The relationship between physical activity level and cardiovascular disease biomarkers in healthy, normal weight 3-6 year old children and their parents

Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2016-0023.R1
Manuscript Type:	Brief communication
Date Submitted by the Author:	24-Mar-2016
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Keyword:	physical activity < exercise, body mass index < body composition, Cardiovascular disease biomarkers, Preschool children, Parent-child triad

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The relationship between physical activity level and cardiovascular disease biomarkers in healthy, normal weight 3-6 year old children and their parents

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ABSTRACT

To determine if physical activity is linked to cardiovascular biomarkers in preschool children at risk, we need information on these biomarkers in healthy normal weight children. In this population, multi-level modeling analyses found no correlation between accelerometer recorded physical activity and fasting lipids, adiponectin, or insulin sensitivity. Exploratory analyses found positive correlations between adiponectin and time spend in light physical activity, and between triglyceride and time spent in sedentary behavior, findings to be confirmed in longitudinal prospective studies.

Key words: physical activity, preschool children, cardiovascular disease biomarkers, parent-child triad, body mass index.

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INTRODUCTION

Cardiovascular disease (CVD) is one of the most important causes of morbidity and mortality in modern society, and risk factors for CVD include obesity, insulin resistance, hypertension, and dyslipidemia. Many of these CVD risk factors are present in childhood, especially in school aged obese children and adolescents, and they often persist into adulthood (Jensen et al. 2014). Adiponectin is a CVD biomarker with insulin sensitizing, anti-atherosclerotic, and anti-inflammatory effects and it forms trimeric (LMW), hexameric (MMW), and oligomeric (HMW) complexes in the circulation; HMW adiponectin is the physiologically active isoform with the best predictive value for CVD risk and type 2 diabetes (Araki et al. 2006, Jensen et al. 2014). In overweight and obese older children, serum lipid and total adiponectin levels are modifiable by lifestyle programs (Cambuli et al. 2008). However, little is known about these biomarkers in pre-school age children of normal weight. Further, as physical activity is negatively associated with adiposity (Collings et al. 2013), it is pertinent to consider its relationship with CVD biomarkers in normal weight children of preschool age. Using data collected as part of a longitudinal observational study on physical activity in pre-school aged children and their parents (Cantell et al., 2012), this study investigated the relationships between level of physical activity and serum levels of CVD biomarkers in a group of healthy, normal weight preschool children.

METHODS

Participants

As part of the *Y-Be-Active* study, a community sample of 54 families with preschool aged children were recruited. The sociodemographic characteristics of the sample are

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reported in Supplementary Table S1 (Cantell et al., 2012).

To address the purpose of the current study, the baseline data on CVD biomarkers and anthropometry from parent-child triads was used. Families met the following criteria: 1) a healthy 3 to 6 year old child with no physical/mental disability that would interfere with participation in physical activity; and 2) two-parent family with both parents residing in the home. In families with more than one child participating in *Y-Be-Active*, one child was randomly selected. As the *Y-Be-Active* study was interested in examining possible genetic association between parents' and children's CVD biomarkers, adopted children were excluded. The study was approved by the Conjoint Health Research Ethics Board of the University of Calgary.

Measurements

CVD Biomarkers. Fasting blood samples were obtained. Serum total cholesterol, triglycerides, and high density lipoprotein (HDL) was measured by the CHOD-PAP enzymatic colorimetric assay, the enzymatic colorimetric assay, and the HDL-C Plus Colorimetric Assay (Roche Diagnostics, Cobas 6000, Rotkreuz, Switzerland), respectively. Apolipoprotein A1, apolipoprotein B1, and lipoprotein(a) were measured by the nephelometric assay (Dade Behring, Deerfield, USA). Total and high molecular weight adiponectin levels and insulin levels were determined with the Adiponectin (multimeric) or the Insulin ELISA kits (ALPCO, Salem, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from the insulin and fasting glucose measure using the following formula: $(\text{Glucose (mmol/L)} * \text{Insulin (}\mu\text{IU/ml)})/22.5$.

