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Physical Activity, Television Viewing Time, and DNA Methylation in Peripheral Blood

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ABSTRACT

VAN ROEKEL, E. H., P.-A. DUGUÉ, C.-H. JUNG, J.H. E. JOO, E. MAKALIC, E. M. WONG, D. R. ENGLISH, M. C. SOUTHEY, G. G. GILES, B. M. LYNCH, and R. L. MILNE. Physical Activity, Television Viewing Time, and DNA Methylation in Peripheral Blood. *Med. Sci. Sports Exerc.*, Vol. 51, No. 3, pp. 490–498, 2019. **Introduction:** Physical activity may affect health via DNA methylation. The epigenetic influences of sedentary behaviors such as television viewing are unknown. We performed a genomewide study of DNA methylation in peripheral blood in relation to physical activity and television viewing time. **Methods:** DNA methylation was measured using the Illumina Infinium HumanMethylation450K BeadChip array in blood samples collected at baseline ($N = 5513$) and follow-up ($N = 1249$) from participants in the Melbourne Collaborative Cohort Study. At baseline, times per week of leisure-time physical activity were self-reported. At follow-up, the International Physical Activity Questionnaire was used to assess MET-hours per week of total and leisure-time physical activity and hours per day of television viewing time. Linear mixed models were used to assess associations between physical activity and television viewing measures and DNA methylation at individual CpG sites, adjusted for potential confounders and batch effects. **Results:** At follow-up, total physical activity was associated with DNA methylation at cg10266336 ($P = 6.0 \times 10^{-9}$), annotated to the *SLA2* gene. Weaker evidence of associations ($P < 1.0 \times 10^{-5}$) were observed for an additional 14 CpG sites with total physical activity, for 7 CpG sites with leisure-time physical activity, and for 9 CpG sites with television viewing time. Changes in leisure-time physical activity between baseline and follow-up were associated with methylation changes ($P < 0.05$) at four of the seven CpG sites with weaker evidence of cross-sectional associations with leisure-time physical activity. **Conclusion:** Physical activity and television viewing may be associated with blood DNA methylation, a potential pathway to chronic disease development. Further research using accelerometer data and larger sample sizes is warranted. **Key Words:** PHYSICAL ACTIVITY, TELEVISION VIEWING, DNA METHYLATION, EPIGENETIC, PERIPHERAL BLOOD

Physical activity is associated with a reduced risk of many chronic diseases, including cardiovascular disease, type 2 diabetes, and cancer (1,2). Sedentary behavior (sitting

or lying while awake, with a low-energy expenditure) is also associated with an increased risk of cardiovascular disease, diabetes, and cancer (3,4). Importantly, sedentary behavior is different to physical inactivity, which is defined as an insufficient level of moderate- to vigorous-intensity physical activity (5). The contribution of sedentary behavior to chronic disease risk appears to be largely independent of moderate-to-vigorous physical activity (6). Understanding the biological mechanisms through which physical activity and sedentary behavior affect health can provide insight into the causal nature of associations and inform the development of mechanism-tailored prevention strategies.

One mechanism through which physical activity may influence health is by modulating gene expression via DNA methylation, an epigenetic mechanism (7). DNA methylation is the addition of a methyl group to a cytosine base neighboring a guanine base (CpG site) (8) and plays a critical role in normal human development (9). The epigenome changes

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over time (10), with ageing (10), and has been linked to genetic, environmental, and lifestyle factors (11,12). Aberrant DNA methylation has been associated with the development of inactivity-associated diseases, including cancer, Alzheimer's disease, and type 2 diabetes (9,13). Thus, DNA methylation may present a mechanistic pathway between physical activity, sedentary behavior, and health.

Short- and long-term exercise interventions can result in DNA methylation changes in skeletal muscle, heart and brain tissues, and blood (7). Observational studies have mostly focused on DNA methylation in blood from healthy adults, some reporting associations between self-reported physical activity and methylation of cardiovascular and metabolic disease-related genes (14,15), microRNA-encoding genes (16), and global methylation (17,18). However, other observational studies observed no associations between physical activity and blood DNA methylation of specific genes, global methylation, or methylation-based ageing measures (19–22), possibly (partly) due to the generally limited sample sizes ($n < 300$).

To our knowledge, no genomewide study of DNA methylation (also called epigenome-wide association studies [EWAS]) and physical activity has been conducted to date. The genomewide scanning of thousands of CpG sites in an EWAS can identify novel loci and potential mechanistic pathways. There has also been little research on sedentary behavior and DNA methylation. One study of 248 elderly individuals reported associations between sedentary behavior and methylation-based age acceleration measures, which were nonsignificant after adjustment for multiple testing (22).

We performed an EWAS of physical activity and television viewing time using data from the Melbourne Collaborative Cohort Study (MCCS). Cross-sectional associations between physical activity and television viewing time and DNA methylation in peripheral blood at individual CpG sites across the genome were evaluated using 5513 samples taken at baseline and 1249 at follow-up, a median of 11 yr later. We also performed a longitudinal analysis to assess whether changes in leisure-time physical activity between these time points were associated with methylation changes.

METHODS

Study Design and Participants

The MCCS is a prospective cohort study (23). Between 1990 and 1994, 41,513 residents of the Melbourne metropolitan area were recruited (59% women; mean age, 55 yr), and 28,240 participants participated in follow-up measurements between 2003 and 2007. The study was approved by the Cancer Council Victoria's Human Research Ethics Committee, and all participants provided informed consent.

