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Associations of sedentary time patterns and TV viewing time with inflammatory and endothelial function biomarkers in children

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Summary

Objective: Investigate associations of TV viewing time and accelerometry-derived sedentary time with inflammatory and endothelial function biomarkers in children.

Methods: Cross-sectional analysis of 164 7–10-year-old children. TV viewing time was assessed by parental proxy report and total and patterns of sedentary time accumulation (e.g. prolonged bouts) were assessed by accelerometry. C-reactive protein (CRP), homeostasis model assessment of insulin resistance, interleukin-2, -6, -8, -10, tumour necrosis factor alpha, adiponectin, resistin, brain-derived neurotrophic factor, soluble intercellular and vascular adhesion molecule 1, plasminogen activator inhibitor 1 and soluble E-selectin were assessed. Generalised linear models assessed the associations of TV viewing and sedentary time with biomarkers, adjusting for sex, waist circumference, moderate- to vigorous-intensity physical activity and diet density.

Results: Each additional h week^{-1} of TV viewing was associated with 4.4% (95% CI: 2.1, 6.7) greater CRP and 0.6% (0.2, 1.0) greater sVCAM-1 in the fully adjusted model. The association between frequency and duration of 5–10 min bouts of sedentary time and CRP was positive after adjustment for sex and waist circumference but attenuated after adjustment for diet density.

Conclusions: This study suggests that TV viewing was unfavourably associated with several markers of inflammation and endothelial dysfunction. The detrimental association between 5 and 10 min bouts of sedentary time and CRP approached significance, suggesting that further research with a stronger study design (longitudinal and/or experimental) is needed to better understand how the accumulation of sedentary time early in life may influence short and longer term health.

Keywords: Cardiometabolic, paediatric, sedentary behaviour.

Introduction

The prevalence of elevated cardiometabolic risk factor levels are increasing among youth worldwide (1,2). While the clinical manifestation of cardiometabolic diseases (e.g. myocardial infarction, stroke) typically appears in adulthood, the pre-clinical phase of many diseases can persist for decades and certain risk factors (i.e. atherosclerotic lesions) may present during childhood (3). Beyond traditional markers of cardiometabolic risk (e.g. cholesterol, triglycerides and blood pressure), emerging research has shown that non-traditional biomarkers such as inflammatory and endothelial dysfunction markers are implicated in atherosclerosis and metabolic syndrome in youth, and are elevated in overweight and obese compared with normal weight children and adolescents (4–7). Consequently, examining inflammatory and endothelial dysfunction markers will provide a unique insight into the early aetiology and development of cardiometabolic diseases.

Physical activity (PA) is critical for the prevention of elevated metabolic and cardiovascular health risk factors levels in children (8), yet accounts for a small proportion of their day (9). In contrast, sedentary time (activities characterised by a low-energy expenditure [<1.5 METS] in a seated or reclined position (10)) makes up more than 60% of a child's waking hours (9). Interventions that aim to reduce sedentary behaviours might be valuable for improving children's cardiometabolic health. TV viewing time has a detrimental effect on traditional cardiometabolic risk factors among children and adolescents, independent of

PA levels (11,12), but little is known about associations with non-traditional risk factors. One study reported that several biomarkers (soluble intracellular adhesion molecule 1, vascular adhesion molecule 1 and E-selectin) were inversely associated with adolescents' TV viewing time, but not objectively measured sedentary time (13). However, it is unknown whether similar relationships exist for younger children.

To assist with refining the public health guidelines in many countries including Australia, the USA, Canada and the UK, which currently recommend limiting screen time (e.g. TV viewing, video games) to less than 2 h per day and breaking up extended periods of sitting (14), it is also important to consider whether sustained bouts of sedentary time of a certain duration are detrimental to health, or whether it is the total time accumulated (regardless of bout length) that is most strongly related to health outcomes. The primary aim of this study, therefore, was to investigate the associations of TV viewing time and objectively measured sedentary time with inflammatory and endothelial function biomarkers in primary school-aged children. A secondary aim was to evaluate the associations of patterns of sedentary time accumulation (i.e. prolonged sedentary bouts and breaks in sedentary time) with inflammatory and endothelial function biomarkers.

