Modifiable mediators associated with the relationship between adiposity and leukocyte telomere length in US adults: The National Health and Nutrition Examination Survey

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Abstract

Obesity is associated with age-related health conditions and telomere attrition – a marker of cellular aging. Obesity is attributable to adverse modifiable lifestyle factors. Little is known about the mediation effect of lifestyle factors associated with the relationship between obesity and telomere length. Our objective was to examine this association in the US.

Pack years smoked, drinking level per day, physical activity (PA) per week and diet based on Healthy Eating Index (HEI) were assessed as mediators associated with the relationship between

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adiposity measures and leukocyte telomere length (LTL); adiposity measures included body mass index (BMI), % total body fat (TBF) and waist circumference (WC).

Separate adjusted linear regressions and mediation analysis were conducted on a total of 4,919 respondents aged 20–84 years using cross-sectional 1999–2002 data from the US National Health and Nutrition Examination Survey. Inadequate PA correlated with 1.28% shorter LTL and was a factor accounting for 35% of the relationship between BMI and LTL ($\beta = -0.0128$, 95% CI= .0259, .0004, $p = .05$). Smoking 30-59 pack years correlated with 4% shorter LTL and accounted for 21% of the relationship between %TBF and LTL ($\beta = -0.0386$, 95% CI= −.0742, −.0030, $p = .03$). Improvement in diet correlated with 0.11% longer LTL and contributed 25% of the association between %TBF and LTL ($\beta = .0011$, 95% CI= .0004, .0018, $p = .01$). Diet correlated with 0.11% longer LTL and correspond to 28% of the relationship between WC and LTL ($\beta = .0011$, 95% CI = .0004, .0018, $p = .03$).

Interventions to improve modifiable behaviors may ameliorate cellular aging and aging related health conditions due to obesity among US adults.

**Keywords**

obesity; telomeres; adiposity; lifestyle behavior; mediator

**Introduction**

The prevalence of risk factors associated with cardiovascular disease (CVD), such as smoking and hypertension, have been declining overtime among US adults. (1) On the other hand, obesity rates have increased. (2) The rate of obesity has nearly tripled worldwide since 1975. (3) The US has among the highest rates of obesity. (2) More than one-third of US adults are obese. (4) Obesity is a major risk factor for many age-related CVD chronic conditions such as hypertension, type 2 diabetes and dyslipidemia which increases the risk for heart failure, heart attack and stroke. (5) It is the leading cause of preventable deaths, in part, due to adverse modifiable lifestyle behaviors such as sedentary physical activity and unhealthy diet. (3, 5)

Obesity has been associated with the shortening of telomeres. (6) Telomeres are the DNA-protein complex at the ends of chromosomes. (7, 8, 9, 10) It consists of highly conserved tandem hexameric nucleotide repeats (TTAGGG). Telomeres are necessary for the replication of DNA and provides protection to chromosomes from nuclease degradation and cellular senescence which promotes the integrity and stability of chromosomes. During the cellular process, telomeres progressively shorten with each cell division. When telomeres shorten to a critical length, replicative senescence is triggered resulting in cell-cycle arrest. (11) In human peripheral leukocytes, telomere shortening has been demonstrated to be a marker for cellular aging as well as a biomarker for age-related diseases such as CVD. (12, 13) Evidence shows that the pathways through which obesity promotes morbidities include increasing systemic inflammation and oxidative stress; inflammation and oxidative stress have also been linked to telomere attrition. (14, 15, 16)
Although there may be a genetic component, obesity occurs primarily as a result of adverse modifiable health behavior risk factors – such as smoking, excess caloric intake, excess alcohol consumption and sedentary physical activity.\(^3\), \(^5\) Studies demonstrate these factors are linked to telomere shortening.\(^17\), \(^18\), \(^19\), \(^20\), \(^21\) Several studies have also demonstrated a relationship between adiposity and telomere length.\(^6\), \(^8\), \(^22\) However, to date, there are no published reports regarding the effect of modifiable risk factors as mediators contributing to the association between adiposity and telomere length. The objective of our research was to examine a large US racial/ethnic cohort to assess this relationship. We hypothesize that modifiable adverse health behaviors will be mediators associated with the correlation between adiposity and telomere length. Findings will provide important information about the association and contribution of adverse health behaviors with cellular aging and aging related health conditions due to obesity among US adults.