The current study was based on baseline ($N = 5513$) and follow-up data ($N = 1249$) from MCCS participants included in one of seven previously conducted nested case-control

studies of blood DNA methylation and cancer (Fig. 1). All subjects selected as cases were free of cancer when relevant blood and questionnaire data were collected. Controls were selected using incidence density sampling and matched to cases on sex, year of birth (for colorectal cancer study, year of baseline attendance), country of birth (Australia/NZ/UK/other, Greece, and Italy), sample type (dried blood spots, peripheral blood mononuclear cells, and buffy coats), and smoking status (only lung cancer study).

DNA Methylation

Blood samples were stored as peripheral blood mononuclear cells, buffy coat samples, or dried blood spots on Guthrie cards. Samples from each study (seven case-control studies and longitudinal reanalysis at follow-up for a subset of controls; Fig. 1) were assayed in nonoverlapping periods. A detailed description of methods used for DNA extraction, DNA methylation assessment, and data processing is provided in the supplemental text appendix (see Text, Supplemental Digital Content 1, Description of methods for DNA extraction, DNA methylation assessment and data processing, <http://links.lww.com/MSS/B426>). Briefly, DNA methylation was measured using the Illumina Infinium HumanMethylation450K BeadChip (HM450K) array (Illumina, Inc., San Diego, CA), and methylation M -values were calculated for each CpG site.

Physical Activity and Television Viewing Time

At baseline, physical activity was assessed by three questionnaire items about the frequency (times per week) of moderate and vigorous exercise and walking. Participants were classified as doing some (≥ 1 time per week) or no (0 time per week) leisure-time physical activity (moderate and/or vigorous exercise). Similarly, based on the frequency of moderate and vigorous exercise and walking, participants were classified as doing some or no total physical activity.

At follow-up, the long version of the International Physical Activity Questionnaire (IPAQ) was used to assess self-reported frequency and duration of moderate and vigorous leisure-time physical activity and walking. The standard IPAQ recall period of 7 d was changed to 3 months. Metabolic equivalent of task (MET)-hours per week was calculated for leisure-time (moderate and vigorous physical activity) and total physical activity (including also walking), by multiplying the hours per week spent in each activity by their MET value and summing these. Moderate and vigorous physical activities were assigned 4 and 8 METs, respectively, according to the IPAQ scoring protocol. The MET value for walking was based on the reported pace, i.e., slow, 2.8 METs; normal/missing, 3.3 METs; brisk, 4.3 METs; and very brisk, 5.0 METs.

At follow-up, self-reported television viewing time was assessed by questionnaire items asking about total time spent watching television on week and weekend days ($\text{h}\cdot\text{d}^{-1}$). A minority of participants completed instead the standard