Methods

Participants

Participants were children aged 7–10 years involved in the Transform-Us! Study, a 2-year cluster-randomised controlled trial, which investigated the impact of a school and home-based intervention on children's sedentary behaviour and PA (15). Grade 3 children were recruited from 20 schools (primarily in low- and middle-income areas) within a 50-km radius of the Melbourne Central Business District (15). Baseline data were collected from the children and their parents between February and June 2010. Written informed parental consent was obtained from 599 parents for their child to participate in one or more of the Transform-Us! assessments. A subsample consented to blood biomarker assessments (n = 351), with 164 children (87 girls) providing complete blood sample and valid accelerometry data for analyses. The study was approved by the Deakin University Human Research Ethics Committee (EC 141-2009), the Victorian Department of Education and Early Childhood Development (2009_000344) and the Catholic Education Melbourne Office (Project Number 1545).

Exposures

TV viewing

Parents proxy-reported the number of hours per day their child spends watching TV/videos/DVDs during the week and on the weekend using the validated CLASS questionnaire (16). The weekday and weekend responses were summed to provide a weekly estimate of TV viewing time (h week^{-1}).

Sedentary time

Sedentary time was objectively assessed every 15 s for 8 consecutive days using hip-mounted ActiGraph GT3X accelerometers (Pensacola, FL, USA). A recent study recommended the use of 60-min of consecutive zeros to define accelerometer non-wear (17). However, our group (unpublished data) found that when ActiGraph data were compared with activPAL data (a direct measure of sitting time), the 20-min definition resulted in an almost identical wear time and a smaller difference between the sedentary time and sitting time estimates compared with the 60-min definition. Consequently, non-wear time was defined as ≥20 min of consecutive zero counts in this study. Sedentary time was defined as ≤100 counts per minute (18). The frequency and duration of time spent in sedentary bouts of medium (5–10 min) and longer (>10 min) duration were also calculated, as others have demonstrated a relationship between sedentary time spent in bouts of these durations and weight status in youth (19). Breaks in sedentary time were defined as the number of times that the accelerometer exceeded 25 counts per 15-s epoch following a 15-s epoch of <25 counts per epoch (20). Participants were required to have ≥8 h and ≥7 h of wear time on weekdays and weekend days, respectively, on at least 3 days to be included in the analyses. The lower weekend wear time requirement is due to children typically waking later on weekends. In this study, data from 82.6% of participants (400/484) were analysed. Total sedentary time and prolonged bouts in sedentary time were adjusted for wear time using the residuals obtained by regressing the sedentary variables on wear time. Breaks in sedentary time were additionally adjusted for total sedentary time using the same approach.

Outcome measures

Cardiometabolic biomarkers

A fasted morning blood sample was collected at a commercial pathology laboratory (Melbourne Pathology Clinic; n = 206). Plasma insulin, glucose and C-reactive protein (CRP) were assessed at Melbourne Pathology. Insulin sensitivity was derived using the homeostasis model assessment of insulin resistance (HOMA-IR) calculated by fasting insulin multiplied by fasting glucose divided by 22.5 (21). Milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA) were used to simultaneously measure plasma levels of cytokines (interleukin-2 [IL-2], -6 [IL-6], -8 [IL-8], -10 [IL-10] and tumour necrosis factor alpha [TNF-α]), adipokines (adiponectin and resistin), neurodegenerative factors (brain-derived neurotrophic factor [BDNF], soluble intercellular adhesion molecule 1 [sICAM-1], plasminogen activator inhibitor 1 [PAI-1], soluble vascular cellular adhesion molecule 1 [sVCAM-1] and cardiovascular disease factor, soluble E-selectin (sE-selectin). The assay was performed according to the manufacturer's instructions and all samples were run in duplicate. Mean inter-assay coefficient of variation for the 12 factors analysed ranged from 2.4% to 7.6%. Intra-assay coefficient of variation was

determined by replicate analysis ($n = 9$) of the two provided assay quality controls and ranged from 7.3% to 13.5%. Samples that returned a value below the detectable limits of the assay were allocated the minimum detectable concentration of each assay.