**Materials/Subjects and Methods**

**Study design and sampling procedures**

Data was collected from the 1999–2000 and 2001–2002 cycles of the National Health and Nutrition Examination Survey (NHANES). This is a nationally representative cross-sectional survey and physical examination of civilian, noninstitutionalized US population conducted by the US Centers for Disease Control and Prevention (CDC) since 1960.\(^23\) NHANES utilizes a 4-stage sampling design which includes 1) primary sampling units (PSUs) consisting of single counties, 2) area segments within PSUs, 3) households within segment areas, and 4) persons within households. NHANES 1999–2002 oversampled low-income individuals, African Americans and Mexican Americans to obtain more accurate estimates in these populations. All respondents aged ≥20 during this period were asked to provide DNA specimens to establish a national probability sample of genetic material for future research. DNA from the most recent NHANES is only available in the form of crude lysates of cell lines thereby precluding the assay of leukocyte telomere length (LTL). However, DNA collected during 1999–2002 is purified from whole blood thus facilitating the assay of LTL. Pooled data were available for public download (https://wwwn.cdc.gov/nchs/nhanes/default.aspx).

Of the 10,291 respondents eligible to provided DNA, 7,825 provided DNA and consented to future genetic research. We excluded 653 respondents whose self-reported race/ethnicity was “other” or “other Hispanic,” since our goal was to examine a cohort with more discrete self-reported race/ethnicity (i.e. non-hispanic white, non-hispanic black, Mexican American) hereafter referred to as White, African American, and Mexican American. We also excluded 225 respondents aged ≥85 because of survival bias among the extreme elderly.\(^24\) An additional 2,037 were excluded due to missing data on one or more variables in the models - resulting in a final sample size of 4,919. There were no significant sociodemographic differences between the full sample and the final sample. Sampling weights were used to address oversampling and nonresponse bias and to ensure that estimates are representative of the general US population. Research was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant. Human subject
approval was provided by the Institutional Review Board (IRB) at the CDC and the study protocol was approved by the IRB of the National Institutes of Health.

**Outcome**

The outcome was LTL derived from aliquots of purified DNA provided by the laboratory of the CDC. DNA was isolated from whole blood using the Puregene (D-50K) kit protocol and stored at −80°C. The LTL assay was performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco, using the quantitative polymerase chain reaction (PCR) method to measure telomere length relative to standard reference DNA (T/S ratio). This method measures LTL as a ratio (T/S) of telomere repeat length (T) to copy number of a single copy gene, 36B4(S), within each sample. Single-copy gene was used as a control to normalize input DNA human beta-globin. Each sample was assayed twice. T/S ratios that fell into the 7% variability range were accepted; the average of the two was taken as the final value. A third assay was run for samples with greater than 7% variability and the average of the two closest T/S values was used. The inter-assay coefficient of variation was 4.4%.

**Primary predictors**

Body mass index (BMI), estimated % total body fat, and waist circumference were analyzed separately as measures of adiposity. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m²) using a calibrated electronic digital scale and a stadiometer. Estimated % total body fat was assessed using dual-energy X-ray absorptiometry of the whole body that lasted 3 minutes (Hologic scanner, QDR-4500, Bedford, MA, USA). Estimated total % body fat was calculated as total body fat mass divided by total mass x 100. Waist circumference was measured in centimeters using a tape measure around the trunk, at the iliac crest, crossing at the mid-axillary line. The details of these assays have been described elsewhere.

