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8 9	Adiposity in Preadolescent Children: Associations with Cardiorespiratory Fitness
10	Running Title: Adiposity in Preadolescent Children
11	
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28 29	ABSTRACT Lifestyle factors contribute to childhood obesity risk, however it is unclear which lifestyle factors are most strongly
30	associated with childhood obesity. The purpose of this cross-sectional study was to simultaneously investigate the
31	associations among dietary patterns, activity behaviors, and physical fitness with adiposity (body fat %, fat mass, body
32	mass index [BMI], and waist to hip ratio) in preadolescent children. Preadolescent children (N=392, 50% female, age: 9.5
33	\pm 1.1year, BMI: 17.9 \pm 3.3 kg/m ²) were recruited. Body fat (%) and fat mass (kg) were measured with bioelectrical
34	impedance analysis. Cardiorespiratory fitness (VO2 max), muscular strength (hand-grip strength), activity, sleep, and
35	dietary pattern was assessed. Multivariable analysis revealed that cardiorespiratory fitness associated most strongly with
36	all four indicators of adiposity (body fat (%) (β = -0.2; p < .001), fat mass (β = -0.2; p < .001), BMI (β = -0.1; p < .001) and
37	waist to hip ratio (β = -0.2; <i>p</i> < .001). Additionally, fruit and vegetable consumption patterns were associated with body fat
38	percentage, but the association was negligible (β = 0.1; p=0.015). Therefore, future interventions should aim to promote
39	the use of cardiorespiratory fitness as a means of reducing the obesity epidemic in children.
40	KEY WORDS

41 Lifestyle factors; Childhood Obesity; Obesity Risk; VO₂ max

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3

43	INTRODUCTION
44	Obese children are at heightened risk for developing early-onset cardiometabolic diseases such as type 2 diabetes
45	and hypertension (1). Considering the global rise in childhood obesity, this is a major public health concern (2). For
46	example, in New Zealand an estimated 1 in 10 children are overweight or obese (3) and prevalence has increased from
47	8% in 2006-07 to 14.9% in 2017-2018 (4). Emerging evidence suggests the rise in obesity is likely attributed to lifestyle
48	factors such as unhealthy dietary pattern and activity (physical activity [PA], sedentary [SB], sleep) behaviors (5) coupled
49	with poor physical fitness (low cardiorespiratory fitness [CRF] and low muscular strength). However, it is unclear how
50	simultaneous associations of lifestyle factors and adiposity present in preadolescent children. Identifying the lifestyle
51	factors from a comprehensive investigation of potential risk factors that most strongly associated with childhood obesity is
52	crucial for informing the design of public health interventions.
53	Obesity is a multidimensional disease in children (5-12 years old). Lifestyle factors such as insufficient daily PA
54	(6,7), high SB (6,7), low physical fitness (8–10), poor sleep (11–13), and unhealthy dietary patterns (14) all likely
55	contribute in both independent and interactive ways. Lifestyle factors interact with one another(15), where one activity
56	behavior influences time spent, or not spent, in the other behaviors. For example, when children engage in SB (any
57	waking behavior in a seated or reclined posture with low energy expenditure <1.5 metabolic equivalents(16)) such as
58	screen time, they are not only being physically inactive, but may also be snacking on unhealthy foods(15). While our data

