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Is replacing sedentary time with bouts of physical activity associated with inflammatory biomarkers in children?

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Conflict of Interest Statement

The authors have no conflict of interests to declare.

Abstract

This study aimed to investigate the theoretical impact of reallocating a specific amount of sedentary time with an equal amount of (a) total, and (b) ≥1min-bout-accumulated time spent in different activity intensities, on inflammatory biomarkers in 8-9 year old children. Accelerometry and inflammatory biomarker baseline data from the Transform-Us! Study (complete cases n=149) was utilized. Isotemporal linear models with Gaussian distribution and identity link functions were used to assess associations between the activity replacements and seven individual inflammatory biomarkers, including C-reactive protein (CRP), and Interleukin (IL)-2, 6, 8, and 10, as well as combined inflammatory and pro-inflammatory composite scores. Eighty-five percent of children met physical activity recommendations. Replacing 10 min of sedentary time per day with VPA, regardless of how this was accumulated, was beneficially associated with CRP and both combined composite scores. In contrast, replacing 10 min/day of sedentary time with ≥1-min MPA bouts was detrimentally associated with CRP and the inflammatory composite score. Substitutions with other activity intensities were not significantly associated with any individual inflammatory biomarkers, or combined inflammatory and pro-inflammatory composite scores. In healthy and active school-aged children, evidence of the theoretical impact of replacing sedentary time with physical activity, regardless of intensity or accumulation, on markers of systemic inflammation was limited. Longitudinal research is needed to investigate the long-term impacts of reallocating sedentary time with physical activity, and particularly VPA, for inflammatory biomarkers in children, including those with increased risk of inflammation.

Keywords: Sedentary behaviour; Inflammation; C-reactive protein; Youth; Objectively-measured; Device-based; Accelerometry.

Introduction

Evidence suggests that regular engagement in physical activity (PA) is beneficially associated with cardiometabolic health in children[1]. Conversely, although evidence is equivocal, excessive sedentary behaviour (sitting) may be detrimentally associated with cardiometabolic health[2,3]. To date, research in children has typically focused on associations between the activity spectrum (from sedentary behaviour to vigorous-intensity PA) and adiposity markers or blood lipid levels, with few studies investigating associations with non-traditional health biomarkers, such as inflammatory cytokines[1,2].

Inflammation represents a complex biological response of the immune system to either acute (e.g., infection/injury) or chronic harmful stimuli (e.g., obesity)[4]. Research indicates that overweight or obese children exhibit higher inflammatory protein levels than healthy weight peers, including higher levels of the acute-phase C-reactive protein (CRP)[4-6]. Obesity can also lead to a chronically elevated pro-inflammatory state, which is characterised by increased circulating cytokine concentrations, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)[7]. Increased pro-inflammatory levels have been shown to detrimentally impact insulin resistance and limit efficient lipid storage[4,6], and if this persists into adulthood, can precipitate the development of cardiovascular diseases[4]. Whilst there has been an increasing interest in associations between activity behaviours and child health, research has mainly focused on traditional health markers, such as adiposity, and the potential consequent impact of increased pro-inflammation is unclear. In addition, the limited research that has focused on relations between the activity spectrum and inflammatory biomarkers have used CRP as a marker of inflammation[8,9]. Examining other inflammatory cytokines, including both pro-inflammatory (e.g., TNF-α, IL-6) and anti-inflammatory (e.g., IL-10, adiponectin) biomarkers, which have been implicated in the causal pathway to various chronic diseases[4,6], may provide greater insights into the potential role of PA and sedentary behaviours in the early development of cardiometabolic diseases.

In recent years, interest has grown into whether replacing sedentary time with PA, of different intensities and specific manners of accumulation (i.e., in shorter or longer sustained bouts of various PA intensities), is important for children's health[10]. Such evidence has implications for the modification of guidelines and the design of interventions, as it provides

insights into what PA intensities to target and the manner in which it can be achieved (i.e., patterns of accumulation). One method for studying the effects of replacing sedentary behaviour with different activity intensities and patterns of accumulation on children's health is isotemporal substitution modelling[11]. Whilst the optimal statistical approach to assess patterns is debated in current research[12-14], isotemporal substitution has been previously identified as the optimal method to assess hypothetical replacements of time spent in one intensity (e.g., sedentary) or pattern (i.e., longer bouts) with time in another (e.g., light- [LPA], moderate- [MPA] or vigorous-intensity [VPA] PA; shorter bouts) and associations with different health outcomes[11,13].

