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Associations of cardiometabolic risk factors with heart rate variability in 6–8-year-old children: the PANIC Study

Running head: Metabolic profile and autonomic nervous system

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ABSTRACT

Background: Associations of cardiometabolic risk factors with heart rate variability (HRV) in children are unclear. We examined associations of cardiometabolic risk score (CRS) and individual cardiometabolic risk factors with HRV variables in 6-8-year-olds.

Methods: The participants were a population-based sample of 443 children participating in baseline measurements of the PANIC trial. Cardiometabolic risk factors included waist circumference (WC), insulin, glucose, triglycerides, HDL cholesterol, systolic blood pressure (SBP), and diastolic blood pressure (DBP). CRS was calculated as WC + insulin + glucose + triglycerides – HDL cholesterol + the mean of SBP and DBP. HRV variables (SDNN, RMSSD, HF, LF, LF/HF, Mean RR) were measured using 5-minute electrocardiography at rest and analyzed using the Kubios® HRV software. In this cross-sectional study, associations of CRS and individual cardiometabolic risk factors with HRV were investigated using linear regression analyses adjusted for sex and peak height velocity.

Results: CRS was negatively associated with RMSSD, HF, Mean RR (P value<0.05) and positively with LF/HF (P value=0.005). Insulin was negatively associated with SDNN, RMSSD, HF, LF, and Mean RR (P value<0.05) and positively with LF/HF (P value=0.008). SBP was negatively associated with SDNN, RMSSD, HF, LF, and Mean RR (P value<0.05). DBP was negatively associated with SDNN, RMSSD, and Mean RR (P value<0.05). WC, glucose, triglycerides, or HDL cholesterol were not associated with HRV variables.

Conclusions: Higher CRS, insulin, and blood pressure were associated with smaller HRV, mainly indicating lower parasympathetic activity, in young children. This knowledge may help improving the clinical management of metabolic syndrome and cardiovascular diseases since childhood.

Keywords: metabolic profile, body fat, autonomic nervous system, cardiorespiratory fitness, pediatrics

INTRODUCTION

Cardiovascular diseases are the main cause of premature mortality worldwide[1]. The main pathophysiological mechanism for these diseases is atherosclerosis that starts to develop during the early years of life[2,3]. Metabolic syndrome refers to a cluster of traditional cardiometabolic risk factors, such as central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, low plasma high-density lipoprotein (HDL) cholesterol and hypertension[4]. The definition of childhood metabolic syndrome is problematic due to its multiple definitions. Nevertheless, a large systematic review[5] has been proposed that the prevalence increases in accordance with weight status and the reported rates has been reported to vary from 0-1% in normal weight children, to 12% in overweight children, and 29% in obese children[5]. Thus, one of the main risk factors for metabolic syndrome is childhood obesity[6], which is a growing public health problem worldwide[7]. Moreover, children with overweight are more likely to become overweight adults indicating an increased lifelong risk for cardiometabolic diseases[8]. However, there is limited knowledge on the associations of metabolic syndrome and its components with cardiac autonomic modulation.

Heart rate variability (HRV) is a non-invasive measure of cardiac autonomic nervous system regulation[9], and it is influenced by parasympathetic and sympathetic activity[10]. Reduced HRV is a risk factor for serious health problems, such as coronary heart disease, hypertension, and overall mortality[11]. Recently, it has been suggested that increased HRV reduces cardiovascular risk beyond traditional risk factors in children[12], adolescents[12,13], and adults[14]. On the other hand, children with overweight have been reported to have decreased HRV[15,16], which may be due to the delaying effect of overweight on the natural increase in parasympathetic activity with growth[16]. Yet, there is a lack of studies in early childhood, although such knowledge would help screening the children who may need support the most.

In addition to overweight, there are other cardiometabolic risk factors that have been related to decreased HRV in children aged about 10 years[17]. For example, elevated blood pressure[18,19] and increased fasting plasma insulin[20,21] have been associated with reduced HRV. Furthermore, the use of a cardiometabolic risk score (CRS) as an indicator of clustered cardiometabolic risk instead of a dichotomous variable for metabolic syndrome is preferred in children[6,22,23]. To the best of our knowledge, there are only two previous cross-sectional studies on the association between CRS and HRV in general populations of children or adolescents[24,25]. A higher CRS was associated with a smaller HRV in children 5-6 years of age[24] and in adolescents aged 17 years[25]. However, there have

been differences in calculating CRS, which may have affected the results and made it difficult to compare the observations of earlier studies. Studies in different age groups and using recommended CRS are needed in order to fill in the gap in the current literature.

