Supplemental Information Appendix

1. UNABRIDGED METHODS **SECTION**

Methods

Study Sample

We used data from ALSPAC, a populationbased birth cohort that recruited 14 541 pregnant women with expected delivery dates between April 1, 1991, and December 31, 1992 [\(www.alspac.bris.ac.](http://www.alspac.bris.ac.uk) [uk](http://www.alspac.bris.ac.uk)). ALSPAC has been described in detail elsewhere. 18 From the age of 7 years, surviving offspring have been invited to regular follow-up clinics. In the present analysis, we examined the association between accelerometer data collected at age 11 to 12 years and biomarker data collected in clinics when the children were aged 15 to 16 years. Ethics approval for the study was granted from the ALSPAC law and ethics committee and the local research ethics committees. For participation in the ALSPAC research clinics, children provided written assent and the parents provided written informed consent. In the follow-up clinic (age 15–16 years), adolescents provided their own consent. The study Web site contains details of all the data that are available through a fully searchable dictionary.¹⁹

Measures of ST and Physical Activity

Participants attending clinics at ages 11 to 12 years and 13 to 14 years were asked to wear an accelerometer for 7 consecutive days during their waking hours (model CSA7164, 2.2, and GTM1; ActiGraph LLC). The full accelerometry protocols have been described in detail previously $20,21$ and are summarized here. Accelerometers were initialized for 1-minute epochs and to start recording at 5:00 AM on the day after the clinic visit; participants wore the devices above the right hip. Movement was expressed as counts per minute, and valid wear-time was defined as \geq 3 days of \geq 10 hours per day of data. Customized software was used to analyze the accelerometer data. Periods of \geq 10 minutes of consecutive zero counts were considered nonwear time. We also excluded any day of recording when the average cpm was \leq 150 or $>$ 3 SDs above the mean because this level of physical activity was considered to be behaviorally implausible. $21,22$ ST was defined as time spent at $<$ 200 cpm^{21,23} and MVPA using the internally derived ALSPAC-specific cutoff of 3600 cpm.²⁰ Participants who wore accelerometers were aged 11 to 12 years (mean age: 11.8 \pm 0.24 years) and 13 to 14 years (mean age: 13.9 \pm 0.20 years) on the first day of accelerometer wear.

A total of 5593 participants provided accelerometer data at age 11 to 12 years. Of those participants who did not provide accelerometer data at age 11 years, 876 provided these data at age 13 to 14 years. For these participants, data at age 13 yearswereusedinsteadofdataat age 11 years; thus, the total number of participants with accelerometer data (at age 11 or 13 years) was 6469 (Fig 1). Because the large majority of accelerometer data is derived from age 11 to 12 years,we refer tothe baseline as "age 11 years" from this point on.

Outcome Measures

Among the 6469 participants who provided accelerometer data at age 11 years, biometric data were collected on 99.6% ($n = 6446$) at baseline and 4639

(72%) at follow-up at age 15 to 16 years (mean age: 15.4 ± 0.29 years [from this point forward referred to as "age 15 years"]) during which outcome measures were assessed. Of these, 3368 (73% of the 4639 who attended the 15-year clinic) had complete data on non–blood-based outcomes, and 2963 (64%) had complete data on blood-based outcomes. We defined the eligible sample for this study as the 4639 participants with accelerometer data who attended the clinic before being issued an accelerometer and who also attended the clinic at age 15 years; the main analysis samples were 4639 for non–blood-based outcomes (with imputation used to estimate outcomes for the 27% with missing data) and 2963 for blood-based outcomes (Fig 1 and Supplemental Table 3).

Measurementofthe outcomeshas been described in detail in a previous publication 24 and is summarized here. Weight and height were measured with the child in light clothing and without shoes. Weight was measured to the nearest 0.1 kg by using Tanita scales (Tanita, Tokyo, Japan) and height to the nearest 0.1 cm with a Harpenden stadiometer (Holtain Limited, Pembs, Wales, UK). Weight and height measurements were used to calculate BMI by dividing weight by height squared. A flexible tape was used to measure waist circumference to the nearest 1 mm at the mid-point between the lower ribs and the iliac crest (pelvic bone). A Lunar Prodigy Primo narrow fan beam densitometer (General Electric Healthcare, Little Chalfont, Buckinghamshire, UK) was used to perform whole-body dual-energy x-ray absorptiometry to measure lean and body fat mass. Blood pressure was