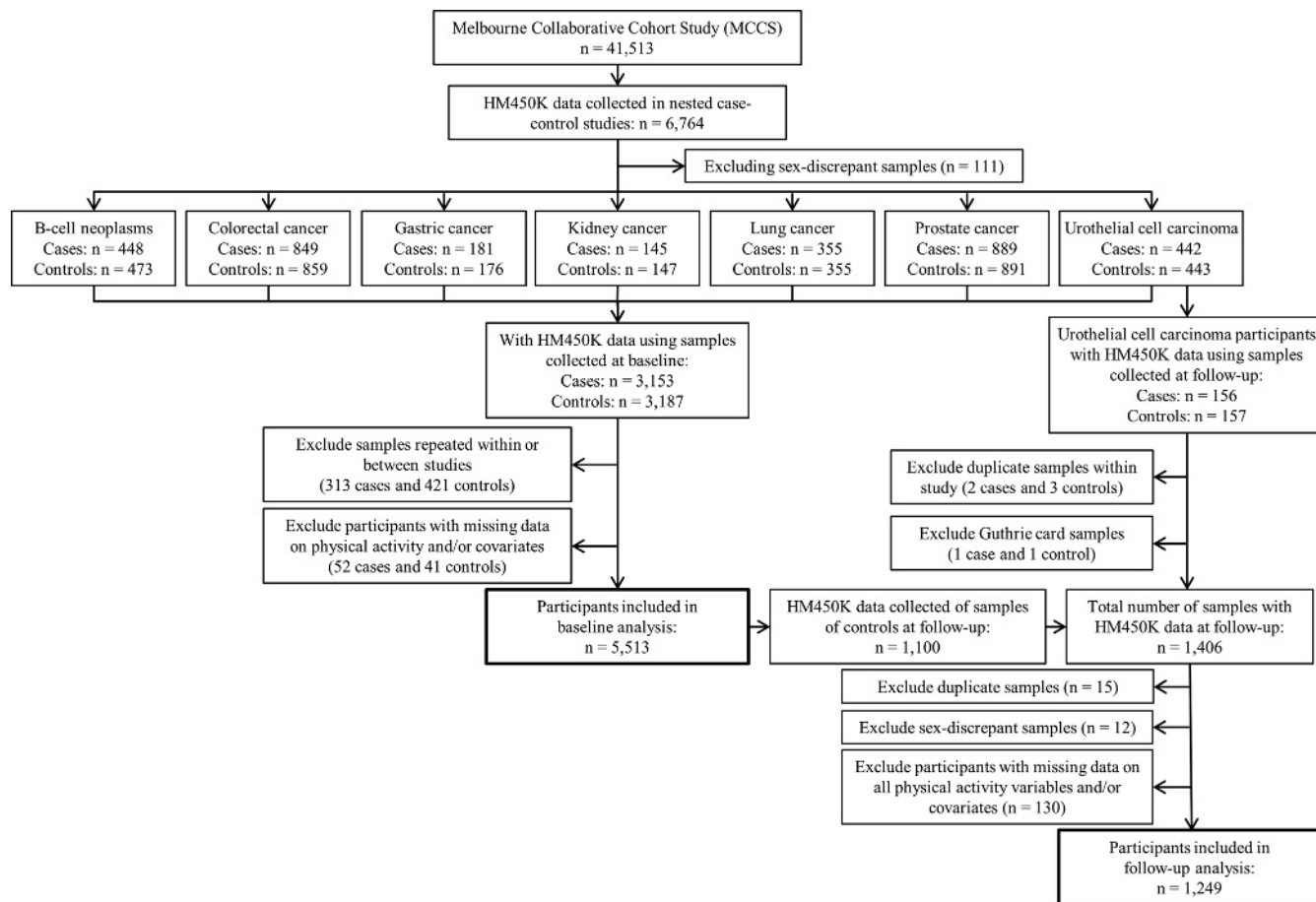


FIGURE 1—Flow chart of participant inclusion for the current analyses using baseline and follow-up data of the MCCS.

IPAQ items of total sitting time on week and weekend days. As reported previously (24), we truncated time spent watching television or sitting to a maximum of 18 h·d⁻¹.

Other Variables

Body mass index (BMI, kg·m⁻²) was calculated from measured weight and height. Smoking status (current, former, and never), cigarettes per day, and time since quitting were self-reported at baseline; these variables were reassessed at follow-up only for those who reported current or former smoking at baseline. Alcohol consumption (g·d⁻¹) was based on beverage-specific frequency and quantity questions within a self-administered food frequency questionnaire. Socioeconomic position was based on the Socio-Economic Indexes for Areas (SEIFA)—Index of Disadvantage, divided into population-based deciles.

Statistical Analysis

The reliability of methylation measures was assessed by calculating intraclass correlation coefficients, as previously described (25).

EWAS. Associations between each physical activity measure and DNA methylation *M*-values at each CpG

site were assessed by linear mixed regression. We studied cross-sectional associations at both baseline and follow-up because the baseline sample included more participants (i.e., greater power), whereas physical activity measures at follow-up were continuous and measured with a validated questionnaire (i.e., more information and probably less measurement error). Physical activity measures included “some” or “no” leisure-time physical activity at baseline and MET-hours per week of total and leisure-time physical activity at follow-up (log-transformed to better approximate a normal distribution). We observed a large peak at 0 MET·h·wk⁻¹ in the distribution of both variables. Therefore, we included in the regression model both the continuous variable as well as a dichotomous variable indicating ≥1 or 0 MET·h·wk⁻¹ and evaluated overall significance using a likelihood ratio test. Models were adjusted for age (yr, continuous), sex, country of birth (Australia/NZ/UK/other, Greece, Italy), SEIFA deciles (continuous), smoking status (never; former, quit ≥15 yr ago; former, quit <15 yr ago; current, <20 cigarettes per day; current, ≥20 cigarettes per day), alcohol consumption (ln-transformed, continuous), sample type (dried blood spots, peripheral blood mononuclear cells, buffy coats), and estimated white blood cell composition (proportion of CD4⁺ T cells, CD8⁺ T cells, NK

cells, B cells, granulocytes, and monocytes, all continuous and calculated using a modified version of the Houseman algorithm) (26) as fixed effects, and study, plate, and chip as random effects. For the follow-up analysis, study (urothelial cell carcinoma study vs longitudinal reanalysis; Fig. 1) was included as a fixed effect; sample type was not included as all urothelial cell carcinoma samples were buffy coats and all other samples were from dried blood spots.

Linear mixed regression was also applied to analyze cross-sectional associations of television viewing time ($\text{h}\cdot\text{d}^{-1}$, continuous) with methylation M -values using follow-up data. Models were adjusted for the same factors as in the follow-up physical activity analysis, and additionally for total physical activity ($\text{MET}\cdot\text{h}\cdot\text{wk}^{-1}$). Because for 149 participants data were available for total sitting time instead of television viewing time, we performed an additional analysis in a larger study sample using quintiles of television viewing or sitting time as explanatory variable (continuous).

To correct for multiple testing, we applied a statistical significance threshold of $P < 1.0 \times 10^{-7}$. The inflation of test statistics was assessed by generating quantile–quantile (Q – Q) plots and by calculating the genomic inflation factor (lambda) (27).

Sensitivity analyses. We performed a sensitivity analysis with additional adjustment for BMI. Further, we performed the baseline analysis separately for case and control participants and compared the regression coefficients for evidence of collider bias because of the sampling of participants based on their subsequent cancer diagnosis (cases).

Longitudinal analysis. Linear mixed regression was applied to assess whether a change in doing some versus no leisure-time physical activity between baseline and follow-up was associated with changes in methylation at CpG sites with evidence of cross-sectional association at $P < 1.0 \times 10^{-5}$ with leisure-time physical activity. For total physical activity, the number of participants in some change categories was too limited to perform a longitudinal analysis (see Table, Supplemental Digital Content 2, Number of participants reporting some versus no leisure-time and total physical activity at baseline and follow-up, <http://links.lww.com/MSS/B427>). We performed two separate longitudinal analyses comparing 1) participants who reported no leisure-time physical activity at baseline and some at follow-up (i.e., increased activity), with those who reported none at both time points, and 2) participants who reported some leisure-time physical activity at baseline and none at follow-up (i.e., decreased activity) with those who reported some at both time points. Because baseline and follow-up samples were assayed at different time points, ComBat was applied to adjust for batch effects (12). Regression models were adjusted for baseline age and change in age (continuous), sex, country of birth, baseline level and change in SEIFA deciles (continuous), smoking status at baseline (as defined previously) and follow-up (current; noncurrent), baseline level and change in log-transformed alcohol consumption (continuous), and baseline methylation M -value as fixed effects, and baseline study as a random effect. A

sensitivity analysis was performed without adjustment for baseline methylation M -value, as baseline adjustment may introduce bias in the analysis of change (28).