Since cardiometabolic risk factors have been found to track from childhood to adolescence[26] and adulthood[27,28], understanding the impact of CRS on cardiac autonomic nervous system regulation could help improving the clinical management of metabolic syndrome and cardiovascular diseases already in young people. The aim of the present study was to investigate the associations of CRS and its components with various HRV variables in a population sample of Finnish children 6–8 years of age. We hypothesized that higher CRS and its components would be associated with smaller HRV in a general population of children.

METHODS

Study design and participants

The present study utilizes baseline data from the Physical Activity and Nutrition in Children (PANIC) study (clinicaltrials.gov NCT01803776) that is an 8-year controlled trial on the effects of a combined physical activity and dietary intervention on cardiometabolic risk factors and associated outcomes in a population sample of children aged 6-8 years at baseline from the city of Kuopio, Finland[29]. The Research Ethics Committee of the Hospital District of Northern Savo approved the study protocol in 2006 (Statement 69/2006). The parents or caregivers of the children gave their written informed consent, and the children provided their assent to participation.

We invited 736 children 6–8 years of age who started the first grade in 16 primary schools of the city of Kuopio in 2007–2009 to participate in the study. Altogether 512 children (248 girls, 264 boys), who accounted for 70% of those invited, participated in the baseline examinations in 2007–2009. The participants did not differ in sex, age, or body mass index - standard deviation score (BMI-SDS) from all children who started the first grade in the city of Kuopio in 2007–2009 based on data from the standard school health examinations performed for all Finnish children before the first grade. Six children were excluded from the study at baseline because of physical disabilities that could hamper participation in the intervention or no time or motivation to attend in the study. We also excluded two children whose parents withdrew their permission to use the data of their children. Complete data on

adiposity and other cardiometabolic risk factors at baseline used in the statistical analyses were available in 232 boys and in 211 girls.

Assessment of adiposity

All children were asked to empty their bladder, and thereafter, body weight was measured twice using a calibrated InBody® 720 bioelectrical impedance device (Biospace, Seoul, South Korea) to accuracy of 0.1 kg. The mean of the two measurements was used for the analyses. Height was measured three times using a wall-mounted stadiometer without shoes to the nearest of 0.1 cm, and the mean of the closest two values was used for the analyses. Body mass index (BMI) was calculated by dividing body weight (kg) by body height (m) squared, and BMI-SDS was obtained using Finnish references[30]. The prevalence of normal weight, overweight, and obesity were defined using the cut-off values provided by Cole and coworkers[31]. Waist circumference (WC) was measured three times at mid-distance between the bottom of the rib cage and the top of the iliac crest after expiration, and the mean of the closest two values was used in the analyses. Body fat percentage (BF%) and lean body mass were measured using the Lunar® dual-energy X-ray absorptiometry device (GE Medical Systems, Madison, WI, USA), as described earlier[32].

Assessment of other cardiometabolic risk factors

A research nurse took blood samples in the morning after a 12-hour overnight fast. Plasma glucose was measured by a hexokinase method, serum insulin by an electrochemiluminescence immunoassay, plasma triglycerides by a colorimetric enzymatic assay, and plasma HDL cholesterol by a homogeneous colorimetric enzymatic assay[33]. A research nurse measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) from the right arm using the Heine Gamma® G7 aneroid sphygmomanometer (Heine Optotechnik, Herrsching, Germany) to accuracy of 2 mmHg. The measurement protocol included a 5-minute seated resting period followed by three measurements with a 2-minute interval in between. The average SBP and DBP of all three values was used in the analysis. Age-, sex-, and height-standardized z-scores were calculated for WC, insulin, glucose, triglycerides, HDL cholesterol, and the mean of SBP and DBP. Thereafter, CRS was calculated using a formula WC + insulin + glucose + triglycerides – HDL cholesterol + the mean of SBP and DBP, a larger score indicating a higher cardiometabolic risk[33].