Covariates

Anthropometry

Height and weight were measured using standardised protocols with a portable stadiometer (SECA 220, Los Angeles, California, USA) and digital scales (Wederburn Tanita, Melbourne, Victoria, Australia) to the nearest 0.1 cm and 0.1 kg, respectively. Waist circumference (cm) was measured using a flexible steel tape at the narrowest point between the bottom rib and the iliac crest, in the midaxillary plane. Where a discrepancy of over 1 cm or 1 kg was observed, a third measure was taken. For the above measures, the average was used in the analyses. Body mass index (BMI; kg m^{-2}) categorised participants as healthy weight, overweight or obese based on the age- and sex-specific International Obesity Task Force classification (22).

Moderate- to vigorous-intensity physical activity (MVPA) and diet density

MVPA was derived from accelerometers using the age-appropriate equation for moderate (4.0–5.9 METs) and vigorous (≥6.0 METs) intensity PA (23). MVPA was standardised for accelerometer wear time using the residuals approach. Children's consumption frequency of key energy-dense foods and beverages was collected via a parental proxy report using items identified as important contributors to children's energy and fat (24). Eight energy-dense foods and drinks were included: salty snacks, chocolate and sweets, cakes, pastries, fast food, chips, fruit juice and soft drinks. Responses for food items ranged on a monthly scale from 'Never or less than once/month' to '6 or more times per day', while beverage items ranged on a daily scale from 'My child does not drink this beverage' to '6 or more serves/day'. Responses were summed to provide a total diet density score (range: 9–42).

Statistical analysis

All analyses were performed using Stata v.12 (StataCorp, College Station, TX, USA). Associations of TV viewing time and total objectively measured sedentary time with inflammatory and endothelial dysfunction biomarkers were assessed using generalised linear models with robust standard errors accounting for clustering at the class level. Gamma variance and logarithmic link functions were selected because of the positive skew of outcomes and residuals and the continuous, positive nature of the data. Initial models were adjusted for clustering within school classes. Model 2 additionally adjusted for the child's sex and waist circumference, model 3 for accelerometry-derived MVPA and model 4 for diet density. The deviance residuals of models were examined to assess the goodness of fit. Generalised linear models were also used to examine associations of patterns of sedentary time accumulation (i.e. prolonged bouts of sedentary time and breaks in sedentary time) with inflammatory and endothelial dysfunction biomarkers. Separate models were fitted for each sedentary time pattern exposure. Four models were estimated as described above. Waist circumference was chosen as a measure of adiposity instead of BMI as a covariate in all models because of stronger correlations with biomarkers in this sample. Interactions of sex and adiposity with sedentary time exposure variables were not statistically significant so data for boys and girls were pooled. Statistical significance was set at $P < 0.05$.

Results

Participant characteristics are presented in Table 1. On average, participants spent 56% of their day in sedentary time, with 20% of sedentary time accumulated in bouts of 5–10 min in duration, 16% accumulated in greater than 10 min in duration and the remainder (64%) accumulated in bouts of less than 5 min.

Table 2 presents the associations of TV viewing and sedentary time with cardiometabolic biomarkers. Several detrimental associations were observed between biomarkers and TV viewing, which persisted after adjustment of accelerometry-derived MVPA and diet density (Supporting Information Fig. S1). Each additional h $week^{-1}$ of TV viewing was associated with 4.4% (95% CI: 2.1, 6.7) greater CRP and 0.6% (0.2, 1.0) greater sVCAM-1. Significant associations were found between objectively measured sedentary time and sE-selectin and IL-6 in Model 1, although these were attenuated after adjustment for waist circumference and sex. No other associations between total sedentary time and the remaining biomarkers were observed ($P > 0.05$).

