Published in final edited form as:
J Phys Act Health. 2014 May ; 11(4): 770–776. doi:10.1123/jpah.2012-0278.

Recent physical activity in relation to DNA damage and repair using the comet assay

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Abstract

Background—Limited evidence suggests that very high-intensity exercise is positively associated with DNA damage but moderate exercise may be associated with DNA repair.

Methods—Participants were 220 healthy, Washington State 50–76 year-olds in the validity/biomarker sub-study of the VITamins And Lifestyle (VITAL) cohort, who provided blood samples and completed questionnaires assessing recent physical activity and demographic and health factors. Measures included nested activity subsets: total activity, moderate- plus high-intensity activity, and high-intensity activity. DNA damage (n=122) and repair (n=99) were measured using the comet assay. Multivariate linear regression was used to estimate regression coefficients and associated 95% confidence intervals (CIs) for relationships between MET-hours per week of activity and each DNA outcome (damage, and 15- and 60-minute repair capacities).

Results—DNA damage was not associated with any measure of activity. However, 60-minute DNA repair was positively associated with both total activity (β=0.21, 95% CI: 0.0057, 0.412; p=0.044) and high-intensity activity (β=0.31, 95% CI: 0.20, 0.60; p=0.036), adjusting for age, sex, BMI, and current multivitamin use.

Conclusions—This study is the first to assess broad ranges of activity intensity levels related to DNA damage and repair. Physical activity was unrelated to DNA damage but was associated with increased repair.

Keywords

Exercise; Body Mass Index; cancer prevention

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Conflicts of Interest: None to disclose

INTRODUCTION

DNA damage is associated with cancer risk[1, 2]; thus, preventing DNA damage and increasing DNA repair may enhance cancer prevention. Of the modifiable factors that may protect against DNA damage or impaired DNA repair, physical activity is of particular interest, since higher levels of physical activity are associated with lower risk of various types of cancers, including breast[3–7], colon[8, 9], and thyroid[10] cancers.

Evidence from some animal studies suggests that regular exercise decreases DNA damage and increases DNA repair[11, 12]. One study found that voluntary chronic physical activity on an exercise wheel was associated with elevated mitochondrial DNA template levels in mature (adult) mice[11]. No effect was observed in senescent mice, which the study authors attributed to lower running speeds of the aged mice[11]. However, another study has shown no association between short-term spontaneous exercise wheel running and DNA damage in the lymphocytes of adult mice[13]. A third study has shown DNA damage in muscle tissue of adult mice two days after spontaneous exercise on a wheel for an entire night (intense exercise)[14]. In terms of DNA repair, at least one study has shown regular exercise is beneficial – eight weeks of treadmill running resulted in increased repair in the muscle tissue of middle- and older-aged rats[12].

Few studies have examined the relationship between physical activity and DNA damage and repair capacity in humans. In one of the first studies, white blood cells of three participants who ran at an increasing speed to exhaustion showed a pattern suggestive of DNA damage in the single cell gel electrophoresis (SCG) assay (comet assay); on the other hand, when the participants were asked to run for 45 minutes at a constant speed, no DNA damage was observed[15]. The authors concluded that physical activity above the aerobic-anaerobic threshold causes detectable white blood cell alterations[15]. However, since the study was so small the ability to draw inferences is limited. Another study examined runners during an ultra-marathon and found DNA damage in lymphocytes mid-race, but the effect subsided two hours post-race[16]. Thus, this evidence suggests that extremely high intensity physical activity may lead to DNA damage, at least in the short term.

The possibility of a threshold effect, whereby intense but not moderate activity may be positively associated with DNA damage, is a consistent theme in the literature. Although an approximately two- to three-fold increase in oxidative damage in muscle tissue has been noted following exhaustive exercise,[17] this is not seen with moderate activity. In a randomized trial of short-term moderate and high intensity exercise programs in colorectal cancer patients, urinary excretions of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a marker of oxidative DNA damage, were significantly decreased in the individuals completing the moderate intensity program and non-significantly increased in individuals completing the high intensity program[18]. Similarly, another study found that urinary 8-oxodG excretions were significantly decreased among individuals performing moderate exercise (<5 hours per week)[19]. In fact, investigators have proposed that exercise is related to DNA damage in a U-shaped fashion, whereby too little exercise may not confer benefit, and excessive intense exercise may cause DNA damage, but moderate exercise may protect against DNA damage[20]. Body mass index (BMI) may also play a role in the relationships

between physical activity and DNA damage and repair. Several cross-sectional studies[19, 21] and at least one longitudinal study[22] have found that BMI and urinary 8-oxdG excretions were inversely associated, but these relationships are still not well-understood.