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59	is not sufficient to explore this, in the aforementioned theoretical example SB is not the only activity increasing obesity
60	risk, instead SB is acting in combination with reduced PA and unhealthy dietary patterns to increase obesity risk.
61	The purpose of this cross-sectional study was to investigate the associations among lifestyle factors (PA, SB, sleep
62	[duration, social jetlag, disturbance], physical fitness (CRF and muscular strength) and dietary patterns with adiposity in
63	preadolescent children.
64 65	METHODOLOGY This observational study was carried out in accordance with STROBE (Strengthening the Reporting of
66	Observational Studies in Epidemiology) guidelines (17). The methodology was prospectively detailed in Castro et al. (18).
67	STUDY DESIGN AND PARTICIPANTS
68	Children aged 8 to 10 years were randomly sampled from schools in three major cities across New Zealand
69	(Wellington, Christchurch, and Dunedin). At invited schools, all children were eligible to participate unless they had an
70	orthopedic injury or surgery that prohibited full physical function within the previous 4 weeks or were currently prescribed
71	any cardiovascular medications. Parental or guardian consent and child assent were obtained prior to participation in
72	accordance with the requirements of the New Zealand Health Disability Ethics Committee (14/CEN/83) and registered
73	with the Australia and New Zealand Clinical Trial Registry (ACTRN12614000433606).
74	Data for this study were collected as part of a larger cross-sectional study (18). The measurements detailed in this
75	article were taken between 9 AM and 12 PM at the child's school. Children were asked to fast for 3 hours and to refrain
	5 This is an accepted version of an article published in PLoS One, available at https://doi.org/10.1371/journal.pone.0275982. © The Authors, 2022

- ⁷⁶ from exercise for 24 hours prior to assessment. Within 7 days of the in-person assessments described below PA/SB,
- dietary patterns, sleep habits, and demographic data were collected using a questionnaire. The questionnaires were
- ⁷⁸ jointly completed at home by the primary caregiver and participant using an online survey.

OUTCOME MEASURES
 The four outcome measures included: body fat (%), fat mass index (FMI), body mass index (BMI), and waist to hip ratio
 (WHR).

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83	ANTHROPOMETRIC
84	To calculate the anthropometric indices height, weight, and waist and hip circumference were measured. Height
85	and weight were measured to the nearest 0.1 decimal, using a calibrated portable stadiometer (Seca 213, Hamburg,
86	Germany) and a calibrated portable scale (Seca 813, Hamburg, Germany), respectively, with shoes and socks removed
87	and head in the Frankfort plane. Using nonelastic tape (Seca 203, Hamburg, Germany), waist and hip circumference were
88	measured to the nearest 0.1 cm according to standard practice to measure WHR(13). For each assessment, participants
89	were measured twice, and the average was recorded (unless the two measurements were more than 0.5 cm apart; then a
90	third measurement was taken and the average of the three was recorded) (13). Age and sex-specific BMI z-scores were
91	calculated using the World Health Organization growth guidelines (19). BMI values were categorized using the
92	International Obesity Task Force's sex and age-dependent cutoff points (20).

6

93	BODY FAT
94	Body fat (%) and fat mass (kg) were measured via multifrequency body impedance analysis (BodyStat Quadscan
95	4000, Isle of Man, UK). The instrument was calibrated in accordance with the manufacturer's instructions, and
96	measurements were conducted according to standardized procedures (21). FMI was calculated by dividing fat mass (kg)
97	by height squared (m ²)(22).
98	INDEPENDENT VARIABLES
99	The ten behavioral variables measured included: PA, SB, sleep (duration, social jetlag, disturbance), dietary
100	patterns (processed foods, fruits and vegetables, breakfast food), and physical fitness (CRF, muscular strength).
101	PHYSICAL ACTIVITY AND SEDENTARY BEHAVIOR
102	The Youth Physical Activity Questionnaire (YPAQ) was used to measure PA and SB(23). To determine how many
103	minutes a day each participant was active and sedentary, participants and their caregiver were asked to jointly complete
104	the 47-item YPAQ. The YPAQ assessed the frequency, duration, and type of PA and SB the participant took part in 7
105	days prior to data collection (24,25). Frequency and duration were used to calculate the total number of active and
106	sedentary minutes on a day-to-day basis, giving each participant a daily average and weekly total of active and sedentary
107	minutes. Types of activities were utilized to classify actions as active movements or SB. For example, playing rugby,
108	walking to school, or skipping were considered being active, whereas reading, watching television, and doing homework
109	were considered being sedentary.
1	7