Relationships between sedentary time accumulation and CRP are presented in Table 3. There was a positive association between the frequency of time spent in sedentary bouts of 5–10 min in length and CRP, independent of sex, waist circumference and MVPA (model 3). Each additional 5–10 min bout of sedentary time was associated with 5.4% (95% CI: 0.4, 10.7) greater CRP. However, this association was attenuated after adjustment for diet density ($P = 0.056$). The duration of time spent in sedentary bouts of 5–10 min in length was positively associated with CRP, independent of sex and waist circumference (model 2). Each additional minute spent in a sedentary bout length of 5–10 min was associated with 0.7% (0.1, 1.4) greater CRP. This association attenuated after adjustment for MVPA ($P = 0.063$) and diet density ($P = 0.084$). No significant associations of the frequency and duration of time spent in sedentary bouts of ≥10 min or frequency of breaks with biomarkers were observed independent of confounders (Table 3 and Supporting Information Table S1).

Discussion

The key finding from this study was that TV viewing time, but not objectively measured sedentary time, was

Table 1 Participant characteristics (n = 164)

Variables	Mean (SD)*
Age (years)	8.7 (0.4)
Height (cm)	132.9 (6.3)
Weight (kg)	30.9 (6.3)
BMI (kg m^{-2})	17.4 (2.5)
Overweight + obese (%)	22.2
Waist circumference (cm)	59.6 (6.3)
Questionnaire-derived variables	
Diet density score	2.3 (4.9)
TV viewing (h $week^{-1}$)	12.1 (6.4)
Accelerometer-derived variables	
Total wear time (min day^{-1})	717.3 (69.2)
Sedentary time (min day^{-1})	401.6 (57.4)
Frequency of sedentary bouts of 5–10 min (no. per day)	11.6 (3.1)
Total duration of sedentary bouts of 5–10 min (min day^{-1})	78.7 (21.5)
Frequency of sedentary bouts of >10 min (no. per day)	4.3 (1.9)
Total duration of sedentary bouts of >10 min (min day^{-1})	62.5 (29.5)
Breaks in sedentary time (no. per day)	315.3 (38.9)
MVPA (min day^{-1})	85.5 (24.8)
Biomarkers	
CRP (mg L^{-1})	0.3 (0.1, 0.6)
HOMA-IR	1.2 (0.8, 1.6)
BDNF (ng mL^{-1})	20.9 (15.6, 25.6)
Adiponectin (mg mL^{-1})	25.7 (18.2, 35.3)
Resistin (ng mL^{-1})	20.2 (16.1, 24.8)
sE-selectin (ng mL^{-1})	60.7 (47.1, 83.5)
sICAM-1 (ng mL^{-1})	141.8 (121.9, 169.3)
sVCAM-1 (ng mL^{-1})	1479.2 (1328.8, 1659.1)
PAI-1 (ng mL^{-1})	237.1 (184.4, 291.8)
TNF-α (pg mL^{-1})	9.8 (7.3, 12.2)
IL-2 (pg mL^{-1})	1.1 (0.3, 3.0)
IL-6 (pg mL^{-1})	0.4 (0.2, 1.3)
IL-8 (pg mL^{-1})	5.6 (4.4, 7.7)
IL-10 (pg mL^{-1})	13.8 (8.9, 25.3)

*Mean (SD, standard deviation) for demographic and sedentary variables, median (IQR, interquartile range) for biomarkers.
BDNF, brain-derived neurotrophic factor; BMI, body mass index; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin; MVPA, moderate-to vigorous-intensity physical activity; PAI, plasminogen inhibitor; sE-selectin, soluble E-selectin; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TNF-α, tumour necrosis factor.

detrimentally associated with several biomarkers, including those related to inflammation (CRP) and endothelial function (sVCAM-1), independent of MVPA and diet density. Likewise, sedentary time bouts lasting 5–10 min were also associated with greater levels of CRP, although this association was attenuated after adjusting for MVPA and diet density.

