binds to AGE and acts as a competitive inhibitor of ligands that activate RAGE. Long-term physical activity and exercise lead to an increase in soluble RAGE, which blocks RAGE activation.(40)

Improvement of AGEs metabolism may be achieved by physical fitness interventions rather than measures focusing on classical risk factors such as BMI and BP reduction. From a pathophysiological point of view it is possible that other sensitive metabolites of the AGEs metabolism, such as RAGE or protein-bound AGEs and markers of dicarbonyl stress, may be

associated with BMI and BP in young children. Subcutaneous AGEs accumulation may occur at later stages compared to increases in circulating serum and urine biomarkers of AGEs metabolism. Future studies will have to analyze blood or urine samples of young children to clarify the clinical relevance and differences of circulating AGEs metabolites as compared to subcutaneous AGEs accumulation.

Strengths and limitations

This is a cross-sectional design and does not investigate temporal development of the associations. However, a significant and independent relationship between CRF and AGEs was found and a long-term follow-up is warranted to proof causal associations between lifestyle-related risk factors and metabolic health in children. Furthermore, only three percent were children with obesity in our cohort. Studies in populations with a higher prevalence of children with obesity may help to further differentiate the association of obesity and AGEs in children. With respect to measuring AGEs, the device cannot be applied in children with dark skin and, therefore, a selection bias is given for technical reasons. One strength of our study includes the large sample size and the use of standardized procedures to measure body composition, BP and CRF in children.

Conclusion

In conclusion, our results demonstrate that physical fitness but not body mass and BP are associated with subcutaneous AGEs in young children. Analysis of skin AGEs is a feasible tool to differentiate the effects of physical fitness on tissue glycation and metabolism. We postulate that exposure times to a higher BMI and BP are too short to affect AGEs deposition in tissue of young otherwise healthy children. Associations may still become apparent at later stages and during adolescents. From a clinical perspective, higher AGEs have been linked with development of diabetes mellitus in children(16,17) but, as our findings demonstrate, this is not the case for childhood obesity and hypertension. In children with increased AGEs and referenced to our normal values, treatment strategies should still focus on reducing AGEs accumulation to counteract the growing prevalence of metabolic disease in childhood and

later in life. Primary prevention programs may need to focus on improving physical activity and fitness as treatment options to achieve this ambitious long-term goal.

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Chapter 6 Synthesis, Discussion and Perspectives

6.1 Summary of the Main Results

This section summarises the main findings of the three publications based on the addressed aims and hypotheses in Chapter 2.

6.1.1 Obesity, Blood Pressure, and Retinal Vessels: A Meta-analysis

The aim of the first publication was to systematically review and meta-analyse associations of BMI, blood pressure and physical activity with retinal vessel diameters in children and adolescents. After an extensive electronic search, a total of 22 studies[61–64,67,68,109–124] (18865 participants) were included in our systematic review and the meta-analysis was performed in 11 studies[61–63,63,64,111,113,115,116,121,122].

Overall, 13 studies published the association of BMI with retinal vessel diameters in in children and adolescents[61,62,64,113–117,121–124]. A higher BMI was associated with higher cardiovascular risk, defined as retinal arteriolar narrowing and venular widening. The meta-analysis of 8 studies[62,64,113,115–117,121,122] showed a pooled effect size of the relationship between BMI and CRAE of -0.37 and between BMI and CRVE of 0.35, whereas the calculated prediction intervals for CRVE do not seem to be consistent (-0.41 to 1.12).

The association of systolic and diastolic blood pressure with retinal vessel diameters was examined in 12 studies[61,63,109,111–113,115,116,118,120,121,123]. Elevated systolic and diastolic blood pressure were consistently associated with arteriolar narrowing. From the available data, it seems that blood pressure did not affect retinal venular diameters in children. Six studies were included in the meta-analysis of blood pressure with vessel diameters[61,63,111,112,115,116]. Between systolic blood pressure and CRAE/CRVE, a pooled estimate of -0.63 and -0.07 respectively was found. With respect to the association of diastolic blood pressure and CRAE/CRVE, we found a pooled estimate of -0.60 and -0.06 respectively.

Three of the published studies investigated the relationship between physical activity/inactivity and retinal vessels[67,68,119]. Siegrist et al. demonstrated a negative association between childhood physical inactivity and AVR due to larger CRVE[119]. In our previous study, we showed that indoor activity affects retinal arteriolar diameters in young children[67]. In contrast to our study in Switzerland, an Australian study found that outdoor, not indoor activity was associated with retinal venular widening[68].