Assessment of heart rate variability

A physician performed a standard clinical examination[32] for each child before the electrocardiographic (ECG) registration. Before the ECG registration, children were told to lay down

still for 10 minutes in order to stabilize their heart rate. Thereafter, the ECG was registered for 5 minutes. For the HRV analyses, 5-minute samples were selected from the ECG. The ECG signals were measured using the Cardiosoft® V6.5 Diagnostic System (GE Healthcare Medical Systems, Freiburg, Germany) at a frequency of 500 Hz. The ECG electrodes were placed according to the conventional 12-lead system, and a chest lead of good quality presenting a high R-wave amplitude was chosen for the HRV analysis. The ECG data were analyzed using the Kubios® HRV software (Kubios Co., Kuopio, Finland), and the details of the techniques and analysis methods employed to assess HRV have been described elsewhere[34]. Briefly, the R-wave peaks were first detected using an adaptive QRS detection algorithm, and the RR interval time series (time intervals between successive R waves as a function of R-wave time instants) were formed. Prior to the analyses, the data were checked for potential ectopic or aberrant beats and, if necessary, such erroneous beats were corrected using interpolation methods. The HRV variables used in the analyses were SDNN, the standard deviation of all RR intervals (ms), a marker of overall HRV and RMSSD, the square root of the mean of the sum of the squares of differences between adjacent RR intervals (ms), a marker of parasympathetic activity as well as Mean RR, the mean of RR intervals[10]. These HRV variables were used to measure HRV in a time domain with a lower value indicating a lowered parasympathetic modulation. In addition, we calculated high frequency power (HF: 0.15 – 0.40 Hz), which represents parasympathetic modulation; low frequency power (LF: 0.04 – 0.15 Hz), which represents a mixture of sympathetic and parasympathetic modulation; and LF/HF, which estimates the ratio between sympathetic and parasympathetic nervous system activity[11].

Covariates

Sex was reported by the parents. Years from peak height velocity was used as an indicator of maturity in children[35], and it was calculated separately for boys and girls using formula provided by Moore et al.[36]. Cardiorespiratory fitness (CRF) was assessed by a maximal exercise test using an electromagnetically braked Ergoselect 200K® bicycle ergometer with a pediatric saddle module (Ergoline, Bitz, Germany). Maximal power output (watt) achieved at the end of the exercise test per lean body mass (kg) was used as the measure of CRF[37].

Statistical methods

All statistical tests were conducted using the two-sided 5% level of significance and performed using SPSS statistical software, Version 24.0 (IBM Corp., Armonk, NY). The characteristics of children are provided as arithmetic means (standard deviations, SD) or frequencies (percentages, %). Before the analyses, HRV variables (SDNN, RMSSD, HF, LF, LF/HF, and Mean RR) were logarithmically

transformed due to skewed distributions. The associations of CRS and individual cardiometabolic risk factors (BF%, WC, insulin, glucose, triglycerides, HDL cholesterol, SBP, and DBP) with HRV variables were investigated using linear regression analyses, and we applied four different models. Firstly, data on the associations of CRS with HRV variables were analyzed without adjustment, since CRS was calculated using age-, sex-, and height-standardized z-scores for cardiometabolic risk factors. Secondly, data on the associations of individual cardiometabolic risk factors with HRV variables were adjusted for sex and peak height velocity. Thirdly, we also included BF% together with sex and peak height velocity in additional linear regression models, since increased BF% has been associated with reduced HRV[15] and it is a key component of clustered cardiometabolic risk. However, due to the multicollinearity, we excluded BF% from the model regarding WC. Finally, we included CRF % together with sex and peak height velocity in further linear regression models, because increased CRF has been associated with increased HRV[12] and decreased cardiometabolic risk factors[38,39]. We also studied whether the associations of CRS and individual cardiometabolic risk factors with HRV variables were different between boys and girls by adding an interaction term for CRS and individual cardiometabolic risk factors in the linear regression models. There was no evidence for the modifying effect of sex on these associations, and the results are thus presented for boys and girls together.

RESULTS

Participants' characteristics are presented in **Table 1.** Boys were taller, had lower BF%, and were further away from peak height velocity than girls. Boys also had higher WC, glucose, HDL cholesterol, LF, and Mean RR as well as lower insulin than girls.

Associations of CRS with HRV variables

CRS was negatively associated with RMSSD, HF, and Mean RR as well as positively associated with LF/HF (**Table 2**). All of these associations remained statistically significant after adjusting for CRF, but they became statistically non-significant after further adjustment for BF% (Table 2).

Associations of BF% and WC with HRV variables

BF% was negatively associated with RMSSD, HF, and Mean RR as well as positively associated with LF/HF adjusted for sex and peak height velocity (Table 2). These associations were no longer statistically significant after further adjustment for CRF ($p>0.05$). WC was not associated with HRV

variables after adjustment for sex and peak height velocity, or after further adjustment for CRF ($p>0.05$).