110	SLEEP
111	Sleep duration, social jetlag (the discrepancy between an individual's circadian clock and social rhythm)(13), and
112	sleep disturbances were recorded to evaluate sleep. To determine average sleep duration, the participant's caregiver(s)
113	was/were asked to note what time their child usually went to bed and what time they usually got up on both school days
114	and weekend days. Single items of habitual school/weekday sleep show reasonable concurrent validity with actigraphy
115	and diary data (26). Average sleep duration was calculated using a ratio of 5 weekdays to 2 weekend days. Social jetlag
116	was calculated as the absolute difference between the midpoints of sleep on weekdays versus weekend days (27). Sleep
117	disturbances were recorded using the 33-item Children's Sleep Habit Questionnaire (CSHQ), which demonstrates
118	adequate internal consistency, acceptable test-retest reliability, and discriminant validity (28). The 33 questions were
119	answered on a 7-point Likert scale from 7 (always) to 0 (never), with higher scores indicative of greater sleep disturbance.
120	The CSHQ includes eight subscales that align with the key sleep complaints relevant for this age group: bedtime
121	resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep-disordered breathing, and
122	daytime sleepiness. A Total Sleep Disturbances score was calculated as the sum of all CSHQ scored questions, with a
123	potential range of 33 to 99. A Total Sleep Disturbances score > 41 was used to indicate significant pediatric sleep
124	disturbance, as this cutoff point has been shown to accurately identify 80% of children with a clinically diagnosed sleep
125	disorder (28). For this study, only the Total Sleep Disturbances score was analyzed (28).

8

126	DIETARY PATTERNS
127	The Physical activity, Exercise, Diet And Lifestyle Study (PEADALS)-Food Frequency Questionnaire (FFQ) was
128	used to assess dietary patterns of participants (14). The 28-item PEDALS-FFQ has been validated in this age group and
129	shows acceptable reliability and validity (29). In this study, these 28 items were aggregated into 21 groups, and principal
130	components analysis (PCA) was conducted to identify components/patterns from these 21 food groups (11,29). PCA
131	restructures large data samples into new combined variables called principal components (30). The principal components
132	account for variation in the sample, enabling the dietary data to be captured with fewer variables. Determining the number
133	of components/patterns to be retained was based on the eigenvalues > 1, identification of the point of inflection in the
134	scree plot, and the interpretability of factors within components/patterns (31). Three dietary components/patterns were
135	identified including i.) processed food, ii.) fruit and vegetables, and iii.) breakfast food.
136	PHYSICAL FITNESS
136 137	PHYSICAL FITNESS CRF (VO ₂ max) was estimated via the 20-meter multistage fitness test (20-MST). The 20-MST has been found to
136 137 138	PHYSICAL FITNESS CRF (VO ₂ max) was estimated via the 20-meter multistage fitness test (20-MST). The 20-MST has been found to be valid and noninvasive, is portable and space efficient, and is popular in school settings as many students can be tested
136 137 138 139	PHYSICAL FITNESS CRF (VO ₂ max) was estimated via the 20-meter multistage fitness test (20-MST). The 20-MST has been found to be valid and noninvasive, is portable and space efficient, and is popular in school settings as many students can be tested simultaneously (32–34). Participants ran in groups of 12-15 children continuously between two lines (20 meters apart) at a
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136 137 138 139 140 141 142	PHYSICAL FITNESS CRF (VO ₂ max) was estimated via the 20-meter multistage fitness test (20-MST). The 20-MST has been found to be valid and noninvasive, is portable and space efficient, and is popular in school settings as many students can be tested simultaneously (32–34). Participants ran in groups of 12-15 children continuously between two lines (20 meters apart) at a running speed (indicated by beep signal) that started at 8 km/hour in the first stage and then incrementally increased pace each minute (1km/hour for the first minute and by 0.5 km/hour in each minute stage following). The final stage was determined when the participants failed to reach the line before the signal on two consecutive occasions (beep signals) or 9

143	when the child voluntarily	y withdrew. VO ₂ max v	was estimated using the	e regression equati	on established by	/ Hamlin et
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144 al.,(33) which has been previously validated on New Zealand children: $[VO_2 \max (ml/kg) = 42.18 + (0.009 \times 20 m) + (-1.009 \times 20 m) + ($

145 0.1762 x body fat%) + (-0.4091 x maturity)]. Anthropometric measures were used to calculate maturity in accordance with

146 Mirwald et al., 2002 (35) using height, sitting height, leg length, chronological age, and their interactions. In accordance

147 with the Cooper Institute (2014) FitnessGram[®] cutoff points (beep signal), a "healthy CRF zone" (high) was reported if

148 females achieved a VO₂ max equal to or greater than 39 mL/kg/min and if boys achieved equal to or greater than 42

149 mL/kg/min(36,37). A VO₂ max below those cutoff points was categorized as "needs improvement fitness zone" (low) for

150 both sexes(37).

