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

3
4 **Running head:** Metabolic profile and autonomic nervous system

5
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23

24 **ABSTRACT**

25

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

29

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

39

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

46

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

50

51

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

54 **INTRODUCTION**

55

56 Cardiovascular diseases are the main cause of premature mortality worldwide¹. The main
57 pathophysiological mechanism for these diseases is atherosclerosis that starts to develop during the
58 early years of life^{2,3}. Metabolic syndrome refers to a cluster of traditional cardiometabolic risk factors,
59 such as central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, low plasma high-
60 density lipoprotein (HDL) cholesterol and hypertension⁴. The definition of childhood metabolic
61 syndrome is problematic due to its multiple definitions. Nevertheless, a large systematic review⁵ has
62 been proposed that the prevalence increases in accordance with weight status and the reported rates
63 has been reported to vary from 0-1% in normal weight children, to 12% in overweight children, and
64 29% in obese children⁵. Thus, one of the main risk factors for metabolic syndrome is childhood
65 obesity⁶, which is a growing public health problem worldwide⁷. Moreover, children with overweight
66 are more likely to become overweight adults indicating an increased lifelong risk for cardiometabolic
67 diseases⁸. However, there is limited knowledge on the associations of metabolic syndrome and its
68 components with cardiac autonomic modulation.

69

70 Heart rate variability (HRV) is a non-invasive measure of cardiac autonomic nervous system
71 regulation⁹, and it is influenced by parasympathetic and sympathetic activity¹⁰. Reduced HRV is a
72 risk factor for serious health problems, such as coronary heart disease, hypertension, and overall
73 mortality¹¹. Recently, it has been suggested that increased HRV reduces cardiovascular risk beyond
74 traditional risk factors in children¹², adolescents^{12,13}, and adults¹⁴. On the other hand, children with
75 overweight have been reported to have decreased HRV^{15,16}, which may be due to the delaying effect
76 of overweight on the natural increase in parasympathetic activity with growth¹⁶. Yet, there is a lack
77 of studies in early childhood, although such knowledge would help screening the children who may
78 need support the most.

79

80 In addition to overweight, there are other cardiometabolic risk factors that have been related to
81 decreased HRV in children aged about 10 years¹⁷. For example, elevated blood pressure^{18,19} and
82 increased fasting plasma insulin^{20,21} have been associated with reduced HRV. Furthermore, the use
83 of a cardiometabolic risk score (CRS) as an indicator of clustered cardiometabolic risk instead of a
84 dichotomous variable for metabolic syndrome is preferred in children^{6,22,23}. To the best of our
85 knowledge, there are only two previous cross-sectional studies on the association between CRS and
86 HRV in general populations of children or adolescents^{24,25}. A higher CRS was associated with a
87 smaller HRV in children 5-6 years of age²⁴ and in adolescents aged 17 years²⁵. However, there have

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

91
92 Since cardiometabolic risk factors have been found to track from childhood to adolescence²⁶ and
93 adulthood^{27,28}, understanding the impact of CRS on cardiac autonomic nervous system regulation
94 could help improving the clinical management of metabolic syndrome and cardiovascular diseases
95 already in young people. The aim of the present study was to investigate the associations of CRS and
96 its components with various HRV variables in a population sample of Finnish children 6–8 years of
97 age. We hypothesized that higher CRS and its components would be associated with smaller HRV in
98 a general population of children.

99

100

101 **METHODS**

102

103 **Study design and participants**

104 The present study utilizes baseline data from the Physical Activity and Nutrition in Children (PANIC)
105 study (clinicaltrials.gov NCT01803776) that is an 8-year controlled trial on the effects of a combined
106 physical activity and dietary intervention on cardiometabolic risk factors and associated outcomes in
107 a population sample of children aged 6-8 years at baseline from the city of Kuopio, Finland²⁹. The
108 Research Ethics Committee of the Hospital District of Northern Savo approved the study protocol in
109 2006 (Statement 69/2006). The parents or caregivers of the children gave their written informed
110 consent, and the children provided their assent to participation.

