

Assessment of biochemical liver markers, physical activity, fitness and body mass index for a cardiometabolic risk model in childhood

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Abstract

Objective

To investigate the effect of liver cell injury markers in clustered risk assessment model for identification of children at risk of cardiometabolic disease

Design/Setting

This cross-sectional observational study was carried out in primary schools in Liverpool and Ulster, UK. Participants were 10-12 year old healthy schoolchildren who underwent anthropometric measurements, phlebotomy, cardiorespiratory fitness and physical exercise assessments.

Main outcome measures

The main outcome measures included assessment of high and low cardiometabolic risk participants through a clustered risk score model, which incorporated covariates implicated in the pathogenesis of the metabolic syndrome: body mass index, waist circumference, blood pressure, proinflammatory cytokines, markers of systemic inflammation, liver cell injury markers, lipid profile, cardiorespiratory fitness and time spent in activity.

Results

Children classified as fit or active have lower cardiometabolic risk than their 'unfit' or 'inactive' peers. This fact remained unchanged whether markers of hepatocyte injury were included in the clustered risk assessment model or not.

Conclusions

The clustered risk score model is a non-invasive and scientifically robust method of cardiometabolic risk assessment in childhood, which reiterates the importance of weight reduction and promotion of cardiorespiratory fitness from childhood. Our study did not show any significant contribution of liver cell injury markers, however larger scale research is

needed so as to fully evaluate the effect of these widely used markers in early cardiometabolic risk stratification in children.

Introduction

According to the recent new worldwide definition by Eckel et al (1), metabolic syndrome (MS) is characterized by accumulation of visceral fat and is associated with the clustering of metabolic and pathophysiological cardiovascular risk factors such as impaired glucose tolerance, dyslipidaemia, and hypertension. MS is a condition with increased morbidity which may have its origin as early as childhood (2, 3).

A growing body of evidence suggests that increased oxidative stress to adipocytes is central to the pathogenesis of cardiovascular disease in MS (4). Increased oxidative stress to adipocytes causes dysregulated expression of inflammation-related adipocytokines; this contributes to obesity-associated vasculopathy and cardiovascular risk, primarily through endothelial dysfunction.

Non-alcoholic liver fatty disease (NAFLD) is a recognised severe hepatic manifestation of MS with increasing prevalence in obese children (5). Higher BMI, significantly and independently of other risk factors, increases the chances of having liver inflammation and fibrosis in children, (6) which may be present but asymptomatic from as early as childhood (7).

Cardiorespiratory fitness (CRF) is a functional measure of the cardiorespiratory system and an independent predictor of cardio-metabolic morbidity in children (8-11). CRF has however received little attention from policy makers in comparison to obesity (12). Levels of cardiorespiratory fitness in children have declined in recent years, independent of changes in body size and/or excessive adiposity (13). Numerous papers have described links between measures of CRF, habitual physical activity (PA) and metabolic risk in children and adolescents (14). Studies have shown that overweight fit children are at lesser risk for MS than overweight unfit children (15).

A number of studies have suggested the potential value of novel serum biomarkers, such as high sensitivity C-reactive protein (hs CRP) (16), adiponectin (17) and homeostasis model of

insulin resistance (HOMA-IR) (18, 19), to detect early stages of MS. Conventional markers of liver cell injury such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) have however not been included in validated assessment models of cardiometabolic risk (20), despite the recognised association between MS and liver pathology (NAFLD).

Aim

The aim of this study was to investigate the clustered cardiometabolic risk scores in healthy 10-12 year olds by using anthropometric characteristics, reference standard measurements of CRF and PA, and laboratory blood markers of metabolic disease. In addition, we aimed to evaluate how inclusion of markers of liver cell injury may affect the clustered cardiometabolic risk assessment model.

Methods

Data were generated by the REACH Year 6 study, which was a collaborative observational study conducted in Liverpool and Ulster UK. The methods for the REACH Year 6 study have been described previously (21). After gaining local NHS and institutional ethical approvals, parental consent and participant assent, participants took part in one laboratory and one school based testing session and wore accelerometers to monitor habitual PA.