Pathway analysis. The “gometh” function of the R package *missMethyl* was used to map CpG sites with the strongest evidence of association ($P < 1.0 \times 10^{-4}$) to genes and to evaluate the overrepresentation of these genes within the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Statistical analyses were performed using R (version 3.4.2), and P values were two-sided.

RESULTS

The majority of included participants were male (68%), and the mean age was 59.2 yr (SD, 7.5 yr) at baseline and 69.1 yr (SD, 8.1 yr) at follow-up (Table 1). At baseline, 48% of participants reported some leisure-time physical activity and 78% some total physical activity, whereas these figures were 42% and 93%, respectively, at follow-up. At follow-up, participants reported a median of 17.3 $\text{MET}\cdot\text{h}\cdot\text{wk}^{-1}$ in total physical activity (interquartile range [IQR], 6.9–34.7) and 0.0 $\text{MET}\cdot\text{h}\cdot\text{wk}^{-1}$ in leisure-time physical activity (IQR, 0.0–12.0). The median reported television viewing time was 2.6 $\text{h}\cdot\text{d}^{-1}$ (IQR, 1.9–3.5).

EWAS. At follow-up, we observed a significant cross-sectional association between more total physical activity and a higher level of methylation at cg10266336 in the TSS200 region (i.e., within 200 base pairs upstream from transcription start site) of *SAAT* ($P = 6.0 \times 10^{-9}$). Weaker evidence of association ($P < 1.0 \times 10^{-5}$) was found for 14 and 7 nonoverlapping CpG sites with total and leisure-time physical activity, respectively (Table 2; Manhattan plots in Figure S1, Supplemental Digital Content 3, Results of genomewide studies of DNA methylation in peripheral blood in relation to total and leisure-time physical activity, <http://links.lww.com/MSS/B428>). For total physical activity, smaller P values were generally observed for the dichotomous variable (≥ 1 vs 0 $\text{MET}\cdot\text{h}\cdot\text{wk}^{-1}$) than for the continuous variable; no clear pattern was observed for leisure-time physical activity (Table 2). There was no clear pattern in direction of associations among CpG sites, and in some cases, the direction of associations was opposite for the dichotomous and continuous variable. We performed an additional analysis for the CpG sites with $P < 1.0 \times 10^{-5}$ for associations with total physical activity where we only included the dichotomous variable without adjustment for the continuous variable and observed significant associations for ≥ 1 versus 0 $\text{MET}\cdot\text{h}\cdot\text{wk}^{-1}$ in total physical activity with higher methylation levels at cg10266336 ($P = 6.9 \times 10^{-10}$) as well as ch.4.134822993R (intergenic probe; $P = 2.9 \times 10^{-8}$). No evidence of associations was observed with $P < 1.0 \times 10^{-5}$ for some versus no leisure-time physical activity at baseline (see Figure, Supplemental Digital Content 4, Results of genomewide study of DNA methylation in peripheral blood

TABLE 1. Characteristics of participants of the MCCS included in the current analysis of baseline and follow-up data.

	Baseline Data (N = 5513)	Follow-up Data (N = 1249)
Age (yr), mean (SD) ^a	59.2 (7.5)	69.1 (8.1)
Male sex, n (%)	3720 (67.5)	868 (67.5)
Country of birth,		
Australia/New Zealand/other	3686 (66.9)	957 (76.6)
Greece	597 (10.8)	51 (4.1)
Italy	803 (14.6)	103 (8.2)
United Kingdom/Malta	427 (7.7)	138 (11.0)
Some leisure-time physical activity (≥1 time per week), n (%) ^b	2642 (47.9)	524 (42.0)
Some total physical activity (≥1 time per week), n (%) ^c	4305 (78.1)	1157 (92.6)
Total MET-hours per week at follow-up, median (IQR) ^d	NA	17.3 (6.9–34.7)
Leisure-time MET-hours per week at follow-up, median (IQR) ^e	NA	0.0 (0.0–12.0)
Television viewing time at follow-up (h·d ⁻¹), median (IQR) ^f	NA	2.6 (1.9–3.5)
Smoking status, n (%) ^a		
Never	2497 (45.3)	605 (48.4)
Former, quit ≥15 yr ago	1112 (20.2)	458 (36.7)
Former, quit <15 yr ago	1082 (19.6)	109 (8.7)
Current, <20 cigarettes per day	314 (5.7)	45 (3.6)
Current, ≥20 cigarettes per day	508 (9.2)	32 (2.6)
Alcohol intake (g·d ⁻¹), median (IQR) ^a	4.3 (0.0–18.7)	2.3 (0.0–3.2)
BMI (kg·m ⁻²), mean (SD) ^a	26.9 (24.5–29.5)	27.1 (4.2)
Sample type, n (%) ^a		
Dried blood spots on Guthrie cards	3938 (71.4)	982 (78.6)
Peripheral blood mononuclear cell	1483 (26.9)	0 (0.0)
Buffy coat	92 (1.7)	267 (21.4)

^aVariables measured at both time points; data reported of time point indicated at top of column.