Associations of insulin and glucose with HRV variables

Insulin was negatively associated with SDNN, RMSSD, HF, LF, and Mean RR as well as positively associated with LF/HF after adjustment for sex and peak height velocity (Table 2). The associations of insulin with SDNN, RMSSD, HF, and Mean RR remained statistically significant after further adjustment for BF% (Table 2) or CRF (SDNN: β= -2.64, P value=0.002; RMSSD: β= -0.14, P value=0.004; HF: β= -0.14, P value=0.003; and Mean RR: β= -0.15, P value=0.001). Glucose was not associated with HRV variables (P value>0.05) (Table 2).

Associations of triglycerides and HDL cholesterol with HRV variables

Triglycerides or HDL cholesterol was not statistically significantly associated with HRV variables (P value>0.05) (Table 2).

Associations of SBP and DBP with HRV variables

SBP was negatively associated with SDNN, RMSSD, HF, LF, and Mean RR after adjustment for sex and peak height velocity (Table 2). These associations were slightly attenuated after additional adjustment for BF%, but further adjustment for CRF had no effect on the magnitude of these associations (P value<0.05). DBP was negatively associated with SDNN, RMSSD, and Mean RR when adjusting for sex and peak height velocity. These associations remained statistically significant after further adjustment for CRF (SDNN: β= -0.10, P value=0.044; RMSSD: β= -0.11, P value=0.026; Mean RR: β= -0.16, P value=0.001) but only Mean RR remained significant after additional adjustment for BF% (Table 2).

DISCUSSION

This is the first study on the association between cardiometabolic risk and autonomic modulation in young children. The novelty of our study is that we investigated the associations of a continuous CRS and individual cardiometabolic risk factors with HRV variables in children. We found that CRS was negatively associated with HRV variables independently of CRF, but these associations were partly explained by BF%. We also observed that fasting plasma insulin was negatively associated with HRV

variables, although these associations were partly accounted by BF%. In addition, SBP and DBP were negatively associated with HRV variables independently of CRF.

A higher CRS was associated with lower parasympathetic activity, as indicated by lower RMSSD and HF and higher LF/HF. This finding is at least partly due to the strong association between insulin and decreased HRV, since insulin resistance has been recognized as one of the key components in the development of metabolic syndrome[40]. To the best of our knowledge, there are only a few previous studies on the associations of CRS with HRV variables in children[17,24]. Vrijkotte et al.[24] found that a higher CRS as well as higher waist-to-height ratio and SBP of its components were associated with lower parasympathetic activity indicating smaller HRV, in children aged 5-6 years, which is in line with the present study. We also found a positive association between CRS and the balance between sympathetic and parasympathetic activity, which was not reported in the study by Vrijkotte et al.[24]. However, direct comparison between the studies is not possible due to the differences in methodology, as in their study, parasympathetic activity was measured by respiratory sinus arrhythmia and sympathetic activity by pre-ejection period[24]. Nevertheless, these findings together suggest that increased CRS is linked to lower parasympathetic activity. Zhou et al.[17] found inverse dose-response relationships of clustered cardiometabolic risk factors with SDNN, RMSSD, LF, and HF in children aged 9–11 years. However, children in their study had elevated levels of cardiometabolic risk factors, and thus, comparison of these results with our observations based on a general population of children needs to be done with caution. When discussing the relationships between CRS and HRV based on studies in different age groups, it is notable that HRV is likely to change during childhood[16] highlighting the need to study the associations in children with varying ages. Finally, we weighted each cardiometabolic risk factor similarly in calculating CRS, and it is therefore difficult to compare their true contribution to the association between CRS. In future studies, it should be further examined whether some of the components of CRS play a bigger role in autonomic nervous system regulation than others.

We found that BF% was negatively associated with RMSSD and HF power, both of which are measures of parasympathetic activity, and positively associated with LF/HF, which reflects the balance between sympathetic and parasympathetic nervous system activity. One explanation for these observations may be that overweight makes cardiac ventricles larger and their walls thicker and thereby worsens ventricular relaxation during diastole that impairs the balance of cardiac autonomic modulation[15]. There is evidence that overweight is associated with impaired cardiac autonomic balance in children[15], but more evidence is needed in young children. Furthermore, BMI has been

used in defining childhood obesity in young children[41], although it is not an optimal measure of pediatric obesity[42]. Therefore, the observed associations of increased BF%, assessed by whole-body DXA, with HRV in our study expands the knowledge on the associations of adiposity with HRV variables in children. Moreover, we found that CRF partly explained the associations of BF% with HRV variables, suggesting that higher CRF might be associated with larger HRV independent of body fat mass among pre-pubertal children. This highlights the beneficial associations of higher CRF with larger HRV already in childhood.