6.1.2 Obesity, High Blood Pressure, and Physical Activity Determine Vascular Phenotype in Young Children: The EXAMIN YOUTH Study

Study 2 was conducted to investigate the association of BMI, blood pressure, physical activity and fitness with retinal vessel diameters and arterial stiffness in 6- to 8- year-old children. Overall, 1171 primary school children were measured for BMI, blood pressure (according to the American Heart Association guidelines), CRAE and CRVE, PWV, cardiorespiratory fitness (20m shuttle run) and 20-meter sprint performance. Physical activity parameters and screen time were assessed in 833 children by a questionnaire fill-out by parents. Overweight and obese children showed narrower CRAE and higher PWV compared to normal weight children ($p<0$-001). One unit increase in BMI was associated with -0.50 µm narrower CRAE and 0.03m/s higher PWV ($p<0$-001). Children categorised as hypertensive had narrower CRAE and higher PWV compared to children with high-normal and normal blood pressure ($p<0.001$). In the regression analysis, one unit increase of systolic blood pressure was associated with 0.34 µm narrower CRAE and 0.02m/s higher PWV ($p<0.001$). Likewise, CRAE was with 0.27µm and PWV with 0.01m/s higher with every unit increase in diastolic blood pressure.

Regarding physical fitness and vascular parameters, we found that cardiorespiratory fitness was associated with 0.51µm wider CRAE ($p=0.06$) and 0.02m/s lower PWV ($p<0.001$), but the associations disappeared after adjusting for BMI and blood pressure. Slower sprint performance was associated with wider CRVE ($p=0.005$), reflecting less microvascular health. One unit increase in screen time was weakly associated with narrower CRAE ($p=0.08$) and higher PWV ($p=0.04$), but not independent of BMI and blood pressure. No associations were found between CRAE, CRVE, PWV and physical activity assessments.

Pearson's correlation analysis demonstrated that CRAE ($r=-0.21$; $p<0.001$) and CRVE ($r=-0.08$; $p=0.005$) were significantly but weakly correlated with PWV. Higher PWV was associated with narrower CRAE ($p=0.005$).

6.1.3 Physical fitness but not body mass index or blood pressure are associated with advanced glycation end products in children

Within the EXAMIN YOUTH study, we additionally assessed the association of BMI, blood pressure, physical activity and fitness with AGEs in young children (aged 7.2±0.4years). In this

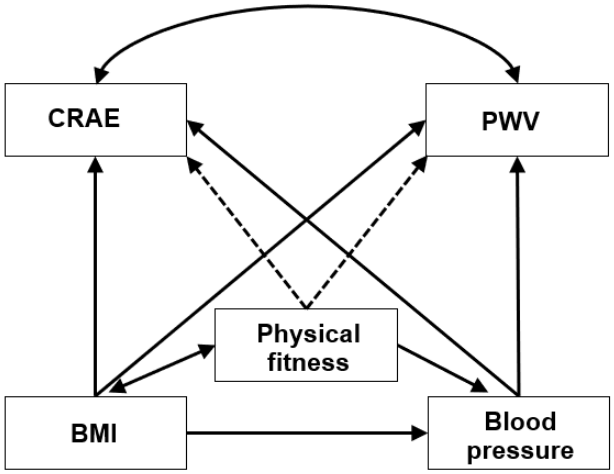
cross-sectional design, 1075 children were additionally screened for subcutaneous AGEs accumulation, measured by SAF. In our cohort, no associations were found between clinically relevant BMI and blood pressure categories and subcutaneous AGEs accumulation. A weak association was found in the regression analysis for BMI, one unit increase in BMI was associated with 0.006 arbitrary units (au) increase in SAF (p=0.042), but the association did not remain significant after adjusting for age and sex. Blood pressure was not associated with SAF.

Cardiorespiratory fitness was associated with 0.03au decreased SAF, independent of BMI and blood pressure (p<0.001). SAF increases by 0.08au for every unit increase in sprint performance (slower sprint performance) (p<0.001). Physical fitness explained 5% of the variance of AGEs accumulation. Physical inactivity, measured by screen time, was independently associated with higher SAF (p<0.001). We found no associations with SAF with respect to Physical activity. Besides, retinal vessel diameters and PWV did not correlate with SAF in young children.