The positive association between TV viewing and CRP (independent of MVPA) are in agreement with a large US nationwide study in 10–16-year-olds, which reported that the odds of having CRP in the highest quartile increased in a dose-response manner with TV viewing, independent of PA and other covariates (11). This is an important finding as CRP is an acute-phase inflammatory marker shown to predict coronary events in adults, independent of conventional risk factors (25), and has been associated with atherosclerosis, endothelial dysfunction, and metabolic syndrome in youth (4–6). To date, the potential physiological mechanisms that may be involved in the association between CRP and sedentary behaviour are currently poorly understood, and further research is needed. However, CRP has been found to be a strong predictor of several diseases, such as cardiovascular diseases, independent of more traditional markers and other inflammatory markers (4,5,25). It is therefore possible that CRP has a higher predictive value for poor health outcomes than the other inflammatory markers assessed in this study. This may partly explain the significant findings observed.

The significant association between TV viewing and soluble cellular adhesion molecules also supports previous adolescent research (13). Cellular adhesion molecules play a role in promoting the adhesion of inflammatory cells on the vascular surface, thereby aiding the development of atherosclerosis (5). Although our findings are modest (4.4% and 0.6% greater CRP and sVCAM-1, respectively, per h $week^{-1}$ of TV viewing), the tracking of low-grade inflammation from childhood through adulthood and associations of markers of inflammation and endothelial function with atherosclerotic lesions (5) suggest that elevated biomarkers early in life may be indicative of cardiometabolic risk later in life. Interestingly, we did not observe associations of TV viewing with other markers of inflammation or endothelial dysfunction. Although we measured several upstream biomarkers of CRP that play a role in systemic inflammation, such as TNF-α and IL-6, their role in the development of cardiometabolic risk is poorly understood (5). Likewise, while other biomarkers measured in the current study have been implicated in the development and progression of cardiometabolic conditions (e.g. PAI-1, adiponectin, HOMA-IR), further work is necessary to identify the clinical relevance of these markers during childhood (4,5).

We failed to observe any association between total sedentary time and inflammatory or endothelial function biomarkers. Similarly, others have failed to observe an association between traditional cardiovascular risk markers (e.g. triglycerides, blood pressure, weight status, vascular function) and total sedentary time in youth after accounting for PA (11,26,27). The lack of associations may

Table 2 Associations of total sedentary time and TV viewing with cardiometabolic biomarkers in school-aged children