Laboratory measures: Stature and sitting stature to the nearest 0.1cm (Seca Ltd. Birmingham, UK), body mass to the nearest 0.1kg (Seca Ltd. Birmingham, UK) and waist circumference were assessed using standard techniques (22). Body mass index, BMI Z-scores(23) and somatic maturation were calculated (years to peak height velocity: YPHV). Blood pressure (BP) was assessed after a 15 minute rest period. After BP measurements, nitric oxide-mediated endothelial function was measured by flow mediated dilation (FMD) using high resolution ultrasonography after a 5-minute ischemic stimulus (Terason, t3000; Aloka, London, UK) (24, 25). FMD % was retained for analysis.

CRF (VO_{2peak}) was assessed using an individually calibrated treadmill (H P Cosmos, Traunstein, Germany) protocol. All participants wore a heart rate monitor (Polar, Kempele, Finland) and an accelerometer (Actigraph, GT1M, Actigraph LLC, Pensacola, FL, USA) throughout the test. To account for differences in biological age and limb length between participants, treadmill speeds were calculated using set Froude (Fr) numbers (21, 25). Participants completed 2 minute stages, with stage 1 speed set at Fr 0.25 and stage 2 at Fr 0.5, each additional speed was then calculated by the difference in speed between the Fr 0.25 and Fr 0.50, ~2 km/hour increases in speed every two minutes. VO_{2peak} was defined as the highest 15 second averaged oxygen uptake, measured by breath by breath gas analysis (Liverpool site: Jaeger Oxycon Pro, Viasys Health Care, Warwick, UK, Ulster site: COSMED, Quark, Italy) when the subjective endpoints were met (respiratory exchange ratio >1.05, and/or heart rate >199 beats/min). Participants were classified as fit or unfit using published thresholds (26).

School-based blood sampling:

After verbal confirmation of overnight fast venous blood samples were drawn between 8.30-10.30 am by experienced phlebotomists. Samples were transported to Alder Hey Children's NHS Foundation Trust or Ulster Hospital for analysis. Total cholesterol, high density lipoprotein cholesterol (HDL-c), triglycerides, glucose, hs CRP, adiponectin, ALT, AST and GGT were retained in this study for analysis.

PA monitoring:

Habitual PA was assessed using a uniaxial accelerometer, which is a valid and reliable method of assessing PA in children (9). Monitors were distributed at school and worn on the right hip for seven days using a five second data collection epoch. Sustained bouts of ≥ 20 minutes of zero counts were subtracted from wear time (27). Minimum wear time was ≥ 9 hours for ≥ 3 days (28). Data were analysed using individually calibrated thresholds. The thresholds were generated from the VO_{2peak} protocol and this approach has been described

previously (29). A sedentary threshold was set at 100 counts per minute (30), and time spent between Fr 0.25 to Fr 0.5 (moderate intensity PA), and \geq Fr 0.5 (vigorous intensity PA) was established. Mean sedentary time and time spent at \geq Fr 0.25 (moderate to vigorous intensity PA: MVPA) were retained for analysis. Accelerometer wear time was retained as a covariate within analysis. Participants were classified as active (MVPA \geq 60 minutes, inactive (MVPA < 60 minutes).

Clustered cardiometabolic risk scores:

Clustered risk scores account for the constellation of factors associated with cardiometabolic disease, are less sensitive to daily fluctuations in individual markers and are widely used in empirical research (13, 21). To calculate clustered risk scores risk markers were standardised by sex and then summed. Data for boys and girls were then re-combined and analysed together to maximise statistical power. Prior to standardisation the following variables were not normally distributed and were log transformed: waist circumference, systolic and diastolic BP (girls only), HDL-c (girls only), glucose, CRP, ALT (girls only), AST (girls only).

Two clustered cardiometabolic risk scores were calculated. Clustered risk score 1 included: waist circumference, systolic BP, diastolic BP, FMD % (inverted), triglycerides, HDL-c (inverted), glucose, CRP and adiponectin (inverted). Clustered risk score 2 included the same variables with the addition of ALT, AST and GGT. Participants with clustered risk score \geq 1SD above the grand mean were classified as at risk.