111

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

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

123

124 **Assessment of adiposity**

125 All children were asked to empty their bladder, and thereafter, body weight was measured twice using
126 a calibrated InBody[®] 720 bioelectrical impedance device (Biospace, Seoul, South Korea) to accuracy
127 of 0.1 kg. The mean of the two measurements was used for the analyses. Height was measured three
128 times using a wall-mounted stadiometer without shoes to the nearest of 0.1 cm, and the mean of the
129 closest two values was used for the analyses. Body mass index (BMI) was calculated by dividing
130 body weight (kg) by body height (m) squared, and BMI-SDS was obtained using Finnish references³⁰.
131 The prevalence of normal weight, overweight, and obesity were defined using the cut-off values
132 provided by Cole and coworkers³¹. Waist circumference (WC) was measured three times at mid-
133 distance between the bottom of the rib cage and the top of the iliac crest after expiration, and the mean
134 of the closest two values was used in the analyses. Body fat percentage (BF%) and lean body mass
135 were measured using the Lunar[®] dual-energy X-ray absorptiometry device (GE Medical Systems,
136 Madison, WI, USA), as described earlier³².

137

138 **Assessment of other cardiometabolic risk factors**

139 A research nurse took blood samples in the morning after a 12-hour overnight fast. Plasma glucose
140 was measured by a hexokinase method, serum insulin by an electrochemiluminescence immunoassay,
141 plasma triglycerides by a colorimetric enzymatic assay, and plasma HDL cholesterol by a
142 homogeneous colorimetric enzymatic assay³³. A research nurse measured systolic blood pressure
143 (SBP) and diastolic blood pressure (DBP) from the right arm using the Heine Gamma[®] G7 aneroid
144 sphygmomanometer (Heine Optotechnik, Herrsching, Germany) to accuracy of 2 mmHg. The
145 measurement protocol included a 5-minute seated resting period followed by three measurements
146 with a 2-minute interval in between. The average SBP and DBP of all three values was used in the
147 analysis. Age-, sex-, and height-standardized z-scores were calculated for WC, insulin, glucose,
148 triglycerides, HDL cholesterol, and the mean of SBP and DBP. Thereafter, CRS was calculated using
149 a formula $WC + \text{insulin} + \text{glucose} + \text{triglycerides} - \text{HDL cholesterol} + \text{the mean of SBP and DBP}$, a
150 larger score indicating a higher cardiometabolic risk³³.

151

152 **Assessment of heart rate variability**

153 A physician performed a standard clinical examination³² for each child before the
154 electrocardiographic (ECG) registration. Before the ECG registration, children were told to lay down

155 still for 10 minutes in order to stabilize their heart rate. Thereafter, the ECG was registered for 5
156 minutes. For the HRV analyses, 5-minute samples were selected from the ECG. The ECG signals
157 were measured using the Cardiosoft® V6.5 Diagnostic System (GE Healthcare Medical Systems,
158 Freiburg, Germany) at a frequency of 500 Hz. The ECG electrodes were placed according to the
159 conventional 12-lead system, and a chest lead of good quality presenting a high R-wave amplitude
160 was chosen for the HRV analysis. The ECG data were analyzed using the Kubios® HRV software
161 (Kubios Co., Kuopio, Finland), and the details of the techniques and analysis methods employed to
162 assess HRV have been described elsewhere³⁴. Briefly, the R-wave peaks were first detected using an
163 adaptive QRS detection algorithm, and the RR interval time series (time intervals between successive
164 R waves as a function of R-wave time instants) were formed. Prior to the analyses, the data were
165 checked for potential ectopic or aberrant beats and, if necessary, such erroneous beats were corrected
166 using interpolation methods. The HRV variables used in the analyses were SDNN, the standard
167 deviation of all RR intervals (ms), a marker of overall HRV and RMSSD, the square root of the mean
168 of the sum of the squares of differences between adjacent RR intervals (ms), a marker of
169 parasympathetic activity as well as Mean RR, the mean of RR intervals¹⁰. These HRV variables were
170 used to measure HRV in a time domain with a lower value indicating a lowered parasympathetic
171 modulation. In addition, we calculated high frequency power (HF: 0.15 – 0.40 Hz), which represents
172 parasympathetic modulation; low frequency power (LF: 0.04 – 0.15 Hz), which represents a mixture
173 of sympathetic and parasympathetic modulation; and LF/HF, which estimates the ratio between
174 sympathetic and parasympathetic nervous system activity¹¹.