^bDefined as participating in vigorous and/or moderate exercise ≥1 time per week.

^cDefined as participating in vigorous and/or moderate exercise and/or walking ≥1 time per week.

^dDefined as total MET-hours spent on vigorous and/or moderate exercise and/or walking; data missing for seven participants included in the follow-up analysis (0.6%).

^eDefined as total MET-hours spent on vigorous and/or moderate exercise.

^fData missing for 167 participants included in the follow-up analysis (13.4%).

relation to doing some versus no leisure-time physical activity at baseline, <http://links.lww.com/MSS/B429>).

A total of nine and five nonoverlapping CpG sites showed weaker evidence of associations ($P < 1.0 \times 10^{-5}$) with television viewing time and quintiles of television viewing and sitting time, respectively (Table 3; Manhattan plots in Figure, Supplemental Digital Content 5, Results of genomewide studies of DNA methylation in peripheral blood in relation to television viewing time and quintiles of television viewing and sitting time, <http://links.lww.com/MSS/B430>). For most CpG sites (11 of 14), greater television viewing time was associated with higher methylation levels. The median intraclass correlation coefficient based on follow-up replicates for methylation M -values at CpG sites with $P < 1.0 \times 10^{-5}$ for physical activity and/or television viewing/sitting time variables was 0.07 (IQR, 0.00–0.34). The $Q-Q$ plots indicated no inflation of test statistics (see Figure, Supplemental Digital Content 6, $Q-Q$ plots of genomewide studies of DNA methylation in peripheral blood in relation to tested physical activity and television viewing and sitting time variables, <http://links.lww.com/MSS/B431>), except for total physical activity (λ , 1.12). This was not observed in $Q-Q$ plots based on P values for each of the dichotomous and continuous variable (λ , 0.97 and 1.03, respectively; results not shown).

Sensitivity analyses. The regression coefficients obtained from models with and without BMI adjustment were very similar (Pearson's $r > 0.99$; see Figure, Supplemental Digital Content 7, Comparison of regression coefficients obtained from linear mixed regression models evaluating associations of tested physical activity and television viewing and sitting time

variables with DNA methylation, with and without adjustment for BMI, <http://links.lww.com/MSS/B432>). We found no evidence for systematic differences between results from the separate analysis of baseline data for cases and controls (see Figure, Supplemental Digital Content 8, Comparison of regression coefficients obtained from linear mixed regression models evaluating associations of some versus no leisure-time physical activity with DNA methylation at baseline in cases versus controls only, <http://links.lww.com/MSS/B433>).

Longitudinal analysis. In total, 121 participants (13%) reported no leisure-time physical activity at baseline, and some leisure-time physical activity at follow-up (i.e., increase), and 264 individuals (27%) reported some leisure-time physical activity at baseline but none at follow-up (i.e., decrease; see Table, Supplemental Digital Content 2, Number of participants reporting some versus no leisure-time and total physical activity at baseline and follow-up, <http://links.lww.com/MSS/B427>). Of the seven CpG sites with $P < 1.0 \times 10^{-5}$ for cross-sectional associations with leisure-time physical activity at follow-up, associations ($P < 0.05$) were observed between changes in leisure-time physical activity (increase or decrease) and changes in methylation for cg27283993 (gene body of *KLF6*), cg05896042 (5' untranslated region of *RAB3D*), cg05467523 (TSS200 of *SV2A*), and cg17757602 (intergenic probe) (Table 4), with the directions of associations consistent with the cross-sectional results (Table 2). The regression coefficients in models without adjustment for baseline methylation were similar, albeit less statistically significant (Table 4).

TABLE 2. EWAS of total physical activity ($N = 1242$) and leisure-time physical activity ($N = 1249$) using follow-up data of the MCCS,^a showing associations with P values $<1.0 \times 10^{-5}$.

CpG site	CHR ^d	Gene ^d	Location ^d	Likelihood Ratio Test ^b	Continuous MET-Hours per Week ^c		≥ 1 vs 0 MET-Hours per Week ^c	
				P	Coeff.	P	Coeff.	P
Total physical activity								
cg10266336	11	SAA2	TSS200	6.0×10^{-9}	0.013	0.50	0.385	1.5×10^{-5}
ch.4.134822993R	4		Intergenic	2.4×10^{-7}	0.007	0.77	0.418	4.1×10^{-5}
cg00318606	11	MS4A14	TSS1500	1.1×10^{-6}	0.0003	0.98	-0.212	4.5×10^{-5}
cg10868567	8		Intergenic	1.3×10^{-6}	0.013	0.43	-0.329	6.9×10^{-6}
cg00939211	1	SNIP1	TSS200	1.6×10^{-6}	-0.011	0.58	0.386	1.5×10^{-5}
cg06104007	15	GALK2	5'UTR	1.8×10^{-6}	0.050	9.0×10^{-3}	0.153	0.078
cg00174845	18		Intergenic	2.8×10^{-6}	-0.009	0.25	-0.109	2.0×10^{-3}
cg18954163	12	DUSP16	Body	3.6×10^{-6}	-0.076	8.5×10^{-5}	0.007	0.94
cg09531019	5		Intergenic	4.1×10^{-6}	-0.060	5.6×10^{-4}	-0.048	0.54
cg15543490	3	BSN	Body	5.1×10^{-6}	-0.024	0.017	-0.084	0.066
cg19610267	10	NUDT9P1	TSS1500	6.6×10^{-6}	0.014	0.10	-0.179	3.6×10^{-6}
cg12819676	2		Intergenic	8.4×10^{-6}	-0.042	3.5×10^{-3}	-0.074	0.25
cg27317150	6	AKD1	5'UTR	8.6×10^{-6}	0.059	1.2×10^{-6}	-0.179	1.1×10^{-3}
cg23177576	2		Intergenic	8.8×10^{-6}	0.004	0.75	-0.190	8.2×10^{-5}
cg26334299	5	C5orf32	5'UTR	8.8×10^{-6}	-0.0001	0.99	-0.339	1.9×10^{-4}
Leisure-time physical activity								
cg16363970	17	CCDC144C	TSS1500	1.5×10^{-6}	0.115	2.1×10^{-7}	-0.322	9.2×10^{-7}
cg17332422	5	ADAMTS2	Body	6.1×10^{-6}	-0.076	9.6×10^{-6}	0.162	1.5×10^{-3}
cg27283993	10	KLF6	Body	6.2×10^{-6}	-0.214	8.5×10^{-4}	0.859	6.5×10^{-6}
cg05896042	19	RAB3D	5'UTR	6.2×10^{-6}	0.004	0.89	-0.160	0.035
cg05467523	1	SV2A	TSS200	6.9×10^{-6}	-0.010	0.64	-0.092	0.13
cg11031064	8	C8orf73	Body	8.7×10^{-6}	0.073	1.7×10^{-6}	-0.182	5.3×10^{-5}
cg17757602	5		Intergenic	9.3×10^{-6}	0.045	0.054	0.002	0.98