Statistical Analysis:

One way ANOVA was completed to assess differences in markers between girls and boys. For the main analysis MANCOVAs were completed to assess differences in BMI Z-score,

VO_{2peak}, MVPA and sedentary time by clustered risk group, this was completed in two models, one for clustered risk score 1 and one for clustered risk score 2, controlling for sex, maturation and accelerometer wear time. ANCOVA was completed to assess differences in clustered risk scores by fitness status (controlling for sex and maturation) and activity status (controlling for sex, maturation and accelerometer wear time). Partial correlations were conducted to assess the relationship between clustered risk score, BMI Z-score, VO_{2peak}, MVPA and sedentary time controlling for sex, maturation and accelerometer wear time.

Results

Table 1 shows the descriptive characteristics of the study participants by sex. One way ANOVA revealed that VO_{2peak} and MVPA were significantly greater in boys than girls ($p < 0.05$). CRP and triglycerides were significantly higher in girls than boys. Non significant differences between sexes were observed for BMI Z-score, waist circumference, cholesterol, AST, ALT, adiponectin, sedentary time and clustered risk scores. The prevalence of obesity and overweight according to Cole et al (31) is demonstrated in Table 2.

Main analysis

Tables 3&4 show the results of MANCOVA between low and high risk participants, controlling for YPHV, sex and accelerometer wear time. In both clustered risk models similar differences were observed; BMI Z score and VO_{2peak} were significantly different between the risk subgroups. MVPA and sedentary time did not differ between the high and low risk groups.

Table 5 displays the results of the ANCOVA. Fit participants had significantly lower clustered risk scores than their unfit counterparts ($p < 0.01$). Similarly, active children displayed significantly lower clustered cardiometabolic risk scores than the inactive group.

Both clustered risk scores demonstrated positive, moderate correlations with BMI Z score ($r=+0.622$ and $r= +0.522$ respectively). The negative correlation between clustered risk scores 1&2 and VO_{2peak} was weaker ($r=-0.241$, $r=-.293$) (Tables 6&7).

Sedentary time was negatively correlated with VO_{2peak} ($r=-0.45$, $p<0.001$). On the contrary MVPA was positively correlated ($r=+0.442$, $p<0.001$) with VO_{2peak} (Table 6). Correlations were weaker when liver function tests were included in the clustered risk assessment model (Table 7).

Discussion

With regards to baseline differences between the two sexes, MVPA and VO_{2peak} were significantly higher in boys ($p<0.05$), which is in line with results from recent longitudinal studies (32) (33). Laboratory parameters such as triglycerides and CRP were significantly higher in girls ($p<0.05$). This finding may be characteristic of our sample population; notably the prevalence of overweight/obese females in our study population was higher than in males. Despite above differences, clustered risk scores were comparable between the two sexes.

Statistically significant differences for both types of clustered risk score were noted between the active and inactive subgroups, and the fit and unfit groups. This reiterates that both fitness and physical activity are important in cardiometabolic risk assessment, and that more active or fit children are at significantly lower risk of cardiometabolic morbidity. Interestingly MVPA and sedentary times were not significantly different between high and low risk subgroups, when analyses were controlled for sex, maturation and accelerometer wear time. These results are in keeping with findings from recent studies by Ried-Larsen et al (34) and Lopez Martinez et al (35). These studies additionally advocate that high volume or more time spent in vigorous physical activity may be independently associated with lower metabolic risk, and may compensate for time spent in sedentary, low or moderate intensity activity. The inclusion of markers of liver cell injury tests, namely AST, ALT and GGT as

additional components in the clustered risk model did not significantly alter the findings in our risk assessment model. Therefore AST, ALT and GGT values were not indicative of increased risk in our study population and did not significantly add to the predictive value of our clustered risk assessment model. This finding can be interpreted in more than one ways. Firstly, our sample size may not be large enough to allow us to detect possible effect of liver function tests on the clustered risk assessment model. Another possible explanation is that cardiometabolic risk may be independent of liver cell injury markers in mid childhood.

It is important to emphasise that the clustered risk values estimated in both models were consistently and more strongly correlated with BMI Z-score than any other parameter investigated. This is not proof of causality, but reiterates the fact that BMI Z-scores play a definitive role in mid childhood risk assessment of cardiometabolic disease, therefore childhood weight reduction interventions are of high importance.