175

176 **Covariates**

177 Sex was reported by the parents. Years from peak height velocity was used as an indicator of maturity
178 in children³⁵, and it was calculated separately for boys and girls using formula provided by Moore et
179 al.³⁶. Cardiorespiratory fitness (CRF) was assessed by a maximal exercise test using an
180 electromagnetically braked Ergoselect 200K® bicycle ergometer with a pediatric saddle module
181 (Ergoline, Bitz, Germany). Maximal power output (watt) achieved at the end of the exercise test per
182 lean body mass (kg) was used as the measure of CRF³⁷.

183

184 **Statistical methods**

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

189 transformed due to skewed distributions. The associations of CRS and individual cardiometabolic
190 risk factors (BF%, WC, insulin, glucose, triglycerides, HDL cholesterol, SBP, and DBP) with HRV
191 variables were investigated using linear regression analyses, and we applied four different models.
192 Firstly, data on the associations of CRS with HRV variables were analyzed without adjustment, since
193 CRS was calculated using age-, sex-, and height-standardized z-scores for cardiometabolic risk
194 factors. Secondly, data on the associations of individual cardiometabolic risk factors with HRV
195 variables were adjusted for sex and peak height velocity. Thirdly, we also included BF% together
196 with sex and peak height velocity in additional linear regression models, since increased BF% has
197 been associated with reduced HRV¹⁵ and it is a key component of clustered cardiometabolic risk.
198 However, due to the multicollinearity, we excluded BF% from the model regarding WC. Finally, we
199 included CRF % together with sex and peak height velocity in further linear regression models,
200 because increased CRF has been associated with increased HRV¹² and decreased cardiometabolic
201 risk factors^{38,39}. We also studied whether the associations of CRS and individual cardiometabolic risk
202 factors with HRV variables were different between boys and girls by adding an interaction term for
203 CRS and individual cardiometabolic risk factors in the linear regression models. There was no
204 evidence for the modifying effect of sex on these associations, and the results are thus presented for
205 boys and girls together.

206

207

208 **RESULTS**

209

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

213

214 **Associations of CRS with HRV variables**

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

218

219 **Associations of BF% and WC with HRV variables**

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

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

225

226 **Associations of insulin and glucose with HRV variables**

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

233

234 **Associations of triglycerides and HDL cholesterol with HRV variables**

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

237

238 **Associations of SBP and DBP with HRV variables**

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

247

248

249 **DISCUSSION**

250

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

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

258

259 A higher CRS was associated with lower parasympathetic activity, as indicated by lower RMSSD
260 and HF and higher LF/HF. This finding is at least partly due to the strong association between insulin
261 and decreased HRV, since insulin resistance has been recognized as one of the key components in
262 the development of metabolic syndrome⁴⁰. To the best of our knowledge, there are only a few previous
263 studies on the associations of CRS with HRV variables in children^{17,24}. Vrijkotte et al.²⁴ found that a
264 higher CRS as well as higher waist-to-height ratio and SBP of its components were associated with
265 lower parasympathetic activity indicating smaller HRV, in children aged 5-6 years, which is in line
266 with the present study. We also found a positive association between CRS and the balance between
267 sympathetic and parasympathetic activity, which was not reported in the study by Vrijkotte et al.²⁴.
268 However, direct comparison between the studies is not possible due to the differences in
269 methodology, as in their study, parasympathetic activity was measured by respiratory sinus
270 arrhythmia and sympathetic activity by pre-ejection period²⁴. Nevertheless, these findings together
271 suggest that increased CRS is linked to lower parasympathetic activity. Zhou et al.¹⁷ found inverse
272 dose-response relationships of clustered cardiometabolic risk factors with SDNN, RMSSD, LF, and
273 HF in children aged 9–11 years. However, children in their study had elevated levels of
274 cardiometabolic risk factors, and thus, comparison of these results with our observations based on a
275 general population of children needs to be done with caution. When discussing the relationships
276 between CRS and HRV based on studies in different age groups, it is notable that HRV is likely to
277 change during childhood¹⁶ highlighting the need to study the associations in children with varying
278 ages. Finally, we weighted each cardiometabolic risk factor similarly in calculating CRS, and it is
279 therefore difficult to compare their true contribution to the association between CRS. In future studies,
280 it should be further examined whether some of the components of CRS play a bigger role in
281 autonomic nervous system regulation than others.