153

151 Muscular strength (isometric handgrip measured in kg) was assessed using a handgrip dynamometer (Camry,

152 EH101). This method is rapid, noninvasive, simple to use, inexpensive, and of minimal risk (38). The participants were

154 participant was given three attempts with each hand, alternating hands, and with a minute recovery time between each

seated with shoulders adducted and neutrally rotated, elbow flexed to 90 degrees, and wrist in a neutral position. Each

155 attempt. The highest score for each hand was recorded for analysis.

156	COVARIATE MEASUREMENTS
157	Demographic data collected included participants' date of birth, age, sex, ethnicity, and school address. The

158 ethnicity data were applied to categorize participants into four classifications: (a) New Zealand European and Others

159	(NZEO), (b) Māori, (c) Pacific, and (d) not specified. The majority of schools in New Zealand are publicly funded and
160	classified by the predominance of students attending, giving the schools a decile classification. Schools with a greater
161	proportion of student from low socio-economic communities (decile 1) receive more funding than those fewer students
162	from low socio-economic communities (decile 10). To achieve a cohort representing different levels of socio-economic
163	position (SEP), schools within the selected cities were stratified as low (1-5) or high (6-10) decile and then randomly
164	sampled. Statistical models were adjusted for sex, ethnicity, age, and school decile as an index of SEP.
165	STATISTICAL ANALYSIS
166	The corresponding author had full access to the data in the study and was responsible for the integrity of the data
167	set and the data analysis. Anonymized data will be shared upon reasonable request. Only participants who had complete
168	data for each independent and outcome variable were included in the analyses. Statistical analyses were performed using
169	R Statistical Software, version 4.0.0. Raw data are presented as mean (standard deviation) and regression outcomes as
170	unstandardized (b) and standardized (β) betas (effect sizes). Using the β , the effect was adjudicated as trivial (<0.2), small
171	(0.2-0.5), moderate (0.5-0.8), or large (>0.8). Additionally, point (two-sided p-value) and interval (95% confidence interval)
172	estimates of statistical significance are presented, with two-sided p-values of <0.05.
173	Linear mixed-effects models, with children nested within schools, were used to identify relationships among the
174	independent- (physical fitness and lifestyle factors) and outcome- (adiposity) variables (39). Model 1: univariable analysis,

175	in which each indep	pendent variable was	regressed against	t each adiposity	outcome. For each	independent variable,
1,0						

- 176 linearity was explored by specifying the quadratic term. In the event of non-linearity, to minimize collinearity the
- 177 independent variable was centered and then used to create the quadratic term. An independent variable was omitted from
- 178 Model 2 if it did not significantly associate at alpha < 0.10 with one of the adiposity outcomes. Model 2: unadjusted
- 179 multivariable analyses, in which all significant independent variables were regressed against each adiposity outcome.
- 180 Model 3: the multivariable models (i.e., Model 2) were adjusted for sex, ethnicity, age, and school decile. All regression
- 181 models were assessed by examination of the model residuals plotted against their normal scores. The assumptions of
- 182 normality and homoscedasticity were assessed via visual inspection of the frequency and residual distributions,
- 183 respectively. To test for multicollinearity, variance inflation factors were compared to the recommended cut-point of 10.

RESULTS PARTICIPANTS Of the 392 participants who took part in the study, only 324 participants had complete data sets (Table 1, Figure 1). Participant characteristics including age, anthropometrics, ethnicity, school decile, obesity classification, CRF classification, PA, SB, and sleep are reported (Table 1).