	Model	Sedentary time (h day^{-1})	TV viewing (h week^{-1})
		b (95% CI)	b (95% CI)
CRP (mg L^{-1})	1	1.139 (0.859, 1.509)	1.043 (1.017, 1.070)[a]
	2	1.180 (0.926, 1.504)	1.032 (1.010, 1.054)[b]
	3	1.163 (0.850, 1.592)	1.034 (1.013, 1.056)[c]
	4	1.083 (0.782, 1.502)	1.044 (1.021, 1.067)[a]
HOMA-IR	1	1.076 (0.896, 1.291)	1.009 (0.996, 1.023)
	2	1.016 (0.881, 1.170)	1.003 (0.989, 1.018)
	3	0.959 (0.806, 1.142)	1.004 (0.989, 1.018)
	4	0.937 (0.794, 1.107)	1.002 (0.987, 1.017)
BDNF (ng mL^{-1})	1	0.998 (0.919, 1.084)	1.005 (0.996, 1.014)
	2	0.999 (0.926, 1.079)	1.003 (0.994, 1.011)
	3	0.970 (0.878, 1.072)	1.003 (0.994, 1.011)
	4	0.972 (0.875, 1.080)	1.003 (0.995, 1.012)
Adiponectin (mg mL^{-1})	1	0.960 (0.853, 1.082)	1.004 (0.992, 1.016)
	2	0.959 (0.854, 1.076)	1.006 (0.994, 1.019)
	3	0.922 (0.801, 1.062)	1.007 (0.994, 1.019)
	4	0.930 (0.811, 1.067)	1.005 (0.993, 1.018)
Resistin (ng mL^{-1})	1	1.024 (0.935, 1.122)	1.007 (0.998, 1.017)
	2	1.016 (0.926, 1.115)	1.007 (0.997, 1.016)
	3	0.993 (0.850, 1.159)	1.007 (0.997, 1.017)
	4	0.997 (0.862, 1.153)	1.007 (0.997, 1.018)
sE-selectin (ng mL^{-1})	1	1.105 (1.004, 1.216)[d]	1.003 (0.991, 1.014)
	2	1.094 (0.997, 1.201)	0.998 (0.988, 1.008)
	3	1.051 (0.935, 1.182)	0.999 (0.989, 1.009)
	4	1.054 (0.923, 1.204)	0.998 (0.988, 1.008)
sICAM-1 (ng mL^{-1})	1	1.007 (0.931, 1.090)	1.002 (0.997, 1.009)
	2	1.000 (0.924, 1.081)	1.001 (0.995, 1.007)
	3	0.989 (0.891, 1.096)	1.001 (0.995, 1.008)
	4	0.987 (0.889, 1.095)	1.001 (0.994, 1.008)
sVCAM-1 (ng mL^{-1})	1	0.991 (0.955, 1.028)	1.005 (1.001, 1.009)[e]
	2	0.995 (0.959, 1.032)	1.007 (1.003, 1.011)[c]
	3	0.995 (0.938, 1.054)	1.007 (1.003, 1.011)[c]
	4	0.999 (0.940, 1.062)	1.006 (1.002, 1.010)[f]
PAI-1 (ng mL^{-1})	1	1.084 (0.982, 1.120)	1.004 (0.995, 1.013)
	2	1.084 (0.988, 1.191)	1.003 (0.994, 1.011)
	3	0.987 (0.880, 1.106)	1.004 (0.995, 1.012)
	4	0.974 (0.866, 1.095)	1.005 (0.996, 1.014)
TNF-α (pg mL^{-1})	1	1.063 (0.948, 1.192)	1.008 (0.998, 1.018)
	2	1.063 (0.948, 1.192)	1.007 (0.997, 1.018)
	3	1.129 (0.936, 1.362)	1.006 (0.996, 1.017)
	4	1.146 (0.941, 1.394)	1.007 (0.996, 1.017)
IL-2 (pg mL^{-1})	1	0.962 (0.680, 1.362)	1.041 (0.989, 1.096)
	2	0.998 (0.701, 1.422)	1.047 (0.998, 1.098)
	3	0.744 (0.494, 1.121)	1.046 (1.000, 1.095)[g]
	4	0.937 (0.633, 1.386)	1.025 (0.988, 1.063)
IL-6 (pg mL^{-1})	1	1.772 (1.112, 2.826)[h]	1.024 (0.979, 1.071)
	2	1.268 (0.899, 1.788)	1.039 (0.998, 1.082)
	3	0.894 (0.571, 1.400)	1.030 (0.996, 1.066)
	4	0.778 (0.476, 1.271)	1.015 (0.980, 1.052)
IL-8 (pg mL^{-1})	1	0.660 (0.349, 1.251)	1.027 (0.953, 1.108)
	2	0.826 (0.549, 1.242)	1.026 (0.977, 1.078)
	3	0.994 (0.613, 1.612)	1.023 (0.980, 1.068)
	4	0.998 (0.606, 1.641)	1.021 (0.974, 1.071)
IL-10 (pg mL^{-1})	1	1.151 (0.884, 1.498)	1.025 (0.992, 1.059)
	2	1.124 (0.869, 1.454)	1.029 (0.997, 1.062)
	3	0.841 (0.589, 1.201)	1.028 (0.998, 1.059)
	4	0.772 (0.552, 1.080)	1.022 (0.991, 1.055)