The hypothesis of the present study was that physical activity is associated with reduced baseline DNA damage and increased DNA repair as measured by the comet assay in healthy older adults. Secondarily, an exploratory analysis investigated if these associations varied with BMI.

MATERIALS AND METHODS

Study population and data collection

The VITamins And Lifestyle (VITAL) study is a prospective cohort study of 77,738 men and women in Washington State, aged 50–76 years at baseline, designed to examine the role of vitamins and other dietary supplements in relation to cancer risk. Baseline data were collected from October 2000 to December 2002 via a 24-page mailed, self-administered, sex-specific questionnaire. Questions assessed use of supplements, diet, physical activity, and health history[23]. In addition, a sub-study was conducted among 220 participants and included a repeat baseline questionnaire, an in-home interview to obtain more detailed information on certain factors and collection of blood and other biospecimens for biomarkers[24]. Data from this subsample assessment are used for the present analysis. The VITAL study was approved by the Fred Hutchinson Cancer Research Center institutional review board and is in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Service and all participants signed written informed consent.

Assessment of physical activity

Physical activity data were collected via the repeat baseline questionnaire completed before the in-home visit. This was a version of the VITAL baseline physical activity questionnaire[25], modified to assess recreational physical activity over the prior month. Participants estimated by category how many days per week (1–2, 3–4, 5–7) and minutes per day (10–25, 30–40, 45–55, 60+) they participated in each of 13 types of activity, plus an “other” category. For analyses, the midpoint value of each category was assigned (e.g., 35 was assigned to the 30–40 minutes per day category), and a value of 65 was assigned to the category of 60+ minutes per week[25]. For walking, participants estimated pace as follows: casual (30 minutes per mile or more), moderate (20–29 minutes per mile), or fast (19 minutes per mile or less). For missing data (<5%), age- (50–64, 65–75) and sex-specific values for minutes per day and days per week were imputed based on the most common response (the mode) for each strata[25]. The number of flights of stairs climbed each day was also ascertained.

Metabolic equivalent task (MET) values were assigned to each of the activities (e.g., walking, running, swimming), based on Ainsworth et al.’s Compendium of Physical Activities[26]. Activity-specific MET-hours per week for the 1-month time span were calculated: [(days per week)*(minutes per day)*(MET for activity)]□[(60minutes/hour)].

MET-hours per week were then summed across all activities for total MET-hours per week. Physical activity predictors were quantified as MET-hours per week of the following five groupings: walking, stair climbing, moderate plus high-intensity activity (for activities with MET values of 4 or higher, excluding walking), high-intensity activity (for activities with MET values of 6 or higher), and total activity (walking, stair climbing, moderate, and high intensity activities combined).

Assessment of baseline DNA damage and repair

Semifasting (≥ 6 hours) blood samples were collected from participants by phlebotomists at the in-home interviews. All specimens were transported to the Fred Hutchinson Cancer Research Center Specimen Processing Laboratory and processed within two hours of collection. Viable leukocytes were isolated by Ficoll-gradient centrifugation, re-suspended, and underwent controlled step-rate freezing. The cryovials with these specimens were placed in a −70°C freezer overnight and were transferred to liqu id nitrogen storage vessels within 48 hours.

Baseline DNA damage was assessed using a single gel electrophoresis (SCG) assay (comet assay), which is a rapid, sensitive, and reliable technique to detect single and double strand breaks in DNA from individual cells[27, 28]. Measurement of damage varies, but one common technique is the Olive tail moment, which is in measured in arbitrary units but is expressed as the difference between the tail mean and the head mean multiplied by the percent of DNA and divided by 100[29]. The Olive tail moment is a measure used commonly in studies of DNA damage and repair[27, 30–32] and has been shown to be a reliable measure of radiation-indcued damage compared to other measures derived from comet assay results[33]. A variation of the standard comet assay was used in this study to enable measurement of baseline DNA damage, response to oxidative stress-induced DNA damage, and repair capacity at two time points[34]. Measuring both DNA damage and repair is an important feature, as it is ultimately the loss of equilibrium between damage and repair that promotes carcinogenesis[35, 36].