CRF was negatively correlated with risk and fit children were at reduced metabolic risk. This finding emphasises the independent role of this parameter in risk assessment process and the attention that should be drawn to fitness promotion from childhood.

PA activity promotion is essential, as the modifiable component of CRF is the product of recent MVPA, whereas adiposity may be significantly reduced by MVPA through energy expenditure. A recent systematic review has also highlighted the beneficial effect of direct delivery of physical activity on fitness and cardiometabolic markers in children and adolescents, through school based interventions (36).

This study is limited by a number of factors. Primarily this is a cross-sectional study with a limited sample size. Findings may not be generalizable before larger scale implementation in different populations with genetic and cultural variations, and matched controls.

The major strength of this study is the implementation of a previously validated clustered risk assessment tool which incorporates relevant anthropometric, functional and laboratory parameters. All measurements were performed in a reproducible, scientific manner, with the

use reference standard methods. The novel parameters assessed in this study were conventional markers of liver cell injury which clearly did not appear to significantly add to the cardiometabolic risk stratification of healthy 10-12 year old schoolchildren. To our knowledge this is the first study to address the effect of inclusion of standard markers of liver cell injury in a cardiometabolic clustered risk score model in mid childhood. In alignment to other studies highlighting the importance of physical activity and fitness, further longitudinal cohort studies are required to investigate the impact of physical activity in prevention of the metabolic syndrome and associated co-morbidities.

Conclusion

Children classified as fit and active exhibited lower clustered cardiometabolic risk scores when compared to their unfit or inactive peers; this fact remained unchanged whether markers of liver cell injury were or were not included in the clustered risk assessment model. Emphasis must be given to weight optimisation and cardiorespiratory fitness promotion through physical activity from childhood, to effectively decrease the risk of cardiometabolic disease in life.

Author Contributions:

Contributors: AK, MA and LMB analysed the data and wrote the manuscript. LMB and GS; designed and conceived the REACH Y6 study. MA: expert input on cardiometabolic and liver markers. LMB, LF, RG, LEFG and NH: acquisition and analysis of REACH Y6 data in Liverpool, MHM & CC acquisition and analysis of data in Ulster. All authors approved the article prior to submission.

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What is already known on this topic?

Cardiometabolic disease begins in childhood, and early detection of those at increased risk would be of significant public health benefit.

Cardiorespiratory fitness and body mass index are independent predictors of cardiometabolic morbidity in children.

Clustered risk scores are used in empirical research and provide a robust methodology for pragmatic cardiometabolic risk stratification in childhood.

What this study adds

Children classified as fit or active have lower cardiometabolic risk scores compared to their unfit and inactive peers.

This is the first study to incorporate liver cell injury markers within a cardiometabolic risk score in children, which did not significantly alter observed relationships.

This study provides more evidence regarding the importance of physical activity promotion to improve cardiorespiratory fitness, body size and subsequent cardiometabolic disease risk in children.

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Tables

Variable	Boys		Girls		p
	Mean	Number	Mean	Number	
Waist circumference	62.98 [6.2]	45	65.60 [9.4]	54	0.11
BMI Z-score	0.28 [0.94]	45	0.49 [1.28]	54	0.36
VO _{2peak}	47.34 [9.93]	43	41.19 [8.59]	52	<0.01*
Systolic BP	104.32 [12.25]	44	102.86 [12.21]	51	0.56
Diastolic BP	62.66 [5.66]	44	62.94 [7.59]	51	0.84
FMD%	7.78 [3.36]	39	8.32 [4.06]	45	0.51
CRP	0.40 [0.40]	42	0.88 [1.27]	51	0.02†
AST	25.57 [4.47]	42	24.10 [5.0]	51	0.14
Triglycerides	0.62 [0.20]	42	0.79 [0.30]	51	0.01†
ALT	16.14 [3.35]	42	16.84 [4.96]	51	0.44
Cholesterol	4.07 [0.65]	42	4.22 [0.57]	51	0.25
HDL-c	1.56 [0.29]	42	1.48 [0.39]	51	0.33
Glucose	4.68 [0.32]	42	4.63 [0.30]	51	0.42
Adiponectin	11.12 [5.21]	42	11.22 [6.27]	51	0.94
Sedentary Time	494.07 [78.00]	39	520.05 [59.77]	49	0.08
MVPA	59.70 [29.71]	39	47.24 [21.28]	44	0.03*
Clustered Risk 1	0.05 [3.90]	36	-0.29 [3.85]	44	0.70
Clustered Risk 2	0.02 [4.63]	36	-0.26 [4.08]	44	0.78