There are a few studies that used an isotemporal substitution approach to investigate associations with health outcomes in youth[15-18], however these focused mainly on adiposity markers and lipids. No studies have investigated the potential effects for pro- and anti-inflammatory biomarkers. Consequently, the aim of this study was to investigate the theoretical impact of reallocating a specific amount of sedentary time with an equal amount of (a) total, and (b) ≥1min-bout-accumulated time spent in different activity intensities, on inflammatory biomarkers in children aged 8-9 years.

Materials and methods

Study sample

This study utilised baseline data from the Transform-Us! study, which was collected in 2010. The study protocol has previously been published[19]. Ethics approval was provided by the Deakin University Human Research Ethics Committee (EC141-2009), the Victorian Department of Education and Early Childhood Development (2009-000344), and the Catholic Education Office Melbourne (Project Number 1545). In total, 1606 children from Melbourne, Australia, were invited to take part in the study. Parental consent was provided for 599 children aged 8-9 years. Further consent for the collection and assessment of blood samples was provided for 351 children.

Inflammatory biomarkers

Fasted morning blood samples were taken at local commercial Melbourne Pathology Clinics by trained phlebotomists. Sample concentrations of high-sensitive CRP, cytokines (serum IL-2, IL-6, IL-8, IL-10 and TNF-α), and adipokines (adiponectin) were determined via multiplex immunoassay (Millipore Corp., Billerica, MD, USA). The assay was performed according to the manufacturer's instructions and all samples were run in duplicate to enable determination of the variation within the assay. Average intra-assay coefficients of variation (CV) ranged from 2.7% to 7.6% across the seven inflammatory biomarkers. Inter-assay CV was determined by replicate analysis (n = 9) of the two provided assay quality controls and ranged from 7.3% to 12.9%. Sample concentrations observed below the detection threshold were reported as the minimum value for the assay's lowest detectable limit. Outliers, defined as those participants with data >3SD above or below the mean (on any inflammatory biomarker variable) were removed from further analysis. To aid comparisons between inflammatory biomarkers, all biomarkers were transformed to z-values (z-value = [value-mean]/SD). Consequently, an overall inflammatory composite score was created by summing the z-values for all inflammatory biomarkers. IL-10 and adiponectin were multiplied by -1 as they are classified as anti-inflammatory biomarkers (i.e., inflammatory composite score = [z_IL2 + z_IL6 + z_IL8 + (-1*z_IL-10) + z_TNFa + (-1*z_adiponectin)])[20]. In addition, a pro-inflammatory composite score was created using the pro-

inflammatory biomarkers only. For both combined composite scores (i.e., inflammatory and pro-inflammatory), higher scores indicated higher inflammatory levels[20].

Accelerometry exposure variables

Participants wore an ActiGraph GT3X accelerometer (ActiGraph, Pensacola, FL, USA) for eight consecutive days on the right hip during waking hours. The 15-second epoch data were downloaded and processed using a customized Excel macro. Non-wear time was defined as ≥20 min of consecutive epochs with zero counts[21]). Total min/day in sedentary, LPA, MPA and VPA were identified using age-specific count cut-points for <1.5 METs, ≥1.5-3.99 metabolic equivalents (METs), 4-5.99 METs, and ≥6 METs, respectively[22,23]. The total min accumulated in ≥1-min bouts of LPA, MPA, VPA was also determined, using the same count cut-points and without bout tolerance (i.e., allowed proportion in another intensity within a bout). Participant data were included if ≥4 valid days, defined as ≥8 hours and ≥7 hours of wear time on weekdays and weekend days, respectively, were provided.

Covariates

Sex and age were included as demographic covariates. These were both self-reported during school visits or via parent proxy-report. Diet was also included as a covariate. Main carers proxy-reported their child's consumption of eight high-energy and high-fat items such as soft drinks, chocolate and salty snacks. Responses were rated on an eight or nine-point scale (depending on item) ranging from 'never or less than once a month' to '6 or more times/serves per day'. Based on these responses, a diet density score was calculated (potential range: 8-70). Previous research has indicated that parent proxy-report of usual food intake are sufficiently accurate[24]. Waist circumference as an index of obesity was also considered as a covariate. This was measured at school by trained research staff at the narrowest point between the bottom rib and iliac crest in the mid-axillary plane. However, as children who are overweight have higher CRP levels than their normal weight peers[4-6], it is likely that weight status is in the pathway between PA and inflammation, and adjusting for this variable would therefore be

inappropriate[25]. Socio-economic status (derived from the Socio-Economic Indexes for Areas [SEIFA] using school postal code[26]) was considered as a covariate. However, as SEIFA and school clustering are highly correlated, including SEIFA may result in over-adjustment and attenuate the association. This is in line with previous studies in this age group[8,9].