Physical activity. Physical activity was measured using the ActiGraph 7146 accelerometers, which has been validated for children <5 years of age. The accelerometer

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was worn by children and parents for five consecutive days (3 weekdays, 2 weekend days), a duration necessary to achieve a reliability level of .80 (Cliff et al. 2009). Accelerometer data was validated against an activity diary and included only if the accelerometer was worn for at least 9 hours for weekdays and 6 hours for weekend days. On average, children wore the accelerometers 11.8 ± 1.3 hours/weekday and 10.6 ± 2.4 hours/weekend day. The accelerometers sampled physical activity at one-minute intervals to ensure that our measure of physical activity was the same for parents and children. Levels of physical activity were categorized as sedentary (SPA) (< 500 counts of movement/minute), light (LPA) (≥ 500 but < 1680 counts of movement/minute) and moderate or vigorous physical activity (MVPA) (≥ 1680 counts of movement/minute) (Cantell et al. 2012). Time spent in vigorous physical activity and moderate physical activity were combined and reported as MVPA to address the potential misclassification of vigorous activity as moderate activity when using 1-minute epochs (Metcalf et al. 2009, Cliff et al. 2009).

Anthropometry. Height, weight, and waist girth were measured as previously described (Cantell et al. 2012). Briefly, waist circumference was measured with the child standing, horizontally at the midpoint between the 10th rib and the iliac crest. All measurements were taken 3 times and the mean of the two closest values was recorded. Body Mass Index (BMI) ($\text{weight (kg)/height (m}^2\text{)}$) for age and gender was calculated for the children and their parents (Supplementary Table S1).

Questionnaires. Parents completed questionnaires on family sociodemographics (Supplementary Table S1). Socioeconomic status (SES) was measured using the Blishen Index (Blishen et al. 1987), which is a Canadian measure of socioeconomic status. For each

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family, the occupation of the parent with the highest SES on this index was used as the indicator of family SES.

Statistical Analyses

Multi-level modeling was conducted to examine the relationships among physical activity and biomarkers, while controlling for individual factors (e.g., sex, age) and the family of each participant. Exploratory analyses of relationships between CVD biomarkers and physical activity in preschool children and relationship between CVD biomarker levels of children and their mothers and fathers were performed using a series of Pearson r correlations and Spearman correlations where appropriate. Statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS version 19, Chicago, IL, USA). Results significant at $p < 0.05$.

RESULTS

Findings on physical activity level of the children and the relationship between parent-child physical activity have been published and baseline results are summarized in Supplementary Table SI (Cantell et al., 2012). This group of children spent most of their time in SPA or LPA, and most attained 60 min of daily MVPA on weekdays. Only 60% of fathers and approximately one-half of mothers attained 30 min of daily MVPA. Although only 22% of children were enrolled in full-time daycare, the level of physical activity attained did not differ between children cared for at home or in daycare.

In the children, the multi-level modeling analysis revealed no significant relationships between CVD biomarkers and MVPA (Supplementary Table SII). As multi-level modeling could have masked associations that may be of interest, given our small sample size, we conducted exploratory analyses of these relationships. As shown in

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Supplementary Figure S1a, for the children, a positive correlation was found between total adiponectin and percentage of time spent in LPA on weekdays ($r=0.417$, $p<0.05$); the association between HMW adiponectin and LPA on weekdays ($r=0.310$, $p=0.09$) is also shown (Supplementary Figure S1b). Higher triglyceride levels were correlated with more time spent in sedentary behavior during weekdays ($r=0.307$, $p<0.05$) (Supplementary Figure S1c). When the effects of children's age and BMI were partialled out, there was a positive correlation between higher apolipoprotein A1 and percentage of time spent in MVPA ($r=0.629$, $p<0.05$). For boys, fasting blood glucose negatively correlated with LPA ($r=-0.762$, $p<0.05$) and positively correlated with sedentary behavior ($r=0.579$, $p<0.05$). No significant correlations were found between the children's biomarkers, MVPA, BMI, and waist circumference (Supplementary Table SIII).