measured with the child at rest and his or her arm supported; a Dinamap 9301 Vital Signs Monitor (Morton Medical, London, UK) was used for testing. Two readings of SBP and DBP were taken, and the mean of the 2 measures was used. Fasting blood sample measures comprised triglycerides, HDL-C, C-reactive protein, glucose, insulin, total cholesterol, and LDL-C. Once the fasting blood samples were taken, they were immediately spun and frozen at -80° C. Plasma lipid concentrations (triglycerides, HDL-C, and total cholesterol) were measured by modification of the standard Lipid Research Clinics Protocol by using enzymatic reagents for lipid determination. LDL-C concentrations were determined from these by using the Friedewald equation (LDL $=$ total cholesterol $-$ HDL triglycerides \times 0.45). Insulin was measured with an enzymelinked immunosorbent assay (Mercodia, Uppsala, Sweden), and plasma glucose was measured with an automated assay.

Covariables

Potential confounding factors accounted for in the analyses included: gender; birth weight; maternal BMI; paternal occupationalsocialclass (I, II, III [manual], III [nonmanual], IV, and V); total energy intake at age 10 years; Tanner puberty stage at age 11 years; age (months) at Tanner measurement; age (months) at the time of assessment of cardiometabolic and adiposity markers; accelerometer wear time (minutes) at age 11 years; time in months between accelerometry at age 11 years and measurement of cardiometabolic and adiposity outcomes at age 15 years; baseline adiposity (BMI, body fat mass, and waist circumference); and SBP and DBP measurements at age 11 years.

Dealing With Missing Data

Participants had varying amounts of missing data for covariables within the 2 main analysis samples. Taking into account only those with complete data on all covariables, the complete case sample sizes were 1075 for the non– blood-based outcomes (20% of the 4639 eligible subjects for those outcomes) and 944 for the blood-based outcomes (35% of the 2967 eligible subjects for those outcomes) (Fig 1).

To increase efficiency and minimize selection bias, missing values of all outcomes and covariates were imputed,withthe exclusionofblood-based outcomes. For non–blood-based outcomes, the main analysis sample comprised respondents who provided accelerometry data at age 11 years and also attended the clinics at ages 11 and 15 years ($n = 4639$). Within this sample, missing values for all outcomes and covariates were imputed (accelerometermeasured sedentary/MVPA time had no missing values). For blood-based outcomes, the main analysis sample was further limited to the 2963 subjects who provided a blood sample (excluding 4 subjects with missing insulin values). Figure 1 provides further clarification.

Missing values were imputed by using the multiple imputation procedure in SPSS version 21, with linear regression as the type of imputation and the generation of 20 imputation data sets. All outcome, exposure, and covariate data were included as predictors in the imputation procedure.

Data Handling and Statistical Analysis

Several outcome variables were logged to improve normality of residuals (waist circumference, triglycerides, HDL-C, C-reactive protein, glucose, and insulin). Due to the range of ages at which height and weight were measured, BMI was converted to an ageand gender-specific SD score (SDS) with the Cole formula we have recently used¹⁰ and is detailed elsewhere.²⁵ Because we found no evidence of an interaction between gender and ST or MVPA in terms of the outcomes, analyses were adjusted for gender but were not gender-specific.

Multiple linear regression was used to examine associations between ST and MVPA and cardiometabolic and adiposity outcomes, adjusting for covariables. Cardiometabolic and adiposity outcomes consisted of BMI SDS, waist circumference, body fat mass, DBP, and SBP (non–blood-based sample) and fasting triglycerides, HDL-C, total cholesterol, LDL-C, C-reactive protein, glucose, and insulin (blood-based sample). Results were expressed as mean differences per 10 minutes per day in either ST or MVPA. Multivariate models were run for each combination of outcome and exposure: model 1 was adjusted for gender, age at measurement, and accelerometer wear time; model 2 was also adjusted for time between the accelerometry measurement and outcome measurement, paternal social class, birth weight, maternal BMI, Tanner puberty stage, age at Tanner measurement, and daily energy intake at age 10 years; and model 3 was also adjusted for MVPA at age 11 years (in models with ST time as the exposure only). A final model further adjusted for each baseline adiposity marker (BMI, body fat mass, and waist circumference analyses) or blood pressure (SBP and DBP analyses) at age 11 years, as appropriate. Analyses with blood-based outcomes (which were not measured at age 11 years) were adjusted for baseline BMI as a broad indicator of baseline cardiometabolic risk. Models were checked for collinearity, and variance inflation factors in all the models were \leq 2. Residual plots and statistics were checked for normality, heteroscedasticity, and independence.