189 **TABLE 1.** Summary of Participant Characteristics

	Total (N =	324)
	Mean or <i>n</i>	SD/%
	12	
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Auth	ors, 2022	

Age (years)	9.56	1.14
Ethnicity		
NZEO	265	82
Māori	37	11
Pacific	18	6
NR	4	1
School year		
4	82	21
5	114	29
6	127	32
7	69	18
School Decile (SEP)		
Low (<u><</u> 5)	163	50
High (>5)	161	50
Adiposity		
Body Fat (%)	19.9	9.39
FMI (kg/m²)	3.61	2.37
WHR	0.84	0.05
BMI (kg/m²)	17.9	3.25
Overweight	89	27
Non-Overweight	235	73
Physical Activity & Sedentary Behavior		
Physical Activity (min/day)	166	136
Sedentary (min/day)	284	208
Cardiorespiratory Fitness		
VO ₂ max (ml•kg-1•min-1)	42.9	4.36
Low	150	45
High	174	52

Sleep			
•	Average Sleep Duration (hours)	10	1
	Social Jetlag (hours)	1	1
	Sleep Disturbances	40.3	5.94

Abbreviations: SEP, socio-economic position; NZEO, New Zealand European or other; NR not recorded; FMI, fat mass index; WHR, waist-to-hip ratio; BMI, body mass index; kg/m²: kilogram/meters; VO2max: maximum volume of oxygen uptake; ml•kg-1•min-1: milliliters/kilogram/minute

190 Participant characteristics including demographics summarized in table 1. Data reported as the mean (or N) with standard 191 deviation.

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Figure 1. Study design and protocol. Includes information regarding recruitment, data collection, <u>This is an acceptec</u> and analysis timeframe. <u>ne.0275982. © The</u>

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197 198	Fig 1. Study design and protocol. Includes information regarding recruitment, data collection, and analysis timeframe.
199	LINIVARIATE MODELS
200	Model 1, Table 2 presents the independent variables that significantly associated with at least one of the adiposity
201	outcomes. PA, SB, and handgrip strength did not significantly associate with one of the adiposity outcomes and were
202	omitted from multivariable analysis. The association of body fat (%) with VO2 max was non-linear, therefore VO2 max and
203	the associated non-linear/polynomial (VO ₂ max _{Poly}) were used to account for non-linearity (Figure 2).

204 **Table 2.** Linear Association Between Adiposity and Lifestyle Factors

		Univariable					Multivariable										
		Model 1					Model 2 (school adjusted)					Model 3 (adjusted)					
	β	b	95%LCI	95%UCI	Р	β	b	95%LCI	95%UCI	Р	β	b	95%LCI	95%UCI	Р		
Body Fat (%)																	
VO₂max	-0.8	-1.6	-1.8	-1.5	<.001	-0.2	-1.6	-1.8	-1.5	< .001	-0.2	-1.6	-1.8	-1.5	< .001		
VO₂max Poly	0.3	0.1	0.1	0.1	<.001	0.3	0.1	0.1	0.1	< .001	0.0	0.1	0.1	0.1	< .001		
Sleep Duration	0.0	0.3	-0.8	1.5	0.595	0.1	0.6	-0.1	1.4	0.103	0.1	0.7	-0.1	1.4	0.085		
Social Jetlag	0.1	2.6	0.8	4.4	0.005	0.0	0.5	-0.7	1.7	0.421	0.1	0.4	-0.8	1.6	0.518		
Sleep Disorders	0.1	0.2	0.0	0.4	0.013	0.0	0.1	0.0	0.2	0.182	0.0	0.1	0.0	0.2	0.189		
Processed Foods	0.1	0.5	-0.1	1.1	0.088	0.0	0.1	-0.3	0.4	0.766	0.0	0.1	-0.3	0.4	0.725		
Fruits/Vegetables	-0.1	-0.3	-1.0	0.3	0.279	0.1	0.5	0.1	0.9	0.012	0.1	0.5	0.1	0.9	0.015		
Breakfast Foods	-0.1	-0.9	-1.7	-0.2	0.018	0.0	-0.4	-0.9	0.1	0.154	0.0	-0.4	-0.9	0.1	0.150		
Fat Mass Index (kg/m ²)																	
VO₂max	-0.8	-0.4	-0.4	-0.4	< .001	-0.2	-0.4	-0.4	-0.4	< .001	-0.2	-0.4	-0.5	-0.4	< .001		
VO₂max Poly	0.4	0.0	0.0	0.0	< .001	0.4	0.0	0.0	0.0	< .001	0.0	0.0	0.0	0.0	< .001		