Cells were tested for viability using Trypan blue staining, and cell morphology was examined. Samples were placed on ice to prevent repair until electrophoresis was conducted for each of the measures. Baseline DNA damage wa7s measured for those samples having viable cells, then the cells were subjected to 1.23 gray of gamma radiation at 4 degrees Celcius (9 seconds of exposure) to induce DNA damage (i.e. single strand breaks (SSBs) and double strand breaks (DSBs)). Repair capacity measurements were then taken at 15 minutes and 60 minutes post-induced damage and expressed as a percentage. For this study, 15-minute DNA repair capacity was considered to be 1 minus (the Olive tail moment at 15 minutes divided by the baseline Olive tail moment after irradiation), based on seminal comet assay work[34]. An analogous calculation was made for DNA repair capacity at 60 minutes. Comet assays were conducted at the German Cancer Research Center (DKFZ), and values were measured using the Metafer4 system (MetaSystems, Altlussheim, Germany).

Although serum specimens were obtained for all 220 subsample participants, 35 were excluded from the comet assay analysis due to a previous cancer diagnosis (reported on their baseline questionnaire). There remained 122 participants with valid baseline DNA damage

data after excluding 63 samples with non-viable lymphocytes or samples deemed ineligible for baseline damage analysis because fewer than 60 cells could be scored, ≥50% of cells were 'ghost' cells meaning no viable cells were present 24 hours after thawing. DNA repair capacity was not calculated when either the baseline damage or the residual damage from the induced damage was higher than the induced damage, because these measures were considered unreliable and likely due to laboratory measurement error. Of the 122 participants with baseline damage measures, all had data for at least one physical activity (exposure) variable. Of these 122 participants, 99 had data available for the other two outcome measures, 15- and 60-minute DNA repair capacity. The 23 individuals who had baseline damage measures but not repairmeasures were dropped from analyses due to the aforementioned exclusions at the repair measure time point.

Statistical analyses

Descriptive statistics were used to characterize the study population. Linear regression was used to estimate adjusted β-coefficients and 95% confidence intervals (95% CIs) for associations between MET-hours per week of physical activity and Olive tail moment measures of baseline DNA damage, 15-minute DNA repair, and 60-minute DNA repair. Total physical activity was considered the primary predictor, and components of total activity were also explored. Although there is no consensus on which repair measure (15-minute or 60-minute) is better, it has been observed in at least one study that cells can take as long as 30 minutes or more to repair[37]; thus, the 60-minute repair measure was emphasized *a priori* as the primary repair outcome, in order to capture as much repair as possible.

All models were *a priori* adjusted for age, sex, and BMI (continuous) given that they are known to be independently associated with both physical activity and DNA damage and repair[22, 38]. For participants missing BMI (n=7), the BMI from their original baseline questionnaire was used if available (n=3). Additional covariates were evaluated in groups in order to construct a more parsimonious multivariate model and to have finals models that were more comparable to each other. Groups were formed by clustering similar variables and were included as follows: 1) demographic/behavioral: race (White, non-White), education (college or higher, less), current cigarette smoking (Y/N), current alcohol use (Y/N); 2) current multivitamin use (Y/N); 3) current antioxidant use: vitamin C (mg), vitamin E (mg dL alpha tocopherol), selenium (mcg); 4) current use of minerals/pro-oxidants: iron (mg), zinc (mg); 5) current use of fish oil, EPA, omega 3, or cod liver oil (Y/N), and 6) history of cardiovascular disease or diabetes (Y/N). Dose calculations for each of the vitamins and supplements included amounts supplied by a multivitamin. The correlation matrix for variables within groups was examined to ensure that included variables were not highly correlated with each other. Each covariate group was added to the model fortotal activity and 60-minute DNA repair (main analysis) and evaluated for significance using a Likelihood ratio test. Only significant groups of variables were included in final models. For the main analysis of total activity and 60-minute DNA repair, the only additional predictor was multivitamin use; thus, all "final adjusted" analyses are adjusted for age, sex, BMI (continuous), and multivitamin use (Y/N).