5'UTR, 5' untranslated region; CHR, chromosome; Coeff., regression coefficient; TSS200, within 200 base pairs upstream from transcription start site; TSS1500, within 1500 base pairs upstream from transcription start site.

^aFrom linear mixed models adjusted for age (yr; continuous), sex (males, females), country of birth (Australia/New Zealand/Other, Greece, Italy, United Kingdom/Malta), socioeconomic indices for areas (SEIFA; deciles, continuous), smoking status (never, former quit ≥ 15 yr ago, former quit <15 yr ago, current <20 cigarettes per day, current ≥ 20 cigarettes per day), ln-transformed (alcohol consumption +1; g d⁻¹) in previous week (continuous), sample type (follow-up samples analyzed within urothelial cell carcinoma case-control study; other follow-up samples), white blood cell composition (continuous variables for proportion of B cells, granulocytes, monocytes, NK cells, CD4⁺ T-cells, and CD8⁺ T cells) as fixed effects, and with random effects for plate and chip.

^b P values reported are for likelihood ratio test comparing the models, including both the ln-transformed variable for MET-hours per week +1 and the variable for ≥ 1 vs 0 MET-h-wk⁻¹, with the models without both these variables (all other covariates were similar).

^cCoefficients and P values reported are from models in which both the ln-transformed variable for MET-hours per week +1 and the variable for ≥ 1 vs 0 MET-h-wk⁻¹ were included as covariates (i.e., variables were mutually adjusted).

^dAccording to annotation file for Illumina Infinium HumanMethylation450K BeadChip array.

Pathway analysis. The pathway analysis indicated an overrepresentation ($P < 0.05$) of the KEGG pathways “ECM–receptor interaction” and “riboflavin metabolism” (physical activity) and “microRNAs in cancer,” “RNA degradation,”

and “p53 signaling pathway” (television viewing or sitting time) (Table 5; included CpG sites in Table, Supplemental Digital Content 9, Overview of CpG sites associated with any of the tested physical activity and television viewing/sitting

TABLE 3. EWAS of television viewing time ($N = 1078$) and combined quantiles of television and sitting time ($N = 1227$) using follow-up data of the MCCS,^a showing associations with P values $<1.0 \times 10^{-5}$.

CpG site	CHR ^b	Gene ^b	Location ^b	Coeff.	P
Television viewing time (h-d ⁻¹)					
cg11337915	21	HMGN1	Body	0.029	1.4×10^{-6}
cg26803216	1	TROVE2	5'UTR	-0.055	1.4×10^{-6}
cg03439687	13	AKAP11	3'UTR	0.037	1.5×10^{-6}
cg07109745	11	STIM1	Body	0.047	1.5×10^{-6}
cg00855479	1	VPS13D	Body	0.120	3.0×10^{-6}
cg04872848	14	FLVCR2	1st exon	0.059	5.1×10^{-6}
cg07141142	3	ZMAT3	TSS1500	-0.040	8.3×10^{-6}
ch.14.1202858F	14		Intergenic	0.184	9.6×10^{-6}
cg20114404	19	MORG1	5'UTR	-0.049	9.9×10^{-6}
Combined television and sitting time quintiles (h-d ⁻¹)					
cg26543333	19	NFIC	Body	0.033	1.5×10^{-6}
cg21354789	6	TRIM39	Body	0.110	4.1×10^{-6}
cg24926589	2		Intergenic	0.121	4.1×10^{-6}
cg12444076	21	PFKL	Body	0.026	4.1×10^{-6}
cg05938001	2	DLX1	Body	0.043	8.1×10^{-6}

3'UTR, 3' untranslated region; 5'UTR, 5' untranslated region; CHR, chromosome; Coeff., regression coefficient; TSS1500, within 1500 base pairs upstream from transcription start site.