Since family history is a significant determinant of CVD risk, we correlated children's and parents' CVD biomarkers (Table I). We found significant positive correlations between children's and mothers' total cholesterol, low density lipoprotein (LDL), HDL ratio (i.e. HDL/total cholesterol), Apolipoprotein(A-1), Apolipoprotein(B), and lipoprotein(a). Between the child and the father, we found a significant correlation for fasting glucose levels only. When we adjust for level of physical activity, the results remain unchanged.

DISCUSSION

Many risk factors for CVD originate in childhood, and a better understanding of predictive factors for CVD risk will allow for the design of preventative measures in childhood (Magnussen et al. 2013). This is a unique study because we collected physical activity data using accelerometers on 54 parents-child triads. The small sample size and the

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lean nature of the preschool aged children may have resulted in the multi-level modeling analysis not revealing any relationship between CVD biomarkers and physical activity levels in the children. However, our exploration of potential associations, which could aid in the design of a larger, fully powered study, revealed positive correlations between the time children spent in sedentary behavior and higher triglycerides, and between higher levels of physical activity and higher serum levels of adiponectin.

Adiponectin is secreted by the adipocyte and a low adiponectin level is associated with CVD, hypertension and metabolic syndrome (Jensen et al. 2014). Here, we found a positive correlation between physical activity and total adiponectin, and to a lesser degree, the more physiologically relevant HMW adiponectin. Interestingly, both positive (Cambuli et al. 2008) and negative correlations (Emken et al., 2010; Metcalf et al., 2009) between serum adiponectin levels and physical activity have been reported. Emken et al. (2010) suggested that physical activity up regulates adiponectin receptor, hence reduced the need for high levels of serum adiponectin. Metcalf et al. (2009) found this negative correlation only in the least physically active group, suggesting that when physical activity is too low to maintain insulin sensitivity, adiponectin level increases to compensate. The higher level of MVPA attained by the pre-school children in our study as compared to school age children (Cantell et al. 2012, Colley et al. 2011) may have been sufficient to influence metabolism during LPA and explain the positive correlation between LPA and adiponectin found in our study. These conflicting results are likely due to variations in the age, pubertal stage, and BMI of the study populations.

Relationships among physical activity and other CVD risk markers such as HDL, TG, LDL, glucose and insulin levels have been reported in older children (Raitakari et al.

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1997; Katzmarzyk et al. 1999). Here, we observed a positive correlation between serum triglycerides and time spent being sedentary, suggesting that in normal weight, 3-6 year old children, being sedentary may contribute to adverse lipid profiles.

Interestingly, while studies in obese children have reported a significant relationship between BMI and CVD risk markers (Magnussen 2013), we did not find significant correlations between the children's BMI and CVD biomarkers, or their BMI and physical activity levels. This could be due to the rather lean phenotype of the children in our study. In children with a wider BMI range, Saakslähti et al. (2004) reported a negative correlation between BMI and self-reported physical activity level while Vorwerk et al. (2013) found no correlation between BMI and accelerometer-recorded physical activity levels.

To determine the contribution of family history to serum levels of biomarkers (Al-Daghri et al. 2011), we analyzed the associations among CVD biomarkers in children and their parents. While we found only one significant correlation between children's and fathers' biomarkers, i.e. fasting glucose, we found significant correlations between several of the children's and mothers' biomarkers. Interestingly, our previous report found that higher level of MVPA activity in fathers was associated with higher MVPA in their children (Cantell et al., 2012). Therefore, the lack of a significant relationship between fathers' and children's biomarkers suggests that the association between the children's and mothers' biomarkers are either related to shared diet during the week, or a more pronounced maternal genetic influence on children's biomarkers.

The strength of this study is in the objective measurement of physical activity by accelerometer in the parent-child triad. One limitation is the high SES of the participants, which may limit the generalizability of results, although low SES has been shown to

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associate with more screen time but not lower physical activity (Tandon et al. 2012). The relative racial homogeneity of our sample may also limit the study results to similar racial groups, but it was representative of background population where the study was conducted (Statscan 2007). Another limitation is sample size. Nevertheless, our exploratory analyses revealed correlations between the children's physical activity level and CVD risk markers, and between children's and mothers' lipid levels, information that will be useful in the design of a fully-powered study.