In an exploratory analysis, BMI (<30.0, ≥30.0 kg/m^2) was examined as a potential effect modifier. Multiplicative interaction terms were generated by creating a cross-product term between each physical activity measure and each BMI category and tested for significance in the univariate model using a likelihood ratio test.

All statistical significance levels (*P* values) reported are two-sided. *P* values of ≤0.05 were considered statistically significant. Statistical analyses were conducted using Stata/SE (version 11.0; StataCorp LP, College Station, TX).

RESULTS

Demographic and health information for the 122 participants with complete information on at least one measure of reported physical activity and a measure of baseline DNA damage in the study sample are shown in Table 1 by sex. The majority of participants were non-Hispanic White (95.0%) and non-smokers (93.0%). Men were slightly more physically active than women; differences were most apparent for moderate-intensity, high-intensity, and total activity, while stair climbing and walking were similar between men and women (Table 1). Men had a slightly higher mean BMI, and a higher proportion of men had a history of cardiovascular disease or diabetes (Table 1).

Table 2 displays the results of each of the five physical activity predictors and their relation to baseline DNA damage and 15-minute and 60-minute DNA repair capacity. Associations between baseline DNA damage and total activity, moderate- plus high-intensity activity, and high-intensity activity were small and not statistically significant.

Physical activity was not significantly associated with 15-minute DNA repair; however, total activity and high-intensity activity were each significantly associated with 60-minute DNA repair. When adjusting for age, sex, BMI, and current multivitamin use, total activity was positively associated with 60-minute DNA repair (p=0.044); for each additional MET-hour of physical activity per week, the mean DNA repair capacity was 0.21% higher (β=0.21, 95% CI: 0.0057, 0.42). Similarly, when adjusting for age, sex, BMI, and current multivitamin use, high-intensity activity was positively associated with 60-minute DNA repair (p=0.036); for each additional MET-hour of high-intensity activity per week, the mean DNA repair capacity was 0.31% higher (β=0.31, 95% CI: 0.20, 0.60). Moderate- plus high-intensity activity was associated with a non-significant 25% higher 60-minute DNA repair capacity (β=0.25, 95% CI: −0.0098, 0.51).

Given the small sample size of this study, there was limited power to detect effect modification by BMI. Thus, overall results (not separated by BMI status) have been presented.

DISCUSSION

In this study of generally healthy, older adults in Washington State, physical activity was not associated with overall baseline DNA damage, but was associated with enhanced DNA repair. Meeting physical activity recommendations of 150 minutes per week[39], which is approximately equivalent to 7.5 MET-hours per week, would be associated with a 1.6%

higher DNA repair capacity at 60 minutes. The benefit of total physical activity for DNA repair appears to be driven primarily by higher-intensity activities, such as running, swimming laps, or fast cycling. Given that DNA repair counteracts DNA damage, and excessive DNA damage is involved in carcinogenesis, these results are important because they demonstrate that physical activity may be beneficial for cancer prevention in older, healthy adults.

Previous studies examining physical activity and DNA damage and repair have either used exhaustive treadmill tests to measure physical activity, or they have focused on aerobically trained individuals; however, this study used a measure of usual physical activity that allows for varying intensities of recreational physical activity. While a previous study showed that DNA damage was significantly decreased among individuals performing moderate exercise (<5 hours per week)[19], no association was observed in the present study. This study, however, is the first study to examine physical activity and DNA repair in humans and show that physical activity is associated with increased repair. These results are similar to the aforementioned study showing that eight weeks of treadmill training resulted in increased DNA repair in the muscle tissue of middle- and older-aged rats, which are more likely to perform moderate-intensity activity compared to high-intensity activity[12]. The results of the present study are also consistent with another study showing that levels of 8-oxoG-DNA glycosylase (OGG1), a base excision repair enzyme, increased in moderately- and strenuously-trained rats but not in rats trained at a higher intensity[40]. Biologically, physical activity may impact DNA repair through the creation and stimulation of oxygen radical scavenger enzymes and repair enzymes[19], the main potential repair mechanisms being Nucleotide-excision repair (NER), base-excision repair (BER), and mismatch repair (MMR) for single-strand breaks, and homologous recombination and mismatch repair (MMR) for double-strand breaks[41].