Statistical analyses

Mean (and standard deviation [SD]) time spent sedentary, and in LPA, MPA and VPA, and the total duration of time spent in ≥1-min LPA, MPA and VPA bouts, were estimated. Isotemporal generalized linear models with Gaussian distribution and identity link functions for the individual inflammatory biomarker z-values, the inflammatory composite score and pro-inflammatory composite score (all normally distributed) were used to assess β-coefficients (and 95% confidence intervals) and effect sizes (*r*) for the associations between the activity behaviours/replacements and individual inflammatory biomarkers. In these models, the "replaced activity" was excluded but all other intensities and total wear time were included. The isotemporal models used to specifically assess replacements of sedentary behaviour with ≥1min-bout-accumulated PA time, included both the time in the intensity of interest subdivided in bouted and other time, to keep the "total time" constant.

All total volumes and time in bouts were scaled to 10-min units (total min divided by 10). Given that the sample was relatively active (85% percent of children met moderate-to-vigorous-intensity PA (MVPA) recommendations[27]), the 10-min per day replacement was chosen based on the smallest observed spread in the accelerometry variables, to ensure that replacements were realistic for participants in the sample. Specifically, as 99.7% of data is expected to fall within three standard deviations under and above the mean, and the smallest observed SD was 4.0 min (for ≥1-min MPA bouts), ten min was selected as the closest rounded value to 3*SD = 3*4.0 = 12 min/day. As the models used assume linearity between exposure and outcomes, the regression coefficients represent the estimated change in the outcome associated with the substitution of one unit (i.e., 10 min/day) for one unit of the corresponding remaining behaviours, and thus hypothetical changes from smaller/larger (e.g., 30 or 60 min/day) replacements can be estimated from these models accordingly.

Previous studies using isotemporal substitution modelling for activity behaviours have typically presented three models alongside each other[11]: 1) single-activity models (i.e. separately for time in each activity intensity/type of bouts with no adjustment for other intensities); 2) partition models (i.e., including adjustment for all other intensities but not for wear time); and 3) isotemporal substitution models. Hence, single-activity and partition models were presented in addition to the main isotemporal substitution models.

All models included robust standard errors accounting for clustering at the school level, and adjusted for wear time (either by residual adjustment or as confounder in the model), child sex, age, and diet density. Total sedentary time was highly correlated with wear time and, thus, models that adjusted for wear time used residuals obtained by regressing total daily volume of sedentary time on wear time[28,29]. After this adjustment, all assumptions for generalized linear models were met in all models. Model Z statistics were transformed into effect sizes using $r = Z / \sqrt{N}$[30,31]. Effect sizes of ≥0.1, ≥0.3, and ≥0.5 were considered small, medium and large effects, respectively[31,32]. All analyses were performed using Stata v15.0 (StataCorp, College Station, TX, USA).

A complete case analysis was conducted, so only participants with valid accelerometry, and complete inflammatory biomarkers and covariates were included in the analysis. Data from participants excluded from the analyses and those with complete data were compared. To explore the role of weight status within the relation between sedentary behaviour, PA and inflammatory biomarkers, waist circumference was considered as a moderator variable and as a potential confounding variable. However, as there is currently limited evidence to suggest that the strength of the relation between the activity spectrum and inflammation may depend on waist circumference[33], and as weight circumference may be on the causal path from exposure to outcome[25], this was not examined. Nevertheless, children were categorized as healthy weight or overweight (≥75th percentile[34], including those who were obese ≥90th percentile[35]) based on Australian age- and sex-specific percentile curves[36] and their characteristics were provided separately in addition to the complete sample, to provide a complete description of the dataset.