6.2 General Discussion

In the following two sections, findings of our research will be discussed in more detail.

Figure 1 gives an overview of the interrelations between CRAE, PWV and cardiovascular risk factors of the study.



CRAE central retinal arteriolar equivalent; PWV, pulse wave velocity; BMI, body mass index

Figure 1. Interrelation between CRAE, PWV, body mass index, blood pressure and physical fitness

6.2.1 Retinal Vessel Diameters and Arterial Stiffness

In the large-scale EXAMIN YOUTH study, we found that obesity and overweight were associated with retinal arteriolar narrowing in 6- to 8- year old children. The results of our cohort study are in line with the findings of our previous meta-analysis in children and adolescents. We demonstrated that higher BMI was associated with retinal arteriolar narrowing and venular widening in children and adolescents. From the calculated prediction intervals, it becomes evident that arteriolar narrowing was predominantly associated with BMI, whereas the association between BMI and venular widening may not to be consistent.

With regard to the macrovascular bed, higher BMI was associated with higher PWV in our cohort of young children. The Avon Longitudinal Study of Parents and Children evaluating more than 6000 children aged 10 - 11 years found that obese children had lower arterial stiffness compared to normal weight children[125]. However, several studies have previously demonstrated that obesity and overweight are associated with higher arterial stiffness in children[85,126,127]. It seems that childhood growth and development play an important role in large artery stiffness during childhood and adolescence. Longitudinal follow-up studies are needed to clarify the influence of obesity on arterial stiffness from childhood to adolescents and adulthood.

In our meta-analysis it became evident, that systolic as well as diastolic blood pressure were consistently associated with retinal arteriolar narrowing. Only two studies found no correlation between diastolic blood pressure and CRAE[63,113]. Similar results were found in the EXAMIN YOUTH study. One mmHg increase in systolic and diastolic blood pressure was associated with a narrowing of the arterioles by 0.34 µm and 0.27 µm respectively. An increase in systolic blood pressure was also associated, even though to a lesser extent, with venular narrowing (p=0.02). In our study, we also found a positive correlation between CRAE and CRVE (r=0.59). However, the association of higher blood pressure with venular narrowing was influenced by the collinearity between arteriolar and venular diameters. Children with narrower arterioles tended to have narrower venules as previously described in adults[128]. The association of CRAE with high blood pressure was independent of CRVE. Children with systolic and diastolic hypertension showed the narrowest arterioles in the microcirculation and the highest large artery stiffness compared to normotensive children. PWV increased by 0.02m/s and 0.01m/s per unit increase both systolic and diastolic blood pressure.

In addition, obese children with hypertension showed additive deleterious effects on vascular phenotype. We found a significant but weak collinearity between CRAE and PWV. A cross-talk between large and small vessels has been proposed[129], but it is important to note that both, micro- and macrovascular beds provides separate clinically relevant information on cardiovascular health.

We objectively measured cardiorespiratory fitness by assessing shuttle run stages. Children with the highest fitness showed favourable wider arterioles, narrower venules and lower arterial stiffness compared to children with a low fitness level. In the regression analysis, the association did not remain significant after adjustment for BMI and blood pressure. A previous smaller-sized study showed that cardiorespiratory fitness was associated with narrower CRVE[67]. Sakuragi et al. reported a negative correlation between cardiorespiratory fitness and PWV, but this association was dependent on BMI[85]. In addition to cardiorespiratory fitness, we found that explosive strength as measured by a 20-m sprint performance was independently associated with wider retinal venules, this as a marker for better cardiovascular health. Physical inactivity was related to unfavourable retinal microvascular diameters.

Based on the above findings, we concluded that early exercise programmes to improve physical fitness, reduce obesity and elevated blood pressure may help to improve micro- and macrovascular health in children.