This study is one of only a handful of studies that have considered BMI as a potential confounding factor in the relationship between physical activity and DNA damage and repair. In one of the first small studies, BMI was accounted for via a crossover design; however this study only included three people[15]. Allgayer et al.[18] examined BMI as a potential confounder but it was not associated with their measure of DNA damage and thus was not considered a confounder. Kasai et al.[19] included BMI in their multivariate analysis of moderate physical activity and urinary 8-oxodG excretion, but their study was restricted to men.

This study has several strengths. It is the first study to assess how usual physical activity with a range of intensity and duration relates to baseline DNA damage and repair using the comet assay among older, more “average” activity level individuals. It is also the largest study to date to examine physical activity and baseline DNA damage and repair capacity in both men and women. The comet assay has been used extensively in biomonitoring studies[28, 42, 43] and more recently in epidemiologic investigations with reliable and reproducible results[34, 44].

Nonetheless, this study has several limitations. Data on physical activity, BMI, and other demographic variables and potential confounders were assessed by self-report, and as such

are subject to reporting error, including that due to social desirability[45, 46]. This is an observational, cross-sectional study, and it is possible that the observed association could be due to residual confounding. The sample size was too small to effectively examine effect modification by BMI. Finally, the majority of VITAL participants are non-Hispanic White and non-smokers, so results may not be generalizable to other subgroups of the population; however, the homogeneity offers a degree of control for confounding.

Although there are many assays available to assess DNA damage and the comet assay has its limitations, only one assay was able to be used in the present study, and the ability of the (relatively inexpensive) comet assay to rapidly and reliably simultaneously assess DNA damage and repair was an important feature in the current study. It is also encouraging that at least one study has: (a) used similar measures derived from the comet assay (e.g. % damaged DNA in the tail) and was able to detect effects, (b) successfully replicated results from the alkaline (pH 13.1) version of the comet assay (similar to the present study) using other versions of the comet assay (pH 12.1), and (c) successfully replicated results using other DNA damage measures (e.g. 8-oxo-7,8-dihydroguanine)[47]. The statistical power to assess baseline DNA damage and repair was somewhat limited by the number of individuals with viable lymphocytes suitable for the comet assay. Nevertheless, an association was detected between physical activity and 60-minute DNA repair, and it was determined that participants who were included in the final analytic sample (i.e. those who had at least one comet assay measure) did not differ from participants who were excluded (i.e. those who did not have a comet assay measure) in terms of physical activity, demographics, and other measured characteristics.

In summary, recent physical activity does not appear to be associated with increased DNA damage as has been suggested by limited evidence in the literature. In fact, usual exercise may stimulate DNA repair, possibly though an oxygen radical scavenger enzyme or repair enzyme mechanism. In order to better assess the relationship between physical activity and DNA damage and repair, larger studies are needed, and particularly those that address remaining questions, including whether or not the associations observed vary by BMI. Nonetheless, our results are consistent with US Centers for Disease Control and Prevention physical activity guidelines for adults and older adults that a mix of moderate and vigorous physical activities is beneficial for health, and that some physical activity is better than none[48].

Acknowledgments

The authors would like to thank the participants of the VITAL study who made this investigation possible, and Dr. Peter Schemezer and the German Cancer Research Center (DKFZ) for conducting the comet assay.

FUNDING SOURCE

The work reported here was supported by R01 CA74846 and R03 CA105336 from the National Cancer Institute (NCI) of the National Institutes of Health (NIH), and K05CA154337 from the National Cancer Institute (NCI) and the NIH Office of Dietary Supplements (ODS).