Results

Participant characteristics

Of the 351 children that provided accelerometer and consent for blood collection, 158 (45%) children had complete inflammatory biomarkers, covariate, and valid accelerometer data at baseline. After exclusion of outliers (>3 x SD from mean) for the inflammatory biomarkers (n=9), 149 were included in the analyses. Participant characteristics are presented in Table 1. On average, participants were almost nine years of age and slightly more than half were female. Sixty-four percent of children were a healthy weight, 20% were overweight and 15% obese. Eighty-five percent of the included participants met the MVPA recommendations of the 24-h Australian movement guidelines[27], based on their average MVPA across valid wear days. Only 2 participants had CRP >3 mg/L, which is often considered to reflect increased inflammation. Data from excluded participants (i.e., those with incomplete data), and participant characteristics separated for participants with healthy weight versus those with overweight/obesity are provided in the supplementary Table S1.

Isotemporal substitution of sedentary time with physical activity

Table 2 shows results from the isotemporal substitution models. Replacing 10 min of sedentary time per day with VPA, regardless of how this was accumulated, was beneficially associated with CRP and both combined composite scores. In contrast, replacing 10 min/day of sedentary time with ≥1-min MPA bouts was detrimentally associated with CRP and the inflammatory composite score. All remaining substitutions were not associated with any inflammatory measures (Table 2). Results from the single-activity and partition models are displayed in supplementary Tables S2 and S3.

Discussion

The present study investigated the theoretical impact of reallocating 10 min/day of sedentary time with an equal amount of total (regardless of accumulation pattern) and ≥1min-bout-accumulated time in different PA intensities, on inflammatory biomarkers in children aged 8-9 years. Little evidence was found for associations between hypothetically modelled 10-

min/day substitutions of sedentary behaviour to PA of different intensities and inflammatory biomarkers. The isotemporal models only showed associations between replacing sedentary time with ≥1min-bout-accumulated MPA and total VPA for CRP and the combined composite scores. Although some results were promising for VPA, replacing just 10 min of sedentary behaviour per day with PA, particularly for lower intensities, is unlikely to provide any clinically relevant benefits to systemic inflammation in young healthy and active children.

The results from the current study are somewhat consistent with the findings of previous studies in this age group that used an isotemporal substitution approach to investigate associations with health outcomes, regardless of whether the sample was active or not [15-18]. For example, Aggio and colleagues found in a sample of low active 5-15 year-olds that replacing one hour of sedentary time with MVPA was associated with adiposity benefits, yet no hypothetical effects for replacing sedentary with LPA were observed[15]. Similarly, a recent study of active children also found that substituting sedentary behaviour with ≥1min-bout-accumulated MPA and VPA, but not LPA, was associated with better lipid concentrations[18]. In addition, Huang and colleagues found that allocating 30 min/day of different types of sedentary behaviour (e.g., screen time, academic-related activities) to MVPA resulted in decreased BMI, but did not observe hypothetical changes from allocating sedentary behaviour to LPA[17]. Moreover, Leppänen and colleagues found that substituting five min of sedentary behaviour, LPA or MPA per day with VPA was associated with higher fat-free mass (index of muscle mass) and better cardiorespiratory and motor fitness[16]. Whilst none of these studies focused on inflammatory biomarkers, their results suggest that no consistent associations were observed for with lower-intensity PA[15,17], and sedentary behaviour may need to be replaced with high-intensity PA[16] to benefit health. Further research is needed to establish whether similar findings are observed for inactive populations.

There is a lack of evidence in children on the role of replacing habitual sedentary behaviour with PA on circulating inflammatory biomarkers, specifically, which make comparison

ns with the present study difficult. A previous systematic review and meta-analysis on the effects of physical exercise and high-intensity interval training on inflammatory biomarkers in children with obesity[37] showed that physical exercise, without dietary modification or other lifestyle changes, resulted in increased adiponectin (an anti-inflammatory biomarker) and a

decrease in the pro-inflammatory biomarker IL-6. As our study only found some evidence for replacing 10 min of sedentary time per day with VPA (better CRP and composite scores), future research should examine the effects of high-intensity PA interventions on children's inflammation levels. It has been suggested, however, that physical activity may have little influence on inflammatory markers in children. This may be due to the relatively low levels observed in active healthy samples[38], though it should be noted that some research has indicated that CRP, for example, may be elevated in children who are overweight and obese since adipose tissue (adipocytes) secrete a host of inflammatory factors[5,39]. As it is possible that overweight and obese children may respond differently to exercise programs compared to healthy weight children, future research should examine changes in inflammation in children with different weight statuses specifically.