In a subsample with valid accelerometry data at both 11 and 15 years of age, we examined whether baseline adiposity was associated with ST at follow-up by using multiple linear regression with BMI SDS ($n = 2067$), body fat mass ($n = 1358$) and waist circumference ($n = 1789$) at age 11 years

as exposures and ST at age 15 years as the outcome.

A standardized continuous CMscore was also calculated by using existing methods.^{10,26} The score comprised 10 of the cardiometabolic risk outcome variables (body fat mass, SBP, DBP, triglycerides, HDL-C, C-reactive protein, glucose, insulin, total cholesterol, and LDL-C). Body fat mass was chosen over BMI SDS and waist circumference as a measure of adiposity to include in the CMscore because of our a priori consideration that this variable was the best measure of fatness. After logtransformation of triglycerides, HDL-C, C-reactive protein, glucose, and insulin, and taking the mean of SBP and DBP, z scores were computed for each component $(z = [participant value - sample$ mean]/sample SD), and the sum of these z scores was divided by 9 (the number of components) to compile the final CMscore with units of SD. For HDL-C, the \overline{z} score was multiplied by -1 , reflecting the fact that higher values of HDL-C are associated with reduced cardiovascular risk.

In the Results section, multivariable associations are presented for all cardiometabolic and adiposity outcomes and the CMscore. Because multiple linear regression is sensitive to the distribution of included variables, we also ran a set of sensitivity analyses by using general linear models with tertiles of accelerometer-measured ST and MVPA as the main exposure. Multivariable-adjusted coefficients and 95% CIs were calculated for each outcome with reference to the ST or MVPA

tertile, and we present the results of these sensitivity analyses as estimated marginal means. SPSS version 21 was used for all analyses.

2. SUPPLEMENTAL METHODS: MISSING DATA AND MULTIPLE IMPUTATION

Including all exposures and covariates, the eligible sample size varied by outcome measure, as shown in Supplemental Table 3. The percent excluded through listwise deletion of missing values ranged from 60.8 for blood outcomes to 70.9 for waist circumference. The eligible sample size was restricted to subjects who provided accelerometer data at age 11 or 13 years and who also attended the clinic at age 11 or 13 years and the clinic at age 15 years ($n =$ 4639). For blood-based outcomes, the sample was further restricted to subjects who provided valid blood-based measurements at the clinic at age 15 years, excluding 4 cases with missing insulin values ($n = 2963$).

Multiple imputation was conducted by using SPSS version 21. Variables included in the imputation process are shown in Supplemental Table 4. Among those in the eligible sample for non– blood-based outcomes ($n = 4639$), missing values were imputed for all covariates (excluding age and gender, which had no missing values), and non–blood-based outcomes. Blood-based outcomes, accelerometer data, age, and gender did not have any missing values (with the exception of blood-based outcomes, which were subject to a smaller eligible subsample) and were

included in the model in a predictive capacity. The observed minimum and maximum values were used as constraints for the imputed values; in those instances where variables were logged for the analysis, the logged variables were used in the imputation model.

Linear regression was used for all variables, and 20 cycles of imputation were conducted, resulting in 20 imputed data sets. Results from these 20 imputed data sets were combined by usingthe multiple imputationmodule in SPSS to provide pooled results. Twenty imputations were considered adequate given the proportion of missing values: for the variable with the highest proportion of values missing (57.7%, maternal weight), 20 imputations provided estimates with an efficiency of 99% (calculated by using a formula from $Rubin³⁶$).

Histograms of variables with imputed data were examined visually to ensure that distributions of key variables were similar in each imputed data set and in the observed data. Variable distributions (mean and SD) were similar between the imputed data sets and the observed data (Supplemental Table 5).