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	Sleep Duration	0.0	-0.1	-0.4	0.2	0.533	0.0	0.0	-0.2	0.2	0.945	0.0	0.1	-0.1	0.3	0.496
	Sleep Disorders	0.0	0.1	0.0	0.1	0.006	0.0	0.1	-0.2	0.4	0.624	0.0	0.0	-0.2	0.3	0.767
	Social Jetlag	0.3	0.7	0.2	1.1	0.004	0.0	0.0	0.0	0.0	0.143	0.0	0.0	0.0	0.0	0.095
	Processed Foods	0.1	0.1	0.0	0.3	0.078	0.0	0.0	-0.1	0.1	0.948	0.0	0.0	-0.1	0.1	0.795
	Fruits/Vegetables	-0.1	-0.2	-0.3	0.0	0.067	0.1	0.1	0.0	0.2	0.133	0.0	0.1	0.0	0.2	0.102
	Breakfast Foods	-0.1	-0.2	-0.4	0.0	0.027	0.0	-0.1	-0.2	0.0	0.170	0.0	-0.1	-0.2	0.0	0.227
Body Mass Index	(kg/m²)															
	VO₂max	-0.5	-0.1	-0.2	-0.1	<.001	-0.1	-0.1	-0.2	-0.1	< .001	-0.1	-0.2	-0.2	-0.1	< .001
	VO₂max Poly	0.3	0.0	0.0	0.0	<.001	0.3	0.0	0.0	0.0	< .001	0.0	0.0	0.0	0.0	<.001
	Sleep Duration	0.0	0.0	-0.2	0.1	0.556	0.0	0.0	-0.2	0.1	0.801	0.0	0.0	-0.1	0.2	0.672
	Sleep Disorders	0.0	0.0	0.0	0.0	0.061	0.0	0.1	-0.1	0.3	0.468	0.1	0.1	-0.1	0.3	0.382
	Social Jetlag	0.2	0.3	0.0	0.5	0.037	0.0	0.0	0.0	0.0	0.522	0.0	0.0	0.0	0.0	0.337
	Processed Foods	0.0	0.0	-0.1	0.1	0.890	-0.1	0.0	-0.1	0.0	0.296	0.0	-0.1	-0.1	0.0	0.116
	Fruits/Vegetables	-0.1	-0.1	-0.2	0.0	0.038	0.0	0.0	-0.1	0.1	0.896	0.0	0.0	-0.1	0.1	0.795
	Breakfast Foods	-0.1	-0.1	-0.2	0.0	0.046	-0.1	-0.1	-0.1	0.0	0.241	0.0	0.0	-0.1	0.1	0.429
Waist: Hip																
	VO₂max	0.0	0.0	0.0	0.0	0.014	0.0	0.0	0.0	0.0	0.025	-0.2	0.0	0.0	0.0	<.001
	VO₂max Poly	0.0	0.0	0.0	0.0	0.019	0.0	0.0	0.0	0.0	0.028	0.1	0.0	0.0	0.0	0.064
	Sleep Duration	-0.1	0.0	0.0	0.0	0.220	-0.1	0.0	0.0	0.0	0.245	0.0	0.0	0.0	0.0	0.384
	Sleep Disorders	0.0	0.0	0.0	0.0	0.948	0.1	0.0	0.0	0.0	0.354	0.1	0.0	0.0	0.0	0.229
	Social Jetlag	0.1	0.0	0.0	0.0	0.149	0.0	0.0	0.0	0.0	0.619	0.0	0.0	0.0	0.0	0.782
	Processed Foods	0.0	0.0	0.0	0.0	0.808	0.0	0.0	0.0	0.0	0.693	0.0	0.0	0.0	0.0	0.399
	Fruits/Vegetables	0.0	0.0	0.0	0.0	0.479	0.0	0.0	0.0	0.0	0.881	0.0	0.0	0.0	0.0	0.826
	Breakfast Foods	0.0	0.0	0.0	0.0	0.862	0.0	0.0	0.0	0.0	0.963	0.0	0.0	0.0	0.0	0.782