b (95% CI), exponentiated regression coefficients and 95% confidence intervals. Model 1 adjusted for clustering within school classes. Model 2 further adjusted for sex and waist circumference. Model 3 further adjusted for moderate-to vigorous-intensity physical activity (MVPA). Model 4 further adjusted for diet density. [a]$P < 0.001$, [b]$P = 0.019$, [c]$P = 0.001$, [d]$P = 0.041$, [e]$P = 0.009$,
[f]$P = 0.005$, [g]$P = 0.05$, [h]$P = 0.016$.
BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin; PAI, plasminogen inhibitor; sE-selectin, soluble E-selectin; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TNF-α, tumour necrosis factor.

Table 3 Associations of sedentary time patterns with C-reactive protein (CRP) in school-aged children

	Model	Frequency of sedentary bouts of 5–10 min (no. per day)	Duration of sedentary bouts of 5–10 min (min day^{-1})	Frequency of sedentary bouts >10 min (no. per day)	Duration of sedentary bouts of >10 min (min day^{-1})	Frequency of breaks in sedentary time (no. per day)
		b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)	b (95% CI)
CRP (mg L^{-1})	1	1.046 (0.999, 1.095)	1.006 (0.999, 1.013)	1.034 (0.923, 1.158)	1.002 (0.994, 1.009)	1.003 (0.995, 1.012)
	2	1.055 (1.010, 1.101)[a]	1.007 (1.001, 1.014)[c]	1.085 (0.985, 1.196)	1.005 (0.999, 1.011)	0.996 (0.990, 1.003)
	3	1.054 (1.004, 1.107)[b]	1.007 (1.000, 1.014)	1.074 (0.963, 1.198)	1.004 (0.998, 1.011)	0.993 (0.985, 1.000)
	4	1.050 (0.999, 1.104)	1.006 (0.999, 1.014)	1.034 (0.927, 1.154)	1.002 (0.995, 1.009)	0.994 (0.986, 1.001)

b (95% CI), exponentiated regression coefficients and 95% confidence intervals. Model 1 adjusted for clustering within school classes. Model 2 further adjusted for sex and waist circumference. Model 3 further adjusted for moderate-to vigorous-intensity physical activity (MVPA). Model 4 further adjusted for diet density. [a]$P = 0.015$, [b]$P = 0.033$, [c]$P = 0.024$.

be due to relatively low levels of sedentariness during childhood compared with adulthood; thus, deleterious associations with cardiometabolic health in adulthood are likely to reflect increasing lifelong exposure to sedentary behaviour (13). More detrimental associations may have been observed had our population been more sedentary or we had investigated a population at higher risk of cardiovascular disease.

Relatively, low sedentariness or high MVPA engagement in childhood compared with adulthood cannot explain the stronger adverse relationships seen between health outcomes and subjective reports of children's TV viewing and objectively measured sedentary time. Unfavourable observations between TV viewing and cardiometabolic risk (11,12), but not total sedentary time (11,27,28), may indicate that certain behaviours associated with TV viewing, such as caloric consumption, and not the actual act of being sedentary itself, may explain the negative associations observed (29). TV viewing was positively associated with diet density in our sample ($r = 0.23$, $P < 0.001$), yet significant detrimental associations between TV viewing and inflammation and endothelial dysfunction persisted even after controlling for diet density. Although we used a simple caloric intake measure, this suggests that the relationship between TV viewing and health is not entirely mediated by dietary intake. Future studies using a more sophisticated measure of dietary intake are warranted. Further, the potential mechanisms driving an association between cardiometabolic health and TV viewing, but not total sedentary time, remains unclear. Studies specifically investigating objectively measured sitting time would greatly add to this evidence base, as accelerometers cannot differentiate between sitting and standing and TV viewing may not just reflect time spent sitting as viewers may be standing or moving while watching.