Table 1: Descriptive statistics: Untransformed mean [SD] one-way ANOVAs by sex

	Boys (n = 45)		Girls (n= 54)	
BMI	Total number	Prevalence	Total number	Prevalence
Overweight (85 th -95 th centile)	5	11.1%	15	27.8
Obese (>95 th centile)	1	2.2%	5	9.3%

Table 2: Prevalence of obese and overweight children as defined by Cole et al³¹

Clustered risk score 2	High Risk (n =9)	Low Risk (n = 46)	p value
BMI Z-score	1.30 [0.32]	0.25 [0.14]	0.005
VO _{2peak}	35.74 [2.78]	43.09 [1.18]	0.02
MVPA	45.47 [8.71]	51.01 [3.69]	0.566
Sedentary Time	544.67 [15.05]	519.07 [6.39]	0.130

Table 3: MANCOVA (controlling for sex, YPHV, accelerometer wear time) between high and low risk groups: clustered risk score 2 (includes liver tests). Risk is defined as ≥ 1 SD above the grand mean for the relevant clustered risk score.

Clustered risk score 1	High Risk (n =11)	Low Risk (n = 56)	p value
BMI Z-score	1.26 [0.32]	0.33 [0.13]	0.009
VO _{2peak}	36.38 [2.57]	44.31 [1.08]	0.007
MVPA	42.77 [8.16]	54.15 [3.44]	0.21
Sedentary Time	527.23 [13.66]	510.18 [5.76]	0.26

Table 4: MANCOVA (controlling for sex, YPHV, accelerometer wear time) between high and low risk groups: clustered risk score 1 (no liver tests). Risk is defined as ≥ 1 SD above the grand mean for the relevant clustered risk score.

Analysis	Unfit [SEM]	Fit [SEM]	p value	Inactive[SEM]	Active [SEM]	p value
Clustered risk 2 (ANCOVA)	2.81 [0.79] n = 33	-1.10 [0.87]= 28	0.002	1.87 [0.71] N = 43	-1.15 [1.22] N = 15	0.038
Clustered Risk 1 (ANCOVA)	1.33 [0.57] n = 38	-1.59 [0.57] n = 38	P=0.001	0.57 [0.51]	-1.75 [0.80]	0.018

Table 5 ANCOVA: Clustered risk scores 1&2 by fitness and activity status (controlling for sex and YPHV)

	Clustered Risk 1	BMI Z-score	VO _{2peak}	MVPA	Sedentary Time
Clustered Risk 1		.622	-.241	-.113	-.075
(df = 62) P		<.001	.055	.372	.540
BMI Z-score	.622		-.281	-.075	-.052
P	<.001		.024	.558	.683
VO _{2peak}	-.241	-.281		.442	-.452
P	.055	.024		<0.001	<0.001
MVPA	-.113	-.075	.442		-.633
p	.372	.558	<0.001		<0.001

Table 6: Partial correlations (controlling for YPHV, sex and accelerometer wear time)

	Clustered Risk 2	BMI Z-score	VO _{2peak}	MVPA	Sedentary Time
Clustered Risk 2		.522	-.293	-.166	.022
(df = 50) P		<.001	.035	.239	.879
BMI Z-score	.522		-.230	-.007	-.087
P	<.001		.101	.961	.538
VO _{2peak}	-.293	-.230		.320	-.421
P	.035	.101		.021	.002
MVPA	-.166	-.007	.320		-.663
p	.293	.961	.021		<.001

Table 7: Partial correlations (controlling for YPHV, sex and accelerometer wear time)