Fasting insulin had strong negative associations with SDNN, RMSSD, and HF, which reflect cardiac parasympathetic tone, and a strong positive association with LF/HF, a measure of the balance between sympathetic and parasympathetic activity. Moreover, these associations of fasting insulin with HRV variables were slightly attenuated after controlling for BF%. Consistent with our findings, previous studies have also shown that insulin resistance was associated with reduced HRV in children aged 11 years and that this relationship was partly accounted by body fat mass[20,21]. Thus, the results of this study together with our findings suggest that excess fat mass partly explains the relationship between insulin resistance and decreased HRV in children. On the other hand, Taşçılar et al.[21] demonstrated that obese children with insulin resistance had lower HF and higher LF/HF than obese children without it, suggesting that insulin resistance has an independent association with reduced HRV. Unlike other studies in children, we took CRF into account in the analyses and found that the associations of fasting insulin with HRV variables remained after controlling for CRF.

We found no association of triglycerides or HDL cholesterol with HRV. To the best of our knowledge, there are no previous studies on these associations in young children. However, Rodríguez-Colón and coworkers[25] found that triglycerides was negatively and HDL cholesterol positively associated with HRV in adolescents. Such associations have also been observed in young adults[43]. In line with the results of previous studies in adults[44], our findings suggest that triglycerides and HDL cholesterol do not play a major role in cardiac autonomic nervous system regulation in children. However, further studies are needed to investigate the associations of plasma lipids with HRV in all age groups.

Both SBP and DBP were negatively related to HRV variables SDNN and RMSSD. These associations, particularly that of SBP, became even stronger after controlling for CRF, whereas they weakened after accounting for BF%. These observations indicate that SBP and DBP are associated with cardiac autonomic regulation. The negative association between SBP and HRV has been reported previously in children aged 10-13 years[18,19], yet, our results show that the relationship seems

to exist already in younger children aged 6-8 years. Moreover, we found that SBP was negatively associated with HF and LF and that these associations remained after controlling for CRF but weakened after taking BF% into account. These observations suggest that BF% plays a bigger role in the association between SBP and cardiac autonomic modulation than CRF among children.

Strengths and limitations

The strengths of the present study include a relatively large population sample of children, the comprehensive and valid assessments of cardiometabolic risk factors and HRV variables, the use of a continuous CRS instead of arbitrary cut-offs for single risk factors, and the ability to control for important confounding factors in the statistical analyses. These characteristics of the study provided us sufficient statistical power to investigate the independent associations of cardiometabolic risk factors with HRV variables in children. However, few limitations should be considered when interpreting the present findings. Firstly, the cross-sectional study design limits the conclusion about causality between the observed associations. Furthermore, a large number of analyses may increase the risk of type I errors, and thus some of the observed associations might have been found by change. Finally, although we measured CRF, the possible confounding effects of physical activity was not addressed, and therefore, future studies are encouraged to investigate the role of physical activity.

In conclusion, higher overall cardiometabolic risk, fasting insulin, and blood pressure were associated with smaller HRV, mainly indicating lower parasympathetic activity, in children 6-8 years of age. Most of these associations were independent of CRF, whereas BF% partly explained them. The results of our study suggest that adiposity and other cardiometabolic risk factors, including poor CRF have multifaceted relationships with cardiac autonomic modulation in children, and further, the associations are similar in boys and girls. Furthermore, our results indicate that metabolic syndrome does not only lead to metabolic disturbances but also to reduction in cardiac autonomic modulation, which may in turn have a role in the development of cardiovascular diseases in later life. Such knowledge is essential in improving the clinical management of metabolic syndrome and cardiovascular diseases already in young children. However, the sample in the current study included mainly normal weight children, and therefore, the associations should be studied in populations with a higher prevalence of overweight and obesity in order to increase knowledge of the clinical significance. In addition, there is a need to further study the role of change in cardiometabolic risk factors to HRV during mid-childhood.

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