Little research has examined associations between sedentary time accumulation and children's cardiometabolic health. Experimental research in adults has shown a detrimental effect of prolonged sedentary bouts on metabolic health (30); however, similar results have yet to be demonstrated in healthy children (29). While there is currently no consensus on how to define a 'prolonged' sedentary bout in children, spending more time and having a greater frequency of 5–10 min sedentary bouts was associated with higher levels of CRP. Sedentary bouts of this duration have been detrimentally associated with BMI z-score in children (19). We found no associations with cardiometabolic biomarkers and longer bouts of sedentary time (>10 min), although few children engaged in sedentary bouts of this duration (median of 4 bouts per day of >10 min of sedentary time vs. 11 bouts per day of 5–10 min of sedentary time). Although the precise biological mechanisms remain to be determined, evidence from animal models suggest that the decreased muscle contractile activity associated with prolonged sedentariness may disrupt the body's cardiometabolic regulatory processes, while breaking up prolonged periods of sedentariness increases muscle contractile activity (31). Further study is necessary to

determine whether such behaviours in childhood are related to adverse health outcomes in adulthood.

Our study had several strengths, including the use of an objective measure of sedentary time and the novel investigation of sedentary patterns with respect to multiple cardiometabolic biomarkers. Despite the use of a simple measure of dietary intake, we were able to account for high-density dietary items in our analyses. Limitations of our study include the cross-sectional nature of the study design, which precludes us from making judgments regarding causality. No objective functional measurements of the vasculature were performed, thus structural changes that are markers of early microvascular dysfunction were not examined. Future research should consider utilising such objective assessments. Sedentary time estimates may have included both standing time and some light-intensity PA as hip-mounted accelerometers cannot determine posture (18). Future research would benefit from activity monitoring devices that can also assess posture (i.e. inclinometers). The commonly used non-wear definition of 20 min may have also resulted in reduced sedentary time estimates, which could have influenced the associations observed. Finally, our sample included relatively healthy children and only one-fifth were overweight or obese. Whether these findings persist in a higher risk cohort remains to be evaluated.

Conclusions

TV viewing was unfavourably associated with several markers of inflammation and endothelial dysfunction independent of PA and energy-dense dietary intake. This study is the first to suggest a detrimental association between prolonged bouts of sedentary time and markers of inflammation in healthy children, but whether this would be related to adverse long-term health outcomes into adulthood requires further study. Nevertheless, given recent public health recommendations for children to break up prolonged periods of sitting, public health policies would benefit from further work directed at understanding how the accumulation of sedentary time may influence health outcomes.

Conflict of Interest Statement

The authors declared no conflicts of interest.

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Author contributions

LG had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JS. Acquisition, analysis and interpretation of data: all authors. Drafting of the manuscript: LG. Clinical revision of the manuscript for intellectual content: all authors. Statistical analysis: LG, EC. Obtained funding: JS. Administrative, technical or material support: NDR, LA, PD. Study supervision: JS.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Partial regression plots of TV viewing time and cardiometabolic biomarkers, accounting for sex, waist circumference, accelerometry-derived moderate-to vigorous-intensity physical activity (MVPA), diet density and clustering by school class. (A) C-reactive protein (C RP, mg L^{-1}) and (B) Soluble vascular adhesion molecule-1 (sVCAM-1, ng mL^{-1}). $^{a}P = 0.02$, $^{b}P = 0.03$; b (95% CI), exponentiated regression coefficients and 95% confidence intervals.

Table S1. Associations of sedentary time patterns with cardiometabolic biomarkers in school aged children. $^{a}P = 0.02$, $^{b}P = 0.03$; b (95% CI), exponentiated regression coefficients and 95% confidence intervals. Model 1 adjusted for clustering within school classes. Model 2 further adjusted for sex and waist circumference. Model 3 further adjusted for moderate-to vigorous-intensity physical activity (MVPA). Model 4 further adjusted for diet density.