**Mediators**

Smoking, drinking, physical activity, and diet were assessed as modifiable health behavior risk factor pathway mediators associated with adiposity exposures and LTL outcome. Smoking was measured as cumulative exposure to tobacco smoke in pack-years, calculated as the average number of cigarettes smoked per day times the number of years smoked divided by 20 (number of cigarettes in one pack). Dummy variables were 30-≥59 pack years, <30 pack years, and never smoked was coded as the reference. Drinking was based on daily alcohol consumption defined as heavy, moderate and abstainer. Heavy drinkers were defined as women reporting having drunk ≥2 alcoholic beverages in the past 12 months per day and men reporting having drunk ≥3 alcoholic beverages in the past 12 months per day. Moderate drinkers were defined as women reporting <2 drinks per day in the past 12 months and men reporting <3 drinks per day in the past 12 months. Men and women reporting no alcoholic beverages in the past 12 months per day were the reference and defined as abstainers. Physical activity was based on guidelines provided by the Department of Health and Human Services. Respondents met or exceeded recommended guidelines if they reported >150-≥200 minutes per week of physical activity, such as brisk walking, gardening, and muscle-strengthening based on total number of minutes reported for each
activity. Those reporting <150 minutes of physical activity per week were below the recommended guidelines. Diet was based on The Healthy Eating Index (HEI) developed by the US Department of Agriculture in 2005. The score is the sum of 10 components representing different aspects of a healthy diet. Each component of the index has a maximum score of 10 and a minimum score of zero. The maximum overall score for the 10 components combined is 100. An overall index score ≥80 implies a “good” diet, an index score between ≥51 and 80 implies a diet that “needs improvement,” and an index score <51 implies a “poor” diet. “Abstainer” was coded as the reference for drinking level per day and “meet/exceed” recommended guidelines for weekly physical activity level per week.

Confounding variables

Factors that may impact the relationship between adiposity measures and LTL were included as confounding variables. Race/ethnicity based on self-reported non-Hispanic White, non-Hispanic Black and Mexican American, age in years at the time of the survey, age², sex, socioeconomic status based on Poverty Income Ratio (PIR), adiposity and LTL related health outcomes, markers of inflammation and oxidative stress, and characteristics of the blood from which DNA was extracted were included. The use of age along with an age² term is important when analyzing LTL given the strength of its association with age and the potential for nonlinearity in this association. PIR was calculated as the ratio of income to the poverty threshold for a household of a given size and composition. PIR values below 1.00 was classified as below the official poverty threshold as defined by the US Census Bureau.

Adiposity/LTL related health status was based on respondents answer to the questions “have you ever been told by a doctor or other health professional that you had hypertension, also called high blood pressure and “have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?” Markers of inflammation and oxidative stress included C-reactive protein (CRP) and gamma glutamyltransferase (GGT) measured from serum. Characteristics of the blood samples from which DNA was extracted included white blood cells (μL), lymphocytes (%), monocytes (%), neutrophils (%), eosinophils (%), and basophils (%).

Statistical methods

Leukocyte telomere length was log-transformed by natural logarithm to improve the normality of the distribution prior to modeling. Two multivariate linear regression models were fitted to assess the relationship between LTL and each adiposity measure separately. Model 1 included the confounders. Additional mediators were included in model 2 and was fitted to assess if the relationship attenuated and to assess the association of each mediator with LTL based on separate adiposity measure. The moderating effect of race/ethnicity and sex was entered as interaction terms for each adiposity measure in model 1. The mediation effect of each mediator was calculated via Arioan test using standardized coefficients of the indirect effects of adiposity on LTL through smoking, drinking, physical activity, and diet. A significant Arioan z test suggests a significant indirect effect of adiposity measure and LTL via a candidate mediator. We also calculated the proportion of each of the mediators associated with the individual adiposity measure and LTL. The mathematical equation formula for Arioan z test is:
All analyses were conducted using SAS version 9.3. A two-tailed level of significance was established as $P \leq .05$. We report the percentage change in the average value of telomere length for a one-unit change in a predictor variable based on the beta estimate for telomere length as the outcome. Because the absolute value of all parameter estimates was <.10, the percentage change in the outcome was estimated by exponentiating the logged coefficient, subtracting one and multiplying the parameter estimate ($\beta$) by 100. Both models accommodated the complex sampling design of NHANES by incorporating strata and PSU indicators, as well as sample weights for the genetic subsample. Race/ethnicity, sex, pack years smoked, drinking level per day, physical activity level per week, hypertension status and type 2 diabetes status were entered as categorical variables. Healthy Eating Index, age, age$^2$, PIR, CRP, GGT and the characteristics of blood were entered as continuous variables.