282

283 We found that BF% was negatively associated with RMSSD and HF power, both of which are
284 measures of parasympathetic activity, and positively associated with LF/HF, which reflects the
285 balance between sympathetic and parasympathetic nervous system activity. One explanation for these
286 observations may be that overweight makes cardiac ventricles larger and their walls thicker and
287 thereby worsens ventricular relaxation during diastole that impairs the balance of cardiac autonomic
288 modulation¹⁵. There is evidence that overweight is associated with impaired cardiac autonomic
289 balance in children¹⁵, but more evidence is needed in young children. Furthermore, BMI has been

290 used in defining childhood obesity in young children⁴¹, although it is not an optimal measure of
291 pediatric obesity⁴². Therefore, the observed associations of increased BF%, assessed by whole-body
292 DXA, with HRV in our study expands the knowledge on the associations of adiposity with HRV
293 variables in children. Moreover, we found that CRF partly explained the associations of BF% with
294 HRV variables, suggesting that higher CRF might be associated with larger HRV independent of
295 body fat mass among pre-pubertal children. This highlights the beneficial associations of higher CRF
296 with larger HRV already in childhood.

297

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

310

311 We found no association of triglycerides or HDL cholesterol with HRV. To the best of our knowledge,
312 there are no previous studies on these associations in young children. However, Rodríguez-Colón and
313 coworkers²⁵ found that triglycerides was negatively and HDL cholesterol positively associated with
314 HRV in adolescents. Such associations have also been observed in young adults⁴³. In line with the
315 results of previous studies in adults⁴⁴, our findings suggest that triglycerides and HDL cholesterol do
316 not play a major role in cardiac autonomic nervous system regulation in children. However, further
317 studies are needed to investigate the associations of plasma lipids with HRV in all age groups.

318

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

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

328

329 **Strengths and limitations**

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

341

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

356

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358

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370

371

REFERENCES

- 372 1. World Health Organization. Global status report on noncommunicable diseases 2014. Geneva:
373 World Health Organization; 2015.
- 374 2. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association
375 between multiple cardiovascular risk factors and atherosclerosis in children and young adults. *N Engl*
376 *J Med* 1998;338(23):1650-1656.
- 377 3. McGill HC Jr, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of
378 atherosclerosis in childhood and adolescence. *Am J Clin Nutr* 2000;72(5 Suppl):1307S.
- 379 4. National Cholesterol Education Program, (NCEP). Expert panel on detection, evaluation, and
380 treatment of high blood cholesterol in adults (adult treatment panel III): Third report of the national
381 cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high
382 blood cholesterol in adults (adult treatment panel III) final report. *Circulation* 2002;106(25).
- 383 5. Friend A, Craig L, Turner S. The prevalence of metabolic syndrome in children: A systematic
384 review of the literature. *Metab Syndr Relat Ds* 2013;11(2):71-80.
- 385 6. Eisenmann JC, Welk GJ, Wickel EE, Blair SN. Combined influence of cardiorespiratory fitness
386 and body mass index on cardiovascular disease risk factors among 8-18 year old youth: The Aerobics
387 Center Longitudinal Study. *Int J Pediatr Obes* 2007;2(2):66-72.