6.2.2 Potential Mechanisms

Systemic obesity-related inflammation caused by impaired adipokine secretion, is related to increased insulin resistance and characterised by dyslipidaemia. These metabolic risk factors may be predictors of micro- and macrovascular alterations early in life. Obesity is characterized by a low nitric oxide (NO) level as a main determinant of vasodilation and regulation of vascular perfusion[130]. Childhood high BMI and body fat may reduce NO bioavailability and therefore contribute to narrower retinal arteriolar diameters. In otherwise healthy young children with a short exposure time to obesity and high blood pressure, functional rather than structural adaptations of the vascular bed are more likely to occur. Persistent elevation of blood pressure and mechanical stress induce myogenic

vasoconstriction and lead to a reduced NO bioavailability[131,132]. The combination of aggravated vasoconstriction and reduced NO-induced vasodilation may explain retinal arteriolar narrowing in children with higher blood pressure. Chronic systemic blood pressure elevation may cause structural remodelling of the micro- and macrovascular wall, which may further trigger arteriolar narrowing and increased large artery stiffness. Chronic blood pressure-induced cyclic stress is known to cause elastin degeneration and collagen deposition in the wall of large arteries, thereby facilitating the development of arterial stiffness[133]. Most of the pathophysiological mechanisms involved can be counteracted by regular exercise. There are multiple exercise-induced beneficial effects on the vasculature including improvement of endothelial function and NO bioavailability, anti-inflammatory properties as well as neurohumoral and autonomic factors.

6.2.3 Advanced Glycation End Products

As mentioned in the introduction, AGEs mediate as predictors for microvascular complications and arterial stiffness in adults[89,98,99]. Regular physical exercise has the potential to reduce AGEs accumulation during lifespan[103]. In children, there are very few studies on cardiovascular risk factors and its relation to AGEs accumulation. This is the first study to investigate the association of body composition, blood pressure, physical activity and fitness with AGEs in young children. We found that low cardiorespiratory fitness and time spent in front of a screen are associated with higher accumulation of subcutaneous AGEs in young children. Obesity and high blood pressure do not seem to affect independently AGEs accumulation in skin.

A previous study demonstrated that self-reported physical activity is related to lower subcutaneous AGEs formation during lifespan[134]. In line with this study, we found that objectively measured physical fitness and low screen time were associated with higher AGEs accumulation in skin. So far, two small-sized studies have measured SAF in healthy young children[134,135]. In our large cohort of 1075 young children, one unit increase in CRF was associated with a 0.03au decrease in SAF. In comparison, in adolescents with Type 1 Diabetes one unit increase in haemoglobin A1c (HbA1c) has been associated with a 0.06au increase in SAF[136]. In this cohort, the difference in SAF between Type 1 Diabetes and healthy controls was

0.26au. In our cohort of unselected young children, the difference in SAF between the lowest and the fittest tertile was 0.08au. In light of this evidence, it may be concluded that our findings are of high clinical relevance. Cardiorespiratory fitness alone was explained by 5% of variance of subcutaneous AGEs in our cohort. However, future studies are needed to investigate long-term associations of physical activity and cardiovascular fitness with AGEs and the development of cardiometabolic disease during childhood and later in life.

Soluble forms of RAGE act as decoy receptors for RAGE ligands and inhibit the interaction between AGEs and RAGE. Therefore, higher soluble RAGE levels have the potential to reduce pro-inflammatory pathways and the progression of cardiometabolic disease. Previous studies demonstrated that soluble RAGEs are involved in the development of diabetes in children[106,107]. There is evidence that high BMI and body fat are associated with lower soluble RAGE in adolescents[108]. No independent association between BMI, body fat and SAF was found in our cohort of young children. We assumed that the short exposure time of obesity in young children did not affects subcutaneous AGEs accumulation.

Similar to body composition, systolic and diastolic blood pressure was not associated with SAF. In adults, higher SAF was positively correlated with systolic and diastolic blood pressure[100]. High blood pressure during childhood has been shown to predict development of hypertension and CVD later in life[28,137]. Hypertension and arterial stiffness seem to be related to increased blood concentrations of AGEs in adults[99]. We found no association of micro- and macrocirculation with subcutaneous AGEs in our cohort of children (data not shown). It remains to be determined if elevated blood pressure and vascular alterations are related to AGEs metabolism in older children and adolescents.

Some of the potential mechanisms need to be discussed. AGEs form when proteins or lipids interact with reduced sugars. In a more downstream pathway, AGEs interact with RAGE and induce oxidative cell stress which triggers the development of vascular impairments and chronic inflammation[138]. Sedentary lifestyle is well known to reduce mitochondrial capacity and therefore induce higher oxidative stress. Physical activity and exercise contributes to control oxidative stress levels[139]. At an early stage of glycation, pentosidine is a reversible product, which undergoes further molecular transformations to form cross-linked stable AGEs. Regular physical activity and exercise may have the potential to counteract formation of irreversible AGEs and tissue accumulation of fluorescent subcutaneous AGEs. We

hypothesise that childhood obesity and elevated blood pressure affect AGEs metabolism at an early stage of formation. In young children, the exposure time may be too short to affect AGEs deposition in skin. Other metabolites such as soluble RAGE or protein-bound AGEs in blood serum, may be more sensitive biomarkers to investigate the association of BMI and blood pressure with AGEs in young children.