An investigation of residual diagnostics inspection showed that the models met the assumption of linear regression. We also checked for potential outliers using regression diagnostics, as well as multicollinearity and found no evidence of variance inflation more than 5. All analyses were conducted using SAS version 9.3. A two-tailed level of significance was established as $P \leq .05$.

**Results**

There was no interaction effect between race/ethnicity and sex with each adiposity measure and LTL; therefore, results are presented for the total sample. Table 1 reveal overall mean LTL was 1.06 and mean age was 46 years. The distribution between men and women was equal with a sample of 56% White, 19% African American and 26% Mexican American. Eighty four percent of the sample lived above poverty and 18% lived below poverty. Body mass index, % total body fat and waist circumference was 28kg/m$^2$, 34%, and 96cm, respectively. Over half never smoked cigarettes and an equal amount were moderate drinkers. More than 60% met/exceeded the recommended level of physical activity while mean HEI score suggest improvement in diet is needed. Prevalence of reported hypertension and type 2 diabetes was 25% and 5%, respectively. Mean overall CRP was .39mg/dL and GGT was 31 U/L.

**Adiposity measure and LTL**

Table 2 presents results for the association of separate adiposity measures and LTL without (Model 1) and with mediators (Model 2). Model 1 reveals a 0.18% decrease in LTL for each unit increase in BMI ($\beta$=-.0018, 95%CI= -.0032, -.0004, $p=.01$). An increase in % total body fat was associated with 0.22% decrease in LTL ($\beta$= -.0022, 95% CI = -.0034, -.0010, $p = .001$). Increasing waist circumference correlated with 0.07% decrease in LTL ($\beta$= -.0007, 95% CI = -.0013, -.0002, $p = .007$). The addition of mediators, as indicated in Model 2, slightly attenuated the association between BMI and waist circumference with LTL.
but remained strongly correlated. However, the association between % total body fat and LTL became marginal ($p=.07$).

**Mediator effect between adiposity measure and LTL**

Table 3 presents the results on the association between mediators and LTL for each adiposity measure along with the mediation effect between adiposity indices and LTL. Findings regarding BMI reveal physical activity below weekly recommended guidelines, compared to meet/exceed, was associated with 1.28% shorter LTL ($\beta = -0.0128$, 95% CI = $-0.0259$, 95% CI = $+0.0004$, $p = .05$) and accounted for 35% of the relationship between LTL and BMI ($z_{\text{physical activity}} = -1.93$, $p = .05$). For % total body fat, smoking 30-≥59 pack years, compared to never smokers, was associated with a 4% decrease in LTL ($\beta = -0.0386$, 95% CI = $-0.0742$, 95% CI = $-0.0030$, $p = .03$) and was a significant mediator contributing 21% of the correlation between LTL and % total body fat ($z_{30-59 \text{ pack years}} = -2.10$, $p = .03$). Improvement in diet, measured by increasing HEI, was associated with 0.11 in LTL ($\beta = .0011$, 95% CI = $0.0004$, 95% CI = $0.0018$, $p = .004$) and was responsible for 25% of the relationship between % total body fat and LTL ($z_{\text{HEI}} = -2.33$, $p = .01$). Physical activity below weekly recommended guideline, compared to meet/exceeded weekly guideline, resulted in a marginal 1.26 decrease in LTL ($p = .06$) but contributed 32% of the association between waist circumference and LTL ($z_{\text{physical activity}} = -1.95$, $p = .05$). On the other hand, improvement in diet corresponded to a 0.11 increase in LTL ($\beta = .0011$, 95% CI = $0.0004$, 95% CI = $0.0018$, $p = .003$) and was responsible for 28% of the relationship between waist circumference and LTL ($z_{\text{HEI}} = -2.14$, $p = .03$).