Model 1: univariable Model 2: school only Model 3: gender, ethnicity, age, decile

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205 Linear association between adiposity and lifestyle factors displayed with significant associations for each model bolded.

Abbreviations: UCI: upper confidence interval; LCI: lower confidence interval; kg/m²: kilogram/meters; VO₂max: maximum volume of oxygen uptake; ml•kg-1•min-1: milliliters/kilogram/minute

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- 216 consumption were associated with body fat (%) (β = 0.1, p = 0.012). For Model 3, which adjusted for sex, ethnicity, age,
- 217 and school decile, VO₂ max remained associated with all four adiposity measures: body fat (%), FMI, BMI, and WHR (β =

-0.2, -0.2, -0.1, -0.2, respectively; all p < 0.001, Table 2), and fruit and vegetable consumption remained associated with

219 body fat (%) (β = 0.1, p = 0.015, Table 2).

220 DISCUSSION

221	The purpose of this study was to investigate the associations among activity behaviors including physical fitness,
222	PA, SB, dietary patterns, and sleep with adiposity in preadolescent children. Following adjustments for potential
223	confounders, this study clearly indicated that CRF, as measured VO2 max estimated via the 20-meter multistage fitness
224	test, was associated with high body fat percentage, greater BMI, greater FMI, and high WHR. Fruit and vegetable
225	consumption independently associated with body fat percentage. The effect of the association between fruit and
226	vegetable consumption and body fat percentage was not in the expected direction however, it should be noted the effect
227	as negligible. Our findings suggest that increases in CRF may result in lower levels of adiposity outcomes, and thereby
228	obesity risk in preadolescent children.
229	LIMITATIONS AND STRENGTHS OF THE STUDY
230	This study had several potential limitations which are important to consider prior to contextualizing the results. First,
231	this was a cross-sectional study whereas further longitudinal research is required to better determine the potential causal
232	relationships between activity behaviors with adiposity. However, this initial investigation was necessary prior to allotting
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233	time and resources into costly trials. Second, data collection was conducted at primary schools in group settings;
234	therefore, the noise, facility limitations (e.g., secluded space, tinted windows, and private room), distractions, interruptions,
235	and weather could not be controlled or measured (40). Lastly, this study investigated New Zealand-based preadolescents,
236	which may limit the generalizability of the findings to preadolescent children globally. For instance, dietary intake
237	(Mediterranean diet vs. Western diet), types of PA (rugby vs. American football), and social ecological factors may affect a
238	child's physiology differently, so further research is required to determine if these findings would be consistently
239	associated with preadolescent outcomes globally. However, a considerable strength of this study was the large and
240	diverse group of New Zealand-based preadolescents included in the research from various parts of the country. Similar
241	studies have shown comparable results to this study, but most of those studies analyzed each factor independently with
242	adiposity (10,12,41–44).
243	CARDIORESPRIATORY FITNESS
244	Of the lifestyle and physical fitness factors measured, only CRF associated solely with all four estimates of
245	adiposity (body fat [%], FMI, BMI, and WHR). However, it should be recognized that CRF was nonlinearly associated with
246	body fat percentage. Beyond ~42 ml/kg/min, an increase in VO2 max did not correspond with change in body fat
247	percentage. This suggests that it may be particularly important to focus on improving CRF in children with a VO2 max

248 below 42 ml/kg/min. Compared to normative data (45), a VO₂ max of 42 ml/kg/min in children is classified as "fair" (46).