In conclusion, we found that in healthy normal weight 3-6 years-old children, physical activity levels do not correlate with CVD biomarkers. However, further exploratory analysis revealed that both total and HMW adiponectin were positively associated with physical activity, and children's and mother's lipid levels were correlated. These findings suggest that in very young children of normal weight, both physical activity levels and hereditary factors may be important determinants of their cardiometabolic risk markers. Longitudinal prospective studies are needed to investigate whether interventions that change PA levels positively influence CVD biomarkers and reduce CVD risks in young at risk pediatric populations.

CONFLICT OF INTEREST DISCLAIMER

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

We thank the participating families and our research assistants Nadia Barnieh, Julie Burbidge, Carly McMorris, and Val San Juan for coordinating data collection, and Dr. Martha Lyon for assisting in sample analysis. We appreciate the cooperation of the Alberta Health Services community healthcare centers and childcare centers for assistance in

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recruitment and data collection. As part of the *YB-active* study, the findings in regards to the physical activity level of the parent-child triad have been published in a manuscript by M Cantell, SG Crawford, and D Dewey, titled “Daily physical activity in young children and their parents: A descriptive study” in the journal, *Paediatric Child Health*. Part of the data in Supplementary Table S1, which include the age of the children and their parents, as well as the BMI of the parents, were already published in the aforementioned study.

Draft

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Table I. Biomarkers for Children and Parents

Biomarkers (mean ± SD)	Mothers	Fathers	Child	Correlation between child and mother:	Correlation between child and father:
Fasting glucose (mmol/L)	5.37±2.37	5.15±0.49	4.71±0.35	r=0.342, p=0.088	r=0.70, p=0.002
Fasting insulin* (μIU/mL)	7.28±5.64	7.89 ± 6.92	9.24±17.34	r=0.244, p=0.221	r=-0.142, p=0.562
HOMA-IR	1.98±2.15	1.93±1.29	0.92±0.67	r=-0.087, p=0.766	r=-0.147, p=0.666
Total Cholesterol (mmol/L)	4.62±1.38	4.66±1.05	3.99±0.79	r=0.719, p<0.001	r=0.217, p=0.288
Triglycerides (mmol/L)	1.06±0.81	1.25±0.86	0.72±0.25	r= 0.225, p=0.168	r= 0.326, p=0.104
LDL (mmol/L)	2.63±1.34	2.74±0.91	2.19±0.78	r=0.753, p<0.001	r=0.305, p=0.130
HDL (mmol/L)	1.53±0.37	1.34±0.17	1.47±0.32	r=0.305, p=0.059	r=0.059, p=0.773
HDL ratio (HDL/total cholesterol)	3.17±1.08	3.53±0.96	2.84±0.89	r=0.522, p=0.001	r=0.241, p=0.235
Apolipoprotein (A-1) (g/L)	1.51±0.24	1.41±0.20	1.36±0.19	r=0.374, p=0.023	r=-0.079, p=0.714

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Apolipoprotein (B) (g/L)	0.84±0.28	0.84±0.27	0.72±0.19	r=0.598, p<0.001	r=0.374; p=0.078
Lipoprotein (a) (g/L)	0.19±0.24	0.13±0.16	0.11±0.16	r=0.428, p=0.008	r=.218, p=0.318
Total Adiponectin (µg/ml)	5.86±2.1	4.63±1.77	8.24±2.55	r=-0.091, p=0.667	r=-0.086, p=0.751
HMW Adiponectin (µg/ml)	3.41±1.55	2.22±1.50	4.96±2.02	r=-0.153, p=0.465	r=0.156, p=0.564

Legend: HOMA-IR: Homeostasis Model of Assessment - Insulin Resistance; LDL: low

density lipoprotein; HDL: high density lipoprotein; HMW adiponectin: high molecular weight adiponectin. “*”: Spearman’s correlation was performed for fasting insulin as it is a non-normally distributed variable. Pearson correlation was performed for all other biomarkers.