**Discussion**

Our primary objective was to examine the association of modifiable risk factors as mediators contributing to the relationship between adiposity measures and LTL in a representative US sample population. We found that BMI, % total body fat and waist circumference were all associated with decreased LTL. Except for % total body fat, the relationship remained strong after adjustment for mediators. Obesity is a major risk factor for chronic morbidities and is due, in large part, to adverse modifiable lifestyle factors.(5) The predominant mechanism through which obesity may shorten telomere length and increase risk of aging-related diseases include increased oxidative stress and inflammation which accelerates leukocyte turnover.(14, 15, 16) A non-biological mechanism contributing to obesity and telomere attrition may also include adverse behaviors factors.(21) We are aware of no published reports examining the association between modifiable health behavior risk factors as mediators between the relation between adiposity and LTL.

We found inadequate physical activity was a pathway mediator between BMI and waist circumference and shorter LTL. Inadequate physical activity contributed 35% of the relationship between BMI and decreased LTL; 32% accounted for the association with waist circumference. 30-≥59 pack year smoked was a mediator between % total body fat and shorter LTL and contributed to 21% of the relationship. On the other hand, improvement in diet was a mediator with % total body fat and waist circumference and contributed 25% and 28%, respectively to longer LTL. Level of drinking per day was the only mediator that was
not associated with any of the adiposity measures and LTL. This may be due to the conflicting contribution of alcohol consumption with health status.\(^{(36)}\)

The relationship between adverse lifestyle behavior and obesity has been well established.\(^{(3, 5)}\) Studies have also examined adiposity exposure with LTL. Lee and colleagues, for instance, demonstrated individuals with higher total and abdominal adiposity had shorter telomere length.\(^{(6)}\) Findings from two studies using NHANES data revealed an increase in adiposity measures was associated with a decrease in LTL.\(^{(8, 22)}\) Several studies have also established a parallel relationship between adverse lifestyle factors and telomere length. Patel el al, for instance, found lack of physical activity resulted in shorter telomere length among US adults.\(^{(19)}\) A national sample of US women likewise found smoking, unhealthy diet and lower physical activity was correlated with telomere attrition.\(^{(20)}\) Our investigation is the first to examine the association of modifiable lifestyle factors as mediators between the relationship with adiposity and LTL, thus demonstrating a potential non-biological pathway.

**Limitations**

There are some caveats to our study that require consideration. First, we do not know the direction of the relationship between adiposity and LTL. Some researchers argue that selective adoption may be a causal factor related to telomere length.\(^{(37)}\) Selective adoption could occur either because telomere length directly affects behavior or because behavior affects telomere length, or both are affected by a third variable – such as exposure to early-life adversity. In addition, telomere senescence occurs overtime and may present in some cases with a U-shaped pattern.\(^{(38)}\) The NHANES is a cross-sectional survey and changes in LTL may come before exposure. Therefore, the correlations we observed should not be interpreted as causal. One way to address these important issues is to design longitudinal analysis to measure the bi-directional effect of differences in LTL and adiposity overtime before obesity event. Second, although we adjusted for potential confounders – other unmeasured confounding factors may exist resulting in “omitted variable bias” such as heritability, ancestry, menopausal status, adiposity biomarkers (i.e. leptin and adiponectin) and sex-hormones that may affect our findings. Third, we measured telomere length only in leukocytes. Whether our findings can be extrapolated to other tissues is unclear. However, studies have demonstrated robust correlations between LTL and telomere length in other tissues.\(^{(39, 40)}\) Fourth, our mediation measures are subject to measurement error even though they have been validated and proven to be accurate in other studies.\(^{(41, 42, 43)}\)

Despite, these limitations, our study has many strengths. It is comprised of a representative sample of US adults from which findings can be extrapolated. It is among the largest and first to investigate the association of modifiable lifestyle factors with the relationship between adiposity and telomere length. Finally, our detailed measurements of lifestyle and dietary factors enabled us to make categories that were consistent with current guideline on lifestyle and diet which can subsequently be translated into public health intervention messages.
Conclusion

Telomere length is a measure of cellular aging and is associated with obesity.\(^{(16)}\) Obesity is increasing at an epidemic rate and is associated with several age-related health conditions.\(^{(5, 44)}\) Our findings reveal select adverse lifestyle factors as a mechanism underlying the relationship between adiposity and telomere length which portend modifying such factors may result in improvements in cellular aging and age-related health conditions due to obesity among US adults.