- 388 7. World Health Organization. Obesity 2018. <http://www.who.int/topics/obesity/en/>. Accessed
389 November 15, 2018.
- 390 8. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, Srinivasan SR, Daniels
391 SR, Davis PH, Chen W, Sun C, Cheung M, Viikari JS, Dwyer T, Raitakari OT. Childhood adiposity,
392 adult adiposity, and cardiovascular risk factors. *N Engl J Med* 2011;365(20):1876-1885.
- 393 9. Kleiger RE, Stein PK, Bigger JT. Heart rate variability: Measurement and clinical utility. *Ann*
394 *Noninvasive Electrocardiol* 2005;10(1):88-101.
- 395 10. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the
396 frequency domain. *Circulation* 1991;84(2):482-492.
- 397 11. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public*
398 *Health* 2017;5:258.
- 399 12. Oliveira RS, Barker AR, Wilkinson KM, Abbott RA, Williams CA. Is cardiac autonomic function
400 associated with cardiorespiratory fitness and physical activity in children and adolescents? A
401 systematic review of cross-sectional studies. *Int J Cardiol* 2017;236:113-122.
- 402 13. Oliveira RS, Barker AR, Williams CA. Cardiac autonomic function, cardiovascular risk and
403 physical activity in adolescents. *Int J Sports Med* 2018;39(2):89-96.
- 404 14. Joyner MJ, Green DJ. Exercise protects the cardiovascular system: Effects beyond traditional risk
405 factors. *J Physiol* 2009;587(23):5551-5558.
- 406 15. Liao D, Rodríguez-Colón S, He F, Bixler E. Childhood obesity and autonomic dysfunction: Risk
407 for cardiac morbidity and mortality. *Curr Treat Options Cardio Med* 2014;16(10):1-13.
- 408 16. Eyre ELJ, Duncan MJ, Birch SL, Fisher JP. The influence of age and weight status on cardiac
409 autonomic control in healthy children: A review. *Auton Neurosci* 2014;186:8-21.
- 410 17. Zhou Y, Xie G, Wang J, Yang S. Cardiovascular risk factors significantly correlate with
411 autonomic nervous system activity in children. *Can J Cardiol* 2012;28(4):477-482.
- 412 18. Xie G, Wang J, Zhou Y, Xu H, Sun J, Yang S. Association of high blood pressure with heart rate
413 variability in children. *Iran J Pediatr* 2013;23(1):37.
- 414 19. Fitzgibbon LK, Coverdale NS, Phillips AA, Shoemaker JK, Klentrou P, Wade TJ, Cairney
415 J, O'Leary DD. The association between baroreflex sensitivity and blood pressure in children. *Appl*
416 *Physiol Nutr Metab* 2012;37(2):301-307.
- 417 20. Kaufman CL, Kaiser DR, Steinberger J, Kelly AS, Dengel DR. Relationships of cardiac
418 autonomic function with metabolic abnormalities in childhood obesity. *Obesity* 2007;15(5):1164-
419 1171.
- 420 21. Taşçılar ME, Yokuşoğlu M, Boyraz M, Baysan O, Köz C, Dündaröz R. Cardiac autonomic
421 functions in obese children. *J Clin Res Pediatr Endocrinol* 2011;3(2):60-64.

- 422 22. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk
423 clustering related to syndrome X from childhood to young adulthood: The Bogalusa Heart Study.
424 *Arch Int Med* 1994;154(16):1842-1847.
- 425 23. Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research.
426 *Cardiovasc Diabetol* 2008;7(1):17.
- 427 24. Vrijkotte TG, van den Born BJ, Hoekstra CM, Gademan MG, van Eijsden M, de Rooij
428 SR, Twickler MT. Cardiac autonomic nervous system activation and metabolic profile in young
429 children: The ABCD study. *PLoS One* 2015;10(9):e0138302.
- 430 25. Rodríguez-Colón SM, He F, Bixler EO, Fernandez-Mendoza J, Vgontzas AN, Calhoun S, Zheng
431 ZJ, Liao D. Metabolic syndrome burden in apparently healthy adolescents is adversely associated
432 with cardiac autonomic modulation—Penn state children cohort. *Metabolism* 2015;64(5):626-632.
- 433 26. Bugge A, El-Naaman B, McMurray RG, Froberg K, Andersen LB. Tracking of clustered
434 cardiovascular disease risk factors from childhood to adolescence. *Pediatr Res* 2013;73(2):245-249.
- 435 27. Camhi SM, Katzmarzyk PT. Tracking of cardiometabolic risk factor clustering from childhood
436 to adulthood. *Int J Pediatr Obes* 2010;5(2):122-129.
- 437 28. Juhola J, Magnussen CG, Viikari JS, Kähönen M, Hutri-Kähönen N, Jula A, Lehtimäki
438 T, Åkerblom HK, Pietikäinen M, Laitinen T, Jokinen E, Taittonen L, Raitakari OT, Juonala M.
439 Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood:
440 The cardiovascular risk in young Finns study. *J Pediatr* 2011;159(4):584-590.
- 441 29. Viitasalo A, Eloranta A, Lintu N, Väistö J, Venäläinen T, Kiiskinen S, Karjalainen P, Peltola
442 J, Lampinen EK, Haapala EA, Paananen J, Schwab U, Lindi V, Lakka TA. The effects of a 2-year
443 individualized and family-based lifestyle intervention on physical activity, sedentary behavior and
444 diet in children. *Prev Med* 2016;87:81-88.
- 445 30. Saari A, Sankilampi U, Hannila M, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth
446 references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-
447 length/height, and body mass index-for-age. *Ann Med* 2011;43(3):235-248.
- 448 31. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness,
449 overweight and obesity. *Pediatr Obes* 2012;7(4):284-294.
- 450 32. Tompuri TT, Lakka TA, Hakulinen M, Lindi V, Laaksonen DE, Kilpeläinen TO, Jääskeläinen
451 J, Lakka HM, Laitinen T. Assessment of body composition by dual-energy x-ray absorptiometry,
452 bioimpedance analysis and anthropometrics in children: The physical activity and nutrition in
453 children study. *Clin Physiol Func Imaging* 2015;35(1):21-33.
- 454 33. Viitasalo A, Lakka T, Laaksonen D, Savonen K, Lakka HM, Hassinen M, Komulainen
455 P, Tompuri T, Kurl S, Laukkanen JA, Rauramaa R. Validation of metabolic syndrome score by