The recently-released 24-h Australian movement guidelines[27] provide high level recommendations for sedentary behaviour (break up long periods of sitting as often as possible), LPA (engage in several hours per day), and VPA (incorporate at least three days per week). However, a specific dose is only recommended for the combined MVPA intensity (accumulate at least 60 min daily)[27]. Whilst the guidelines are based on the evidence available, it may be worth considering the inclusion of separate dose recommendations for MPA and VPA in the future. Studies that separate between MPA and VPA are necessary to identify whether there are intensity-specific benefits. For example, the present study showed that a 10-min/day replacement of sedentary behaviour with VPA was beneficially associated with CRP and both combined composite scores; yet, replacing sedentary behaviour with ≥1-min MPA bouts specifically was detrimentally associated with CRP and the inflammatory composite score. More research into the dose-response between these intensities, as well as the manner of accumulating these intensities, could inform future guidelines as to whether separate recommendations for MPA and VPA, and specific recommendations on how best to accumulate PA to benefit health, are needed.

This study has some strengths and limitations that have to be acknowledged. Although inflammatory biomarkers as health indicators amongst children is novel, and could provide insights that have previously not been considered, this was only tested in a small, healthy and

active sample. This may have led to limited significant findings across the tested associations. Future research is needed to determine whether the theoretical impact of reallocating sedentary time to physical activity differs for different population groups (e.g. meeting versus not meeting physical activity guidelines). The lack of findings in isotemporal models may also be explained by the participants' age range and their limited cumulative exposure to unhealthy lifestyle behaviours, such as excessive sedentary behaviour, and comorbidities, including obesity. As only 53 participants (35% of the sample) were overweight or obese, stratified analysis per weight status was not conducted. As previous research has indicated that weight status may impact children's inflammation levels[4-6], future research should specifically investigate the replacement that sedentary behaviour with PA has on weight status using larger samples. In addition, stratification based on level of inflammation, and the inclusion of children whom have various illnesses that may be associated with increased inflammation (e.g., cancer, various forms of arthritis), could provide useful insights in the relationships between the activity pattern and inflammatory biomarkers. Due to the cross-sectional nature of this study, causal relationships cannot be determined. It is thus unclear whether there are any long-term associations between sedentary behaviour, PA and inflammatory biomarkers long-term. Finally, data were collected using waist-worn accelerometers processed by applying thresholds to epoch-level data. Whilst this methodology has acceptable validity for capturing total volumes of PA, research has suggested it may have limited validity for measuring accumulation patterns[40] given it is difficult to discern sitting from standing. Posture-based device should be considered in future studies.

There are also some statistical considerations that should be discussed. Firstly, due to the high number of results within this study, there may be an increased likelihood of false discovery due to multiple testing. As this is a relatively unexplored area of behavioural research, especially in children, the conscious decision was made not to adjust for multiple comparisons[41,42]. It is also acknowledged that there is ongoing debate about the advantages and disadvantages of the present isotemporal substitution approach ("standard") versus the "compositional" isotemporal approach[12,13], and other statistical methods such as multivariate pattern analysis[14]. As the "standard" isotemporal approach used in this paper does not account for the fixed number of hours in a day, it may have been appropriate to use the "compositional" isotemporal substitution. However, as waking hours in the day were not fixed and data on sleep was not

collected in the current study, the "standard" isotemporal approach was used to obtain results in absolute, rather than relative, amounts[13]. Further the "compositional" isotemporal substitution approach is still developing, results can be more difficult to interpret, there may still be high correlation between the predictors included in the model, and it induces "altered and distorted" correlations between PA variables[14]. The authors acknowledge that every modelling approach has limitations[41], including the "standard" isotemporal substitution modelling used in this paper, and therefore results should be interpreted with caution[14].

In conclusion, while replacing sedentary time with VPA showed some beneficial associations with children's inflammatory biomarkers, this study suggest that replacing 10 min of sedentary behaviour per day with PA, regardless of intensity or accumulation, is unlikely to provide meaningful benefits to systemic inflammation in children. Future longitudinal research using posture-based devices are needed to investigate the long-term impacts of reallocating sedentary time with an equal amount of total and ≥1min-bout-accumulated time of PA for inflammatory biomarkers. This can inform public health interventions and policies on whether to include specific recommendations for MPA and VPA, and on how to best accumulate these intensities to influence health outcomes.