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Table 1

Characteristics by sex for 122 participants with at least one physical activity measure and a measure of DNA damage in the VITAL validity/biomarker sub-study

	Males N=67	Females N=55
	N (%)[a]	N (%)[a]
Race		
Non-Hispanic White	63 (94.0)	53 (96.4)
Other	4 (6.0)	2 (3.8)
Education		
Less than college graduate	24 (35.8)	30 (54.6)
College graduate or advanced degree	43 (64.2)	25 (45.5)
Current Cigarette smoker		
Yes	3 (4.5)	5 (9.1)
No	64 (95.5)	50 (90.9)
Alcohol in the past month		
Any	50 (75.8)	37 (67.3)
None	16 (24.2)	18 (32.7)
History of diabetes or cardiovascular disease		
Yes	12 (17.9)	3 (5.5)
No	55 (82.1)	52 (94.5)
Current multivitamin use		
Yes	33 (49.3)	39 (70.9)
No	34 (50.8)	16 (29.1)
	Mean (SD)	Mean (SD)
Age (years)	59.9 (7.5)	62.2 (7.8)
BMI (kg/m^2)	27.6 (4.3)	25.3 (4.5)
MET-hours per week of walking	4.1 (5.0)	4.6 (5.1)
MET-hours per week of stair-climbing	2.3 (1.7)	1.9 (1.9)
MET-hours per week of moderate+high intensity activity	9.9 (14.7)	5.9 (9.2)
MET-hours per week of high intensity activity	6.8 (12.4)	3.3 (7.1)
MET-hours per week of total activity	18.7 (17.7)	13.5 (11.1)
Olive tail moment for baseline DNA damage	3.0 (2.7)	3.5 (3.7)
Olive tail moment for DNA repair capacity (%)	38.4 (12.3)	40.1 (18.7)
Olive tail moment for DNA repair capacity (%)	61.5 (13.9)	64.2 (15.7)

[a] N's may not sum to 122 due to missing data, %'s may not sum to 100% due to rounding

BMI: Body Mass Index

Table 2

Adjusted beta coefficients and 95% confidence intervals for the association between MET-hours per week of physical activity and baseline DNA damage and repair capacity in the VITAL validity/biomarker sub-study

	β (95% confidence interval)	
Average MET-hours per week of physical activity	**Model 1 – *a priori* adjusted [a]**	**Model 2 – final adjusted [b]**
Baseline DNA damage		
Total activity(n=120)	−0.01 (−0.03, 0.02) p=0.70	−0.01 (−0.04, 0.02) p=0.62
High-intensity activity (n=120)	−0.02 (−0.06, 0.02) p=0.29	−0.03 (−0.07, 0.01) p=0.20
Moderate-+high-intensity activity (n=120)	−0.02 (−0.06, 0.02) p=0.28	−0.02 (−0.06, 0.01) p=0.20
Stairs (n=122)	0.14 (−0.18, 0.46) p=0.38	0.14 (−0.18, 0.46) p=0.39
Walking (n=120)	0.08 (−0.08, 0.24) p=0.32	0.08 (−0.08, 0.23) p=0.33
15-minute DNA repair capacity		
Total activity (n=97)	0.02 (−0.20, 0.25) p=0.86	0.03 (−0.20, 0.27) p=0.78
High-intensity activity (n=97)	0.07 (−0.25, 0.38) p=0.67	0.10 (−0.21, 0.42) p=0.53
Moderate-+high-intensity activity (n=97)	0.06 (−0.20, 0.33) p=0.65	0.09 (−0.17, 0.36) p=0.48
Stairs (n=99)	0.35 (−1.52, 2.22) p=0.71	0.40 (−1.44, 2.25) p=0.67
Walking (n=97)	0.24 (−0.33, 0.82) p=0.40	0.26 (−0.27, 0.80) p=0.33
60-minute DNA repair capacity		
Total activity (n=97)	0.19 (−0.07, 0.46) p=0.15	**0.21 (0.01, 0.41) p=0.044**
High-intensity activity (n=97)	0.25 (−0.05, 0.56) p=0.10	**0.31 (0.20, 0.60) p=0.036**
Moderate-+high-intensity activity (n=97)	0.19 (−0.01, 0.39) p=0.065	0.25 (−0.01, 0.51) p=0.059
Stairs (n=99)	1.09 (−0.49, 2.67) p=0.17	1.15 (−0.46, 2.75) p=0.16
Walking (n=97)	0.40 (−0.16, 0.94) p=0.16	0.42 (−0.11, 0.95) p=0.12

Note: Sample sizes vary based on available data for exposure and outcome measurements.

BMI: Body Mass Index

[a] Adjusted for age, sex, and BMI (continuous)

[b] Adjusted for age, sex, and BMI (continuous), and multivitamin use (Y/N)