In summary, our findings demonstrate that low cardiorespiratory fitness and physical inactivity are associated with accumulation of subcutaneous AGEs early in life. The supposed relationship between high BMI, blood pressure and AGEs metabolism during childhood and adolescence will have to be investigated in a longitudinal approach.

6.3 Strengths and Limitations

The EXAMIN YOUTH study is a large-scale cross-sectional design and does not allow to determine causal associations over time. Longer-term follow-up studies are warranted to examine the association of vascular and metabolic health with cardiovascular risk factors during childhood and later in life. It is important to investigate if physical activity and cardiorespiratory fitness during childhood have the potential to counteract cardiometabolic disease in adulthood. In our cohort of predominantly Caucasian children, the prevalence of obese children was only 3%. Similar to the large-scale German KiGGS Study, the prevalence of children with high-normal blood pressure and hypertension was about 9% and 13%, respectively[140]. Future studies in a cohort with a higher prevalence of obese children of different ethnic origin are of relevance to differentiate our current findings. We were able to present reliable normal values of subcutaneous AGEs (mean SAF 1.05±0.20au) in a large cohort of young Caucasian children for the first time. For technical reasons, the device to assess AGEs cannot be applied in children with dark skin. Future studies will have to analyse serum and urine samples of young children to clarify the clinical relevance and differences of circulating AGEs metabolites as compared to subcutaneous AGEs accumulation.

Given that physical activity and sedentary behaviour are often assessed using a variety of questionnaires, a certain bias towards secular trends for physical activity may have occurred. Questionnaire-based duration and intensity of physical activity are often overestimated[141]. Therefore, we also assessed physical fitness by the established and validated 20-m shuttle run

performance test. Future studies may also have to use objective physical activity measurements such as accelerometry.

It is a strength of our study to have investigated a representative large cohort and the use of standardised methods for young children. This large sample-size allows a baseline data set for future longitudinal cohort studies. We were able to phenotype different vascular beds and metabolic associations of cardiovascular risk factors in young children.

6.4 Scientific Relevance and Perspectives

There is a worldwide lack of knowledge on the pathophysiological development of CVD. Population- and individually targeted prevention strategies are needed to counteract the early onset of premature CVD. Alterations in retinal vessel diameters have been shown to be associated with hypertension[57,58,142]; obesity and diabetes[55,143] in adults. Moreover, arteriolar narrowing and venular widening serve as biomarkers for increased cardiovascular morbidity and mortality[144]. In adulthood, an increase of PWV by 1m/s represents a risk increase of about 15% in total CV and all-cause mortality[71]. Therefore, the results of our study are of high clinical relevance. We demonstrated that obesity and elevated blood pressure are associated already with small and large vessel alterations in young children. Furthermore, there is evidence that AGEs mediate arterial structural remodelling in the vessel wall[93–95]. We found that subcutaneous AGEs accumulate in children with low fitness and physical inactivity. Based on our results, it is of scientific and public importance to investigate if physical activity and fitness during childhood may improve cardiometabolic health later in life. A follow-up of my PhD-project is planned to give an extensive knowledge about long-term associations of obesity, blood pressure and physical fitness/activity with vascular and metabolic health during childhood into adolescence. In 2020/2022, the same cohort of children will be recruited again and all measurements will be conducted four years after the baseline assessments. Furthermore, we will expand the medical screening by analysis of urinary metabolomics and dietary intake assessments. Urinary metabolomics analysis may help to identify novel markers or predictors for the development of vascular alterations early in life, especially due to the ongoing technological advances in this field. Multiple samples can be collected non-invasively. It is known that urine contains significant amounts of peptides and amino acids which help to

discover novel biomarkers. Metabolomics are downstream products of numerous genome-wide interactions. They can be sensitive biomarkers of a human phenotype. Obesity and dietary intake affect urinary metabolomics profiles and play an important role in the development of hypertension[145]. Based on my PhD-project, our future research is expected to be a milestone in the diagnosis of early development of cadiometabolic disease.