Results in the main article are presented according to the pooled outcomes of the 20 imputed data sets, whereas key analyses using the observed (nonimputed) data are presented in Supplemental Tables 6 and 7. Where multiple imputation has been conducted, the presentation of imputed results is considered preferable to the presentation of nonimputed results (listwise deletion). 37

3. SUPPLEMENTAL RESULTS TABLES

SUPPLEMENTAL TABLE 3 Listwise Exclusion of Missing Data According to Outcome Measure at Age 15 Years

a Numbers represent the sample size for each outcome variable once missing cases in all exposures and covariates have been excluded.

b The eligible sample size comprises subjects who provided accelerometry data at age 11 years and also attended the clinic at age 11 years and the clinic at age 15 years. For blood-based outcomes, this sample is further limited to subjects who also provided valid blood measurements at the clinic at age 15 years, excluding 4 missing values for insulin.

SUPPLEMENTAL TABLE 4 Variables Used in the Multivariable Multiple Imputation Model

NA, not applicable.

a Included in the model as a scale variable due to problems inputting it as an ordinal variable.

b Blood-based sample limited to subjects with valid data on all blood-based outcomes ($n = 2968$).

SUPPLEMENTAL TABLE 6 Sample Characteristics According to Tertile of ST at Age 11 Years

Nonimputed data. Sample restricted to respondents with valid accelerometry data at age 11 years and/or 13 years who attended the clinics at ages 11 years and/or 13 years and 15 to 16 years. ^a The χ^2 test was used to test the significance of the association between categorical variables and ST tertile; analysis of variance was used for continuous variables.

b Cardiometabolic risk factors and adiposity were measured at a clinic at age 15 to 16 years.

c Accelerometry, baseline cardiometabolic risk indicator, and Tanner stage data at age 13 years were used for subjects with no accelerometry data at age 11 years.

SUPPLEMENTAL TABLE 7 Multivariable-Adjusted Associations Between Time Spent Sedentary at Age 11 Years and Cardiometabolic Risk Markers at Age 15 Years in the Observed (Nonimputed) Data Set

Coefficients represent the mean difference in the CMscore per 10-minute greater time spent in sedentary behavior. Accelerometry and baseline cardiometabolic risk indicator data at age 13 years were used for subjects with no accelerometry data at age 11 years.

a Adjusted for gender, wear time at age 11 years, and age at measurement (months) of cardiometabolic risk indicator.

b Also adjusted for time between accelerometry data collection and measurement of cardiometabolic risk factors (months), paternal social class, birth weight, and maternal BMI. c Also adjusted for time spent in MVPA at age 11 years.

d Also adjusted for baseline cardiometabolic risk indicator at age 11 years (BMI SDS used for blood-based outcomes).

e These variables were log-transformed by using the natural log before analysis.

^f A composite z score compiled from 10 of the outcome variables (body fat mass, SBP, DBP, triglycerides, HDL-C, C-reactive protein, glucose, insulin, total cholesterol, and LDL-C).

Coefficients represent the mean difference in the CMscore per 10-minute greater time spent in MVPA at age 11 years. Accelerometry and baseline cardiometabolic risk indicator data at age 13 years were used for subjects with no accelerometry data at age 11 years.

a Adjusted for gender, wear time at age 11 years, and age at measurement (months) of cardiometabolic risk indicator.

b Also adjusted for time between accelerometry data collection and measurement of cardiometabolic risk factors (months), paternal social class, birth weight, and maternal BMI.

c Also adjusted for baseline cardiometabolic risk indicator at age 11 years (BMI SDS used for blood-based outcomes).

d These variables were log-transformed by using the natural log before analysis.

e A composite z score compiled from 10 of the outcome variables (body fat mass, SBP, DBP, triglycerides, HDL-C, C-reactive protein, glucose, insulin, total cholesterol, and LDL-C).

SUPPLEMENTAL TABLE 9 Multivariable-Adjusted Associations Between Adiposity Markers at Age 11 Years and Time Spent Sedentary at Age 15 Years in the Observed

 $(Mənimnuthəd)$ Data θ

Coefficients represent the mean difference in time (minutes) spent sedentary at age 11 years per unit increase in adiposity marker.

a Adjusted for gender, accelerometer wear time at age 15 years, and time (in months) between measurement of adiposity at age 11 years and the first day of accelerometer wear at age 15 years. Adiposity data at age 13 years were used for subjects with no accelerometer data at age 11 years.

b Also adjusted for ST at age 11 years and accelerometer wear time at age 11 years. Accelerometry data at age 13 used were for subjects with no accelerometry data at age 11 years.