^aFrom linear mixed models adjusted for age (yr; continuous), sex (males, females), country of birth (Australia/New Zealand/Other, Greece, Italy, United Kingdom/Malta), socioeconomic indices for areas (SEIFA; deciles, continuous), total MET-hours per week of physical activity (including the continuous variable for ln-transformed variable for MET-hours per week +1 and the dichotomous variable for ≥ 1 vs 0 MET-h-wk⁻¹), smoking status (never, former quit ≥ 15 yr ago, former quit <15 yr ago, current <20 cigarettes per day, current ≥ 20 cigarettes per day), ln-transformed (alcohol consumption +1; g d⁻¹) in previous week (continuous), sample type (follow-up samples analyzed within urothelial cell carcinoma case-control study; other follow-up samples), white blood cell composition (continuous variables for proportion of B cells, granulocytes, monocytes, NK cells, CD4⁺ T-cells, and CD8⁺ T cells) as fixed effects, and with random effects for plate and chip.

^bAccording to Illumina annotation file for Infinium HumanMethylation 450 K BeadChip array.

TABLE 4. Associations of change in any leisure-physical activity with change in methylation levels (*M*-values) between baseline and follow-up at selected CpG sites with weaker evidence of cross-sectional associations with leisure-time physical activity ($P < 1.0 \times 10^{-5}$), using data of the MCCS ($N = 969$).^a

CpG site	CHR ^e	Gene ^e	Location ^e	Beta-Value Baseline		Beta-Value Difference		Increase: 0 Time per Week of Leisure-Time Physical Activity at Baseline and ≥ 1 Time per Week at Follow-Up ^b				Decrease: ≥ 1 Time per Week of Leisure-Time Physical Activity at Baseline and 0 Time per Week at Follow-Up ^c			
				Mean	SD	Mean	SD	With Baseline Methylation Adj. ^d		Without Baseline Methylation Adj.		With Baseline Methylation Adj. ^d		Without Baseline Methylation Adj.	
				Coeff.	<i>P</i>	Coeff.	<i>P</i>	Coeff.	<i>P</i>	Coeff.	<i>P</i>				
cg16363970	17	<i>CCDC144C</i>	TSS1500	0.245	0.051	0.002	0.040	-0.011	0.73	-0.017	0.61	-0.006	0.80	-0.015	0.57
cg17332422	5	<i>ADAMTS2</i>	Body	0.930	0.018	<0.001	0.023	-0.040	0.26	-0.015	0.79	0.033	0.24	0.056	0.21
cg27283993	10	<i>KLF6</i>	Body	0.110	0.051	-0.058	0.067	0.276	0.044	0.212	0.18	-0.234	0.028	-0.222	0.072
cg05896042	19	<i>RAB3D</i>	5'UTR	0.063	0.023	-0.011	0.024	-0.162	1.8×10^{-3}	-0.141	0.038	0.122	2.9×10^{-3}	0.133	0.013
cg05467523	1	<i>SV2A</i>	TSS200	0.062	0.021	-0.008	0.024	-0.045	0.28	-0.029	0.65	0.140	1.4×10^{-5}	0.142	4.7×10^{-3}
cg11031064	8	<i>C8orf73</i>	Body	0.062	0.014	0.002	0.018	<0.001	1.00	0.014	0.75	-0.015	0.49	0.032	0.36
cg17757602	5		Intergenic	0.045	0.016	0.007	0.020	0.095	0.029	0.040	0.52	-0.139	5.0×10^{-5}	-0.146	2.9×10^{-3}

5'UTR, 5' untranslated region; adj., adjustment; CHR, chromosome; Coeff., regression coefficient; TSS200, within 200 base pairs upstream from transcription start site TSS1500, within 1500 base pairs upstream from transcription start site.

^aFrom linear mixed models adjusted for age at baseline and change in age between baseline and follow-up (yr; continuous), sex (males, females), country of birth (Australia/New Zealand/Other, Greece, Italy, United Kingdom/Malta), socioeconomic indices for areas (SEIFA) at baseline and change in SEIFA between baseline and follow-up (deciles, continuous), smoking status at baseline (never, former quit ≥ 15 yr ago, former quit < 15 yr ago, current < 20 cigarettes per day, current ≥ 20 cigarettes per day), smoking status at follow-up (current, noncurrent), ln-transformed (alcohol consumption +1) in previous week at baseline and change in ln-transformed (alcohol consumption +1) between baseline and follow-up (g·d⁻¹; continuous), baseline levels and changes between baseline and follow-up in white blood cell composition (continuous variables for proportion of B cells, granulocytes, monocytes, NK cells, CD4⁺ T-cells, and CD8⁺ T cells) as fixed effects, and with random effects for as part of which nested case-control study the baseline sample was analyzed (prostate, colorectal, lung, kidney, urothelial cell, and gastric cancer, or mature B-cell neoplasms case-control study).

^bWith no leisure-time activity at both baseline and follow-up as the reference category (i.e., comparing participants changing from no activity to activity versus no activity at both time points).

^cWith leisure-time activity at both baseline and follow-up as the reference category (i.e., comparing participants changing from activity to no activity versus activity at both time points).

^dModel additionally adjusted for the baseline *M*-value of the selected CpG site.

^eAccording to Illumina annotation file for Infinium HumanMethylation 450 K BeadChip array.

time variables at $P < 1.0 \times 10^{-4}$, that were included within the pathway analysis, <http://links.lww.com/MSS/B434>).