6.4.1 Broader Impact

Early cardiovascular risk stratification by use of non-invasive, reliable and easy to apply biomarkers may help to identify children at risk for developing CVD later in life. The assessment of combined macro- and microvascular biomarkers in clinical practice has the potential to improve primary health decision making and intervention programs before maladaptive health effects occur in early childhood. In addition, the EXAMIN YOUTH study will provide the scientific evidence for physical activity/fitness related cardiometabolic health during childhood and adolescence. Further investigation on the cross-link between cardiovascular and metabolic pathways will shed light on the mechanism behind the development of chronic cardiometabolic disease later in life.

6.5 Overall Conclusions

This dissertation demonstrates important and clinically relevant relationships between cardiovascular risk factors and cardiometabolic health in childhood. Obesity and elevated blood pressure were associated with maladaptive small and large artery modification in young children. Physical fitness seems to play a key role in the healthy and disease-free development during childhood. In this dissertation, it is therefore suggested that avoiding physical inactivity and improving physical fitness are main public health goals in order to reduce obesity and high blood pressure during a person's lifespan. Intervention programmes and treatment strategies in school and game settings are needed to reach and prevent children who are at risk to develop CVD later in life. Vascular phenotyping as a tool for cardiovascular risk stratification may help improve primary prevention strategies.

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Appendix A Publication 4: Exercise and Arterial Modulation in Children: The EXAMIN YOUTH Study

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Appendix B Publication 5: Effects of a school-based physical activity program on retinal microcirculation and cognitive function in adolescents

Authors:

Ludyga Sebastian[1]
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Appendix C Curriculum Vitae

SABRINA KÖCHLI

Date of birth:	February 7th 1986
Nationality:	Swiss citizen
Family status:	Single
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EDUCATION

Since 11/2015	**PhD program within the PhD Educational Platform for Health Sciences (PPHS)** University of Basel Department of Sport, Exercise and Health (DSBG) Sports Medicine and Systems Physiology Supervisor: Prof. Dr. med. Henner Hanssen
09/2013-09/2015	**Master program** **Human Movement Sciences** **Major in Exercise Physiology** ETH Zürich Department of Health Sciences and Technology Director of Studies: Prof. Dr. med. Christina Spengler Walder
09/2009 – 09/2013	**Bachelor program** **Human Movement Sciences** Department of Health Sciences and Technology ETH Zürich Director of Studies: Prof. Dr. med. Christina Spengler Walder
09/2006 – 08/2008	**Federal High School for Adults** Maturitätsschule für Erwachsene KME, Typus E, Zürich
08/2001 – 08/2005	**Vocational-technical High School** Technische Berufsmaturitätsschule Zürich
08/2001 – 08/2005	**Vocational Baccalaureate** Baugewerbliche Berufsschule Zürich

WORK EXPERIENCE

Since 11/2015	**PhD Project:** Association of physical fitness, body composition and blood pressure with vascular and pulmonary health in primary school children: The EXAMIN YOUTH Study Supervisor: Prof. Dr. med. Henner Hanssen
09/2014- 09/2015	**Traineeship/Scientific research fellow:** University of Zurich, Institute: Epidemiology, biostatistics and preventive medicine (SPLASHY study)
	Master Thesis: Effect of a physical activity program on preschooler's motor skills: The „SPLASHY HOPPS" intervention Supervisor: Prof. Dr. med. Susi Kriemler
01/2014-09/2014	**Traineeship** Kliniken Valens, sports therapy **Traineeship** Rehazentrum Leukerbad, sports therapy
08/2001 – 08/2005	**Education: building construction** SRT Architekten AG, 8044 Zürich

APPROVED RESEARCH PROJECTS

Since 01/2018	Sportcheck follow-up study (support for medical screening and coordination); SNSF project funding (#32003B_176172 / 1)
Since 04/2017	EXAMIN YOUTH South Africa (teaching of field workers)
Since 09/2015	EXAMIN YOUTH study (study coordinator and scientific researcher)
07/2015-12/2017	Exercise and cognitive function study (supervision of Master students)
09/2014- 09/2015	SPLASHY study (Master thesis)

SUPERVISION OF STUDENTS

Master students (8):	Luca Engler, Marina Trinkler, Luca Nogler, Julia Grenacher, Ramona Steiner, Tim Bartenstein, Morgane Mondoux, Livia Graf
Bachelor students (1):	Marina Capellini