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Highlights

- Inadequate physical activity accounted for 35% of the relationship between BMI and LTL.
- Diet contributed 25% of the association between %TBF and LTL and 28% between WC and LTL.
- Smoking accounted for 21% of the relationship between %TBF and LTL.
- Inadequate PA, smoking and healthy diet were important mediators associated with obesity and LTL in US adults.
Table 1:

Weighted characteristics of select study variables for the total sample, NHANES© 1999–2002

| % | Mean(SD) |
|---|----------|
| **Leukocyte telomere length (t/s ratio)** | 1.06(.01) |
| **Age, years (20–<85)** | 46.0(.42) |
| **Sex** |  |
| Women | 50.8 |
| Men | 49.2 |
| **Race/ethnicity** |  |
| African American | 18.6 |
| White | 55.6 |
| Mexican American | 25.6 |
| **Poverty income ratio** |  |
| Below poverty | 17.6 |
| Above poverty | 82.4 |
| **Body mass index (kg/m^2)** | 27.9(.16) |
| % Total body fat | 33.7(.19) |
| Waist circumference (cm) | 95.6(.38) |
| **Pack years smoked** |  |
| 30–≥59 | 8.7 |
| <30 | 36.8 |
| Never smoker | 54.5 |
| **Drinking level per day** |  |
| Heavy | 20.9 |
| Moderate | 50.1 |
| Abstainer | 29.0 |
| **Physical activity recommendation level per week** |  |
| Below | 38.5 |
| Meet / Exceed | 61.4 |
| **Healthy Eating Index Score (2005)** | 50.7 (.46) |
| Condition                  | Total (N=4919) | Mean(SD) |
|---------------------------|----------------|----------|
| Hypertension              | 25.1           |          |
| Type 2 diabetes           | 5.3            |          |
| CRP$^c$ (mg/dL)           | 0.39 (0.01)    |          |
| GGT$^d$ (U/L)             | 30.7 (0.70)    |          |

$^a$NHANES, National Health and Nutrition Examination Survey
$^b$SD, standard deviation
$^c$CRP, C-reactive protein
$^d$GGT, gamma glutamyltransferase
Table 2:

Adjusted ordinary least squares regression of adiposity measure on log-transformed telomere length (T/S ratio) for the total sample without and with mediator effects, NHANES\textsuperscript{a} 1999–2002

| Adiposity             | Model 1\textsuperscript{b} | Model 2\textsuperscript{c} |
|-----------------------|----------------------------|----------------------------|
|                       | \( \beta \) (95\% CI)      | \( P \)-value              | \( \beta \) (95\% CI)      | \( P \)-value              |
| BMI                   | \(-.0018 (-.0032, -.0004)\) | \(.01\)                    | \(-.0015 (-.0030, -.0002)\) | \(.02\)                    |
| % Total body fat      | \(-.0022 (-.0034, -.0010)\) | \(.001\)                   | \(-.0019 (-.0033, -.0005)\) | \(.07\)                    |
| Waist circumference   | \(-.0007 (-.0013, -.0002)\) | \(.007\)                   | \(-.0006 (-.0011, -.0001)\) | \(.02\)                    |

\textsuperscript{a}NHANES, National Health and Nutrition Examination Survey

\textsuperscript{b}Adjusted for race/ethnicity, age, age\textsuperscript{2}, sex, PIR, Hypertension, Type 2 diabetes, CRP, GGT, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils.