DISCUSSION

In this first EWAS of physical activity and television viewing time, we found a cross-sectional association between total physical activity and methylation at one CpG site, and weaker evidence of association for an additional 14 CpG sites with total physical activity, 7 CpG sites with leisure-time physical activity, and 9 (nonoverlapping) CpG sites with television viewing time. Changes in leisure-time physical activity between baseline and follow-up were associated with methylation changes at four of the seven CpG sites with weaker evidence of cross-sectional associations with leisure-time physical activity. These findings require replication. Nevertheless, our results are in line with observational and intervention research findings that physical activity may affect blood DNA methylation (7) and suggest that television viewing time may also be associated with DNA methylation, independently of moderate to vigorous physical activity.

There is some overlap between our findings and those reported in a gene ontology meta-analysis (29), which summarized results of observational and intervention studies investigating associations of exercise with DNA methylation in humans. For leisure-time physical activity, we found weak evidence of a cross-sectional and a longitudinal association with methylation at cg27283993, annotated to *KLF6*, a tumor suppressor gene involved in colon cancer development (30). The meta-analysis included a study (31) of skeletal muscle samples in men, reporting that a 6-month endurance exercise intervention decreased DNA methylation at two CpG sites annotated to *KLF6*, and increased mRNA expression of *KLF6*. Further, an animal study reported increased *KLF6* expression in adipose tissue after exercise (32). For total physical activity, associations appeared mainly driven by doing some versus no activity. Specifically, doing some versus no total physical activity was associated with methylation at cg10266336, annotated to the *SAA2* gene. This gene codes for serum amyloid A-2 protein, which is secreted in response to inflammatory cytokines (33) and is implicated in cardiovascular disease development (34). One intervention study reported upregulated *SAA2* transcription in skeletal muscle in

TABLE 5. Results of pathway analysis for CpG sites with associations at $P < 1.0 \times 10^{-4}$ with any of the physical activity and television viewing/sitting time variables, showing identified KEGG pathways with *P* values < 0.05 .

KEGG Identifier	Pathway	<i>N</i>	<i>n</i>	<i>P</i>	Genes
Physical activity					
hsa04512	ECM-receptor interaction	82	4	0.0045	<i>COL6A3</i>
hsa00740	Riboflavin metabolism	8	1	0.0489	<i>ACP1</i>
Television viewing/sitting					
hsa05206	MicroRNAs in cancer	286	4	0.0281	<i>CDK6</i>
hsa03018	RNA degradation	77	2	0.0377	<i>LSM4</i>
hsa04115	p53 signaling pathway	68	2	0.0446	<i>CDK6</i>

N, total number of annotated genes in pathway; *n*, number of genes with at least one CpG associated with any of the physical activity and television viewing/sitting time variables at $P < 1.0 \times 10^{-4}$.

response to resistance exercise in postmenopausal women (35). We also observed weaker evidence of total physical activity with methylation at cg00939211, annotated to the *SNIP1* gene, coding for smad nuclear interacting protein 1, which influences transforming growth factor- β (TGF- β) and nuclear factor kappa-B (NF- κ B) signaling (36,37). TGF- β and NF- κ B signaling are known to play an important role in cancer development (38,39) and can be activated in blood by exercise (40,41). Television viewing time showed weak evidence of association with methylation at cg07141142, annotated to the *ZMAT3* gene, which influences p53-mediated growth inhibitory signaling that is associated with cancer development (42). Pathway analysis also identified cancer-related pathways including “ECM–receptor interaction,” “microRNAs in cancer,” and “p53 signaling pathway.” Again, there is some consistency with previous results from the gene ontology meta-analysis on exercise and DNA methylation (29) that identified microRNA-associated and tumor suppressor gene networks to be involved. In our analysis, greater television viewing time was mostly associated with higher methylation levels, whereas for physical activity, no clear pattern in direction of associations was observed. Further research is required to replicate our findings and investigate whether and how these may affect gene expression.

An important strength of our study was the large sample size, although it may still have been insufficient to detect associations at the significance threshold. Because ours is the first EWAS of physical activity and television viewing time to date, we also applied a more liberal cutoff point ($P < 1.0 \times 10^{-5}$) for findings that warrant follow-up. Other strengths included the longitudinal analysis and adjustment for several potential confounders.

A limitation of our study included the use of self-reported physical activity data, which may have resulted in measurement error. We had a crude measure of physical activity at baseline and in our longitudinal analysis, which could have limited statistical power. At follow-up, we used data from the comprehensive IPAQ, which has good test–retest reliability but poor to moderate criterion validity (43). The reliability for the identified CpG sites was relatively low, and measurement error may have reduced statistical power. Nevertheless, in a previous study using the same data, we replicated many previously reported BMI-associated CpG sites and identified hundreds of novel associations that

were replicated in independent populations (12), which is indirect evidence for the validity and reliability of our data. We observed some inflation in test statistics for total physical activity, whereas none was found for the individual continuous and dichotomous variables. Inflation was still observed after adjusting for 10 methylation-based principal components (results not shown) (27); we cannot explain these findings. Some CpG sites with weaker evidence of associations with leisure-time and total physical activity showed opposite directions of associations for the dichotomous and continuous variable, which may indicate spurious findings and require further investigation.

In conclusion, we observed generally weak evidence of cross-sectional associations between physical activity and television viewing time and DNA methylation in peripheral blood, and that changes in physical activity over approximately a decade may be associated with methylation changes. Studies with accelerometer data and with larger sample sizes will be necessary to replicate our findings. Mechanistic research is needed to investigate whether DNA methylation may be a biological mechanism linking physical activity and sedentary behavior to chronic disease development.

The MCCS was made possible by the contribution of many people, including the original investigators, the teams that recruited the participants and continue working on follow-up, and the many thousands of Melbourne residents who continue to participate in the study.

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