TEACHING ACTIVITIES

Since 09/2017	**Lecturer** in «Cardiovascular diagnostics», Department of Sport, Exercise and Health (DSBG), Preventive Sports Medicine and Systems Physiology, University of Basel
Since 09/2017	**Lecturer** of «Hands on - exercise and vascular physiology», Department of Sport, Exercise and Health (DSBG), Preventive Sports Medicine and Systems Physiology, University of Basel
Since 09/2015	**Lecturer** of «Sports and exercise physiology», Department of Sport, Exercise and Health (DSBG), Preventive Sports Medicine and Systems Physiology, University of Basel

ACTIVE MEMBERSHIP IN SCIENTIFIC SOCIETIES

Science 12/2015	European Association of Preventive Cardiology (EAPC), Regular Membership
Science 12/2015	Die Sportwissenschaftliche Gesellschaft der Schweiz (SGS), Membership

AWARDS

04/2018	Nominated for the Young Investigator Award at EuroPrevent 2018
09/2017	Prize winner of the Young Investigator Award at DeGAG (German Speaking Society of Arterial Stiffness) Congress 2017
04/2017	Nominated for the Young Investigator Award at EuroPrevent 2017

LANGUAGUES

German:	mother tongue
English/French:	fluent in writing and speaking
Spanish/Italian:	basic skills

COMPUTER SKILLS

Software MS Office (Word, Excel, PowerPoint)
STATA, basic knowhow in R
Adobe Photoshop, ArchiCAD

PUBLICATIONS IN PEER-REVIEWED SCIENTIFIC JOURNALS

Köchli S, Endes K, Ramona S, Engler L, Grenacher J, Schmidt-Trucksäss A, Zahner L, Hanssen H. Obesity, High Blood Pressure, and Physical Activity Determine Vascular Phenotype in Young Children: the EXAMIN YOUTH Study. *Hypertension.* (**2018**); 73:153-161. doi:10.1161/HYPERTENSIONAHA.118.11872

Ludyga S, **Köchli S***, Phüse U, Gerber M, Hanssen H. Effects of a school-based physical activity program on retinal microcirculation and cognitive function in adolescents. *Journal of Science and Medicine in Sport.* (**2018**). doi:10.1016/j.jsams.2018.11.029

Köchli S, Endes K, Infanger D, Zahner L, Hanssen H. Obesity, Blood Pressure, and Retinal Vessels: A Meta-analysis. *Pediatrics*. (**2018**);141(6). doi:10.1542/peds.2017-4090

Kakebeeke TH, Knaier E, **Köchli S**, Chaouch A, Rousson V, Kriemler S, Jenni OG. Comparison between the Movement ABC-2 and the Zurich Neuromotor Assessment in Preschool Children. *Perceptual and Motor Skills*. (**2016**);123(3):687-701.

SUBMITTED MANUSCRIPTS

Köchli S, Endes K, Trinkler M, Mondoux M, Zahner L, Hanssen H. Physical fitness but not body mass index or blood pressure are associated with advanced glycation end products in children. *Journal of Pediatrics; submitted.* (**2018**)

Endes K, **Köchli S**, Zahner L, Hanssen H. Exercise and Arterial Modulation in Children: The EXAMIN YOUTH Study. *Frontiers in Physiology; submitted.* (**2018**)

WORK IN PREPARATION

Köchli S, Endes K, Bartenstein T, Schmidt-Trucksäss A, Zahner L, Hanssen H. Obesity, physical fitness and lung function in young children: The EXAMIN YOUTH Study. *In preparation (target journal: European Respiratory Journal)*

Köchli S, Endes K, Schmidt-Trucksäss A, Zahner L, Hanssen H. Influence of body composition and physical fitness on central hemodynamics in children. *In preparation (target journal: Atherosclerosis)*

CONTRIBUTION TO INTERNATIONAL CONFERENCES

Köchli S, Endes K, Steiner R, Engler L, Grenacher J, Infanger D, Schmidt-Trucksäss A, Zahner L, Hanssen H. Body composition and blood pressure determine vascular phenotype in young children: The EXAMIN YOUTH Study. European Society of Cardiology (ESC) Congress, Munich (Germany). (**2018**). Poster Presentation