Perspective

Research in children has typically focused on associations between the activity spectrum (from sedentary behaviour to vigorous-intensity PA) and adiposity markers or blood lipids, with few studies investigating associations with non-traditional health biomarkers, such as inflammatory cytokines[1,2]. In this study, we used isotemporal substitution modelling to assess the associations of hypothetical replacements of sedentary behaviour with an equal amount of total and ≥1min-bout-accumulated time spent in different activity intensities, on inflammatory biomarkers in 8-9 year olds. This study is novel as isotemporal substitution allows combined assessment of intensities across the activity spectrum[13] and it looks beyond traditional health markers by examining both pro-inflammatory (e.g., TNF-α, IL-6) and anti-inflammatory (e.g., IL-10, adiponectin) biomarkers. As these markers have been implicated in the causal pathway to various chronic diseases[4,6], they may provide novel insights into the potential role of PA and

sedentary behaviours in the early development of cardiometabolic diseases. Whilst this exploratory study found limited evidence of theoretical impact of replacing sedentary time with PA on markers of systemic inflammation, it contributes to the evidence base informing public health interventions and policies on how to best accumulate these intensities to influence health outcomes.

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493 **Tables**

Table 1. Participant characteristics (n=149)

Demographic characteristics	**Mean (SD)**
Age	8.7 (0.4)
Sex (% girls)	56%
Waist circumference	
Continuous (cm)	59.6 (6.1)
Categorical (% overweight or obese) [A]	36%
Diet density (range: 8-70 [observed range 11-41])	21.3 (5.0)
Inflammatory biomarkers	**Median (IQR)**
C-reactive protein (mg/L)	0.5 (0.1-0.6)
Interleukin-2 (pg/mL)	1.1 (0.3-3.0)
Interleukin-6 (pg/mL)	0.4 (0.2-1.2)
Interleukin-8 (pg/mL)	5.6 (4.4-7.8)
Interleukin-10 (pg/mL)	13.6 (8.8-26.6)
TNFα (pg/mL)	9.8 (7.3-12.0)
Adiponectin (mg/mL)	26.1 (18.8-35.1)
Inflammatory composite score [B]	-0.6 (-1.3-0.0)
Pro-inflammatory composite score [C]	-1.1 (-1.8-0.4)
Accelerometry variables	**Mean (SD)**
Total sedentary time (min/day)	403.7 (55.2)
Total LPA (min/day)	230.6 (36.3)
Total MPA (min/day)	56.7 (13.4)
Total VPA (min/day)	27.8 (13.4)
Total time in ≥1-min LPA bouts (min/day)	95.7 (23.7)
Total time in ≥1-min MPA bouts (min/day)	9.0 (4.0)
Total time in ≥1-min VPA bouts (min/day)	7.6 (7.0)
Meeting 60 min of MVPA/day (25) (%yes) [D]	85%

494 SD: Standard deviation; IQR: Interquartile range; LPA: Light-intensity physical activity; MPA:
495 Moderate-intensity physical activity; VPA: Vigorous-intensity physical activity; MVPA: Moderate-
496 to-vigorous-intensity physical activity.

497 [A] Children were categorized as healthy weight, overweight (≥75th) or obese (≥90th) based on the
498 Australian age- and sex-specific percentile curves [36].

499 [B] The inflammatory composite score was created by summing the z-values for all inflammatory
500 biomarkers. IL-10 and adiponectin were multiplied by -1 as they are classified as anti-
501 inflammatory biomarkers [20].

502 [C] The pro-inflammatory composite score was created using the pro-inflammatory biomarkers
503 only (CRP, IL2, IL6, IL8, TNFα) [20].

504 [D] The recently-released 24-h Australian movement guidelines [27] state that children should engage
505 in 60 min of moderate-to-vigorous-intensity physical activity (MVPA) per day. Adherence to these
506 guidelines is reported based on the average MVPA across valid wear days.

Table 2. Isotemporal model estimated changes (β 95% CI) in standardized inflammatory biomarkers attributable to substituting 10 min of sedentary behaviour per day with an equal amount of time in physical activity and in ≥1-min physical activity bouts (n = 149)

