International Journal of Obesity (2016) **40,** 411–416

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ORIGINAL ARTICLE

FTO association and interaction with time spent sitting

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BACKGROUND/OBJECTIVES: Multiple studies have revealed an interaction between a variant in the *FTO* gene and self-reported physical activity on body mass index (BMI). Physical inactivity, such as time spent sitting (TSS) has recently gained