Köchli S, Endes K, Trinkler M, Mondoux M, Zahner L, Hanssen H. Influence of physical activity and fitness on advanced glycation end product accumulation in children: The EXAMIN YOUTH study. Young Investigator Award, EAPC-EuroPrevent Congress, European Society of Cardiology (ESC), Ljubljana (Slovenia). (**2018**). Oral Presentation

Köchli S, Endes K, Bartenstein T, Zahner L, Hanssen H. Association of lung function with body mass and physical fitness in primary school children: The EXAMIN YOUTH Study. Schweizerische Gesellschaft für Sport Tagung (SGS), Magglingen (Switzerland). (**2018**). Oral Presentation

Köchli S, Endes K, Trinkler M, Mondoux M, Zahner L, Hanssen H. Influence of physical fitness and activity on advanced glycation end product accumulation in children- the EXAMIN YOUTH study. Schweizerische Gesellschaft für Sport Tagung (SGS), Magglingen (Switzerland). (**2018**). Oral Presentation

Köchli S, Endes K, Bartenstein T, Zahner L, Hanssen H. Association of lung function with body mass and physical fitness in primary school children: The EXAMIN YOUTH Study. Clinical Research Day, University hospital Basel, Basel (Switzerland). (**2018**). Poster Presentation

Köchli S, Endes K, Trinkler M, Mondoux M, Zahner L, Hanssen H. Influence of physical fitness and activity on advanced glycation end product accumulation in children: The EXAMIN YOUTH study. Clinical Research Day, University Hospital Basel, Basel (Switzerland). (**2018**). Poster Presentation

Köchli S, Endes K, Engler L, Schmidt-Trucksäss A, Zahner L, Hanssen H. Gefässsteifigkeit im Kindesalter: Die EXAMIN YOUTH Studie. Young Investigator Award, Kongress der deutschen Gesellschaft für arterielle Gefässsteifigkeit (DeGAG), Bad Oeyenhausen (Germany). (**2017**). Oral Presentation

Köchli S, Endes K, Engler L, Schmidt-Trucksäss A, Zahner L, Hanssen H. Prevalence and influence of obesity and hypertension on arterial stiffness in Swiss primary school children: The EXAMIN YOUTH study. Young Investigator Award, EAPC-EuroPrevent, European Society of Cardiology (ESC), Malaga (Spain). (**2017**). Oral Presentation

Köchli S, Endes K, Engler L, Schmidt-Trucksäss A, Zahner L, Hanssen H. Prevalence and influence of obesity and hypertension on arterial stiffness in Swiss primary school children: The EXAMIN YOUTH study. Schweizerische Gesellschaft für Sport Tagung (SGS), Zürich (Switzerland). (**2017**). Oral Presentation

Köchli S, Endes K, Engler L, Schmidt-Trucksäss A, Zahner L, Hanssen H. Prävalenz und Einfluss von Adipositas und Bluthochdruck auf die arterielle Gefässsteifigkeit bei Primarschulkindern: Die EXAMIN YOUTH Studie. Clinical Research Day, University Hospital Basel, Basel (Switzerland). (**2017**). Oral Presentation

GRADUATE EDUCATION (ORDERED BY DATE 2015-2018)

Course	Institution	ECTS
Advanced STATA Programming, Dr. J. Hattendorf	STPH Basel, University of Basel	1
Regression analysis and multi-level modelling, Prof. J. Scholderer	University of Basel	3
Academic Writing in the Health Sciences, Prof. A. Mündermann	University of Basel	1
Good Clinical Practice, Prof. Ch. Burri	University of Basel	1
Essentials in Health Research Methodology Different speakers, Clinical Trial Unit	University of Basel	1
Summer school "CardioLung 2017: Updates in Cardiovascular and Pulmonary Pathophysiology", Prof. C.Palombo	University of Pisa	6
Forschungsmethoden und Statistik III, Prof. M. Stöcklin	University of Basel	4
Advances in Infection Biology, Epidemiology and Global Public Health, Prof. M.Tanner	STPH Basel, University of Basel	1
Systematic Review and Meta-Analysis: A Practical Approach, Prof. M. Egger	SSPH+, ISPM Bern	1
1st Summer School of the European Society for Microcirculation (ESM) and the European Vascular Biology Organization (EVBO), Prof. H. Morawietz	University of Dresden, King's College London	2
1 ETCS equals to 30 hours investment time	**ETCS Total**	**21**