	LPA		MPA		VPA	
	β (95% CI)	*r*	β (95% CI)	*r*	β (95% CI)	*r*
Total duration						
C-reactive protein (mg/L)	1.00 (0.95, 1.04)	-0.01	0.96 (0.90, 1.03)	-0.10	**0.96 (0.93, 0.99)**	**-0.22**
Interleukin-2 (pg/mL)	1.00 (0.99, 1.01)	0.01	1.01 (0.97, 1.06)	0.05	0.98 (0.95, 1.00)	-0.13
Interleukin-6 (pg/mL)	1.00 (0.99, 1.02)	0.03	0.96 (0.92, 1.01)	-0.12	0.99 (0.98, 1.01)	-0.05
Interleukin-8 (pg/mL)	1.00 (0.98, 1.03)	0.02	1.03 (0.96, 1.10)	0.06	0.95 (0.91, 1.01)	-0.14
Interleukin-10 (pg/mL)	0.99 (0.98, 1.01)	-0.08	1.03 (0.98, 1.09)	0.09	1.00 (0.97, 1.03)	-0.01
TNFα (pg/mL)	0.99 (0.97, 1.02)	-0.04	0.98 (0.93, 1.03)	-0.07	1.01 (0.96, 1.06)	0.02
Adiponectin (mg/mL)	0.98 (0.94, 1.03)	-0.06	1.03 (0.91, 1.18)	0.04	0.97 (0.86, 1.09)	-0.04
Inflammatory composite score [A]	0.97 (0.90, 1.06)	-0.05	1.01 (0.87, 1.16)	0.01	**0.87 (0.76, 0.99)**	**-0.17**
Pro-inflammatory composite score [B]	1.00 (0.92, 1.08)	-0.01	0.95 (0.83, 1.08)	-0.07	**0.90 (0.81, 0.99)**	**-0.18**
Accumulated in ≥1-min bouts						
C-reactive protein (mg/L)	1.02 (0.97, 1.07)	0.05	**1.23 (1.02, 1.49)**	**0.18**	0.98 (0.89, 1.07)	-0.04

Interleukin-2 (pg/mL)	1.00 (0.98, 1.01)	-0.05	1.00 (0.88, 1.13)	0.00	0.95 (0.90, 1.01)	-0.15
Interleukin-6 (pg/mL)	1.01 (0.99, 1.03)	0.09	0.98 (0.90, 1.07)	-0.03	1.02 (0.98, 1.05)	0.08
Interleukin-8 (pg/mL)	1.01 (0.96, 1.05)	0.02	1.07 (0.93, 1.25)	0.08	0.98 (0.94, 1.01)	-0.11
Interleukin-10 (pg/mL)	0.98 (0.96, 1.00)	-0.16	1.01 (0.91, 1.13)	0.02	1.00 (0.94, 1.06)	0.00
TNFα (pg/mL)	0.98 (0.94, 1.02)	-0.08	1.05 (0.87, 1.26)	0.04	1.00 (0.94, 1.08)	0.01
Adiponectin (mg/mL)	1.01 (0.89, 1.13)	0.01	1.45 (0.87, 2.41)	0.12	1.15 (0.92, 1.44)	0.10
Inflammatory composite score [A]	0.99 (0.85, 1.16)	-0.01	**2.00 (1.13, 3.52)**	**0.20**	1.07 (0.80, 1.42)	0.04
Pro-inflammatory composite score [B]	1.01 (0.90, 1.12)	0.01	1.36 (0.95, 1.94)	0.14	0.92 (0.78, 1.10)	-0.07

Regression coefficients (β) and 95% confidence intervals (CI). **Bold values** denote statistical significance at the $p<0.05$ level.

Model Z statistics were transformed into effect sizes using $r = Z / \sqrt{N}$. Consequently, effect sizes of ≥0.1, ≥0.3, and ≥0.5 were considered small, medium and large effects, respectively.

LPA: Light-intensity physical activity; MPA: Moderate-intensity physical activity; VPA: Vigorous-intensity physical activity.

To aid comparisons between inflammatory biomarkers, all biomarkers were transformed to standardized z-values (z-value = [value-mean]/SD).

The β-coefficients estimate the associations for replacing 10 min of sedentary time per day with an equal amount of time in a given type of activity. All models included robust standard errors accounting for clustering at the school level, and adjusted for child sex, age, and diet density. The

models used to assess replacements of sedentary behaviour with ≥1min-bout-accumulated PA time, included the time in the intensity of interest subdivided in bouted and other time, to keep the "total time" constant.

[A] The inflammatory composite score was created by summing the z-values for all inflammatory biomarkers. IL-10 and adiponectin were multiplied by -1 as they are classified as anti-inflammatory biomarkers [20].

[B] The pro-inflammatory composite score was created using the pro-inflammatory biomarkers only (CRP, IL2, IL6, IL8, TNFα) [20].