456 confirmatory factor analysis in children and adults and prediction of cardiometabolic outcomes in
457 adults. *Diabetologia* 2014;57(5):940-949.

458 34. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-aho PO, Karjalainen PA. Kubios HRV – heart
459 rate variability analysis software. *Comput Methods Programs Biomed* 2014;113(1):210-220.

460 35. Malina RM, Rogol AD, Cumming SP, Coelho e Silva, Manuel J, Figueiredo AJ. Biological
461 maturation of youth athletes: Assessment and implications. *Br J Sports Med* 2015;49(13):852-859.

462 36. Moore S, McKay H, MacDonald H, Nettlefold L, Baxter-Jones AD, Cameron N, Brasher PM.
463 Enhancing a somatic maturity prediction model. *Med Sci Sports Exerc* 2015;47(8):1755-1764.

464 37. Tompuri T, Lintu N, Savonen K, Laitinen T, Laaksonen D, Jääskeläinen J, Lakka TA. Measures
465 of cardiorespiratory fitness in relation to measures of body size and composition among children.
466 *Clin Physiol Funct Imaging* 2015;35(6):469-477.

467 38. Ekelund U, Anderssen S, Froberg K, Sardinha L, Andersen L, Brage S. Independent associations
468 of physical activity and cardiorespiratory fitness with metabolic risk factors in children: The european
469 youth heart study. *Diabetologia* 2007;50(9):1832-1840.

470 39. Agbaje AO, Haapala EA, Lintu N, Viitasalo A, Barker AR, Takken T, Tompuri T, Lindi V, Lakka
471 TA. Peak oxygen uptake cut-points to identify children at increased cardiometabolic risk - the PANIC
472 study. *Scand J Med Sci Sports* 2019;29(1):16-24.

473 40. Wittcopp C, Conroy R. Metabolic syndrome in children and adolescents. *Pediatr Rev* 2016;37
474 (5):193-202.

475 41. Birch SL, Duncan MJ, Franklin C. Overweight and reduced heart rate variability in British
476 children: An exploratory study. *Prev Med* 2012;55(5):430-432.

477 42. Forsum E, Flinck Carlsson E, Henriksson H, Henriksson P, Löf M. Total body fat content versus
478 BMI in 4-year-old healthy Swedish children. *J Obes* 2013;2013:206715.

479 43. Soares-Miranda L, Sandercock G, Vale S, Santos R, Abreu S, Moreira C, Mota J. Metabolic
480 syndrome, physical activity and cardiac autonomic function. *Diabetes Metab Res Rev*
481 2012;28(4):363-369.

482 44. Pehlivanidis AN, Athyros VG, Demetriadis DS, Papageorgiou AA, Bouloukos VJ, Kontopoulos
483 AG. Heart rate variability after long-term treatment with atorvastatin in hypercholesterolaemic
484 patients with or without coronary artery disease. *Atherosclerosis* 2001;157(2):463-469.

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