\textsuperscript{c}Additionally adjusted for pack years smoked, drinking level per day, physical activity level per week, diet based on Healthy Eating Index.
Table 3: Adjusted ordinary least squares regression of individual mediator on log-transformed telomere length (T/S ratio) per adiposity measure and mediation effect for the total sample, NHANES<sup>a</sup> 1999–2002

| Adiposity/Mediator | β (95% CI) | P-value | MP<sup>b</sup> | Zmediation | P-value |
|--------------------|------------|---------|---------------|------------|---------|
| **BMI**<sup>b</sup> |            |         |               |            |         |
| Pack years smoked  |            |         |               |            |         |
| 30–≥59             | −.0379 (−.0734, −.0023) | .03     | .04           | −.45       | .65     |
| <30                 | .0065 (−.0123, .0255)  | .48     | .01           | .11        | .90     |
| Never smoker       | Reference   |         |               |            |         |
| Drinking level per day |            |         |               |            |         |
| Heavy              | .0232 (−.0110, .0573) | .17     | .09           | −1.05      | .29     |
| Moderate           | .0098 (−.0148, .0344) | .42     | .13           | −.94       | .34     |
| Abstainer          | Reference   |         |               |            |         |
| Physical activity per week |            |         |               |            |         |
| Below              | −.0128 (−.0259, .0004) | .05     | .35           | −1.93      | .05     |
| Meet / Exceed      | Reference   |         |               |            |         |
| Healthy Eating Index | .0011 (.0004, .0018) | .003    | .28           | −1.84      | .06     |
| **% Total body fat**<sup>b</sup> |            |         |               |            |         |
| Pack years smoked  |            |         |               |            |         |
| 30–≥59             | −.0386 (−.0742, −.0030) | .03     | .21           | 2.10       | .03     |
| <30                 | .0055 (−.0138, .0248)  | .56     | .02           | .26        | .79     |
| Never smoker       | Reference   |         |               |            |         |
| Drinking level per day |            |         |               |            |         |
| Heavy              | .0227 (−.0114, .0568) | .18     | .08           | −1.17      | .24     |
| Moderate           | .0099 (−.0144, .0343) | .41     | .07           | −.93       | .35     |
| Abstainer          | Reference   |         |               |            |         |
| Physical activity level per week |            |         |               |            |         |
| Below              | −.0115 (−.0247, .0016) | .08     | .29           | −1.87      | .06     |
| Meet / Exceed      | Reference   |         |               |            |         |
| Healthy Eating Index | .0011 (.0004, .0018) | .004    | .25           | −2.33      | .01     |
| Adiposity/Mediator | β (95% CI)   | P-value | MP | Zmedication | P-value |
|-------------------|--------------|---------|----|-------------|---------|
| Waist circumference<sup>b</sup> |              |         |    |             |         |
| Pack years smoked |              |         |    |             |         |
| 30–<59            | −.0367 (−.0724, −.0011) | .04     | .15 | −1.55       | .11     |
| <30               | .0068 (−.0121, .0257)    | .46     | .004| .08         | .94     |
| Never smoker      | Reference     |         |    |             |         |
| Drinking level per day |        |         |    |             |         |
| Heavy             | .0236 (−.0101, .0573)    | .16     | .06 | −.91        | .36     |
| Moderate          | .0101 (−.0141, .0343)    | .40     | .09 | −.95        | .34     |
| Abstainer         | Reference     |         |    |             |         |
| Physical activity level per week |        |         |    |             |         |
| Below             | −.0126 (−.0258, .0007)   | .06     | .32 | −1.95       | .05     |
| Meet/Exceed       | Reference     |         |    |             |         |
| Healthy Eating Index | .0011 (.0004, .0018)  | .003    | .28 | −2.14       | .03     |

<sup>a</sup>NHANES, National Health and Nutrition Examination Survey

<sup>b</sup>Adjusted for race/ethnicity, age, age<sup>2</sup>, sex, PIR, hypertension, type 2 diabetes, CRP, GGT, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, pack years smoked, drinking level per day, physical activity level per week, diet based on Healthy Eating Index.

<sup>c</sup>MP, mediated proportion