249	Therefore, "fair", "poor", or "very poor" VO2 max classification (46) is associated with higher body fat percentage. These
250	data are similar to other studies which have also demonstrated association between lower VO ₂ max and adiposity(47–50),
251	however, the present study provides continuous data to better elucidate the non-linear association between VO2 max and
252	adiposity, which is lost when VO2 max is dichotomized into classifications. Collectively, children and adolescents with low
253	levels of CRF are at greater risk of myocardial infarction, CVD, and sustaining lower than average physical fitness levels
254	in adulthood (51). Additionally, it is important to note that VO2 max classification differs by biological sex and age (i.e.,
255	puberty stage). Associations of maturation, sex, and body fat with VO2 max have been previously demonstrated (52)
256	where VO2 max is higher in males. In the present study despite the same weight (kg) between males and females, there
257	are differences in body fat percentage between the biological sexes. At the onset of puberty, females tend to have higher
258	adiposity indicating classifications for outcomes such as VO ₂ max should account for biological sex.
259	DIETARY PATTERN AND SLEEP
260	In this study, fully adjusted multivariable analyses indicated no association between any sleep variable with
261	adiposity, and no association of any dietary pattern with BMI, FMI, and WHR. However, there was a small, although
262	statistically significant (0.1, $p = 0.015$) association of the fruit and vegetables dietary pattern with body fat percentage in
263	preadolescent children. More fruit and vegetable consumption was associated with a higher body fat percentage, which
264	challenges our hypothesis that fruit and vegetable consumption would be associated with a lower body fat percentage

265	(53). This contrary association is likely due to collinearity with an additional variable; we should also emphasize that the
266	association was trivial. Additionally, it may be that individuals with higher body fat percentage likely consume more
267	calories, and thus have a higher intake of every type of food including fruit and vegetables. Further, we should emphasize
268	that our findings should not be interpreted to indicate that diet and sleep are insignificant to adiposity. For example, sleep
269	has been associated with other health behaviors, including diet (7,54), and both sleep and diet may be determinants of
270	optimal CRF. While beyond the scope of the current paper, further research is warranted to elucidate the likely complex
271	relationships between sleep, diet and activity behaviors with CRF and adiposity. It is important to note that while diet, SB,
272	sleep, and PA are undoubtably important to the health and development of a child, our findings suggest that CRF is a
273	particularly useful systems physiology target for health-based interventions in this population.
274	IMPLICATIONS
275	CRF had the strongest association with adiposity in preadolescent children. Devoting resources towards prevention
276	and intervention strategies that target improving and maintaining CRF could be one of the essential components for
277	addressing the childhood obesity epidemic, and subsequently making an impact on the deteriorating health and wellness
278	of preadolescent children. CRF is extremely important in potentially mitigating adiposity in preadolescents and should be

- targeted by improving PA levels. In adults, PA contributes to about 30% of VO₂ max whereas genetics controls the
- remaining 70% (55). In comparison, PA contributes to a moderate amount of VO₂ max in children (56). Furthermore,

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- 281 estimating CRF with a shuttle run test is a feasible and reliable measurement schools can use to track CRF as children age. Therefore, participation in PA that increases heart rate, reducing SB, and engaging in sport/physical education 282 283 classes at school should be at the forefront of pediatric health because they are associated with improving CRF. CONCLUSIONS 284 285 CRF is an especially important target for preventing adiposity in preadolescent children. Lifestyle factors inter-relate 286 with one another and additional factors such as unhealthy dietary patterns, poor sleep (i.e., high sleep disturbance or 287 social jetlag), and poor activity behaviors (low PA and high SB) are also important to consider when attempting to mitigate 288 adiposity risk. These preliminary findings suggest that CRF correlates most strongly with adiposity (all four adiposity 289 estimators) and therefore support the development of preventative measures (i.e., policy and access to physical activity in 290 school) to increase CRF to mitigate adiposity risk in preadolescent children. Therefore, future interventions and public health guidelines should strive to improve CRF in preadolescent children to prevent obesity. 291 ACKNOWLEDGEMENTS 292 293 The authors declare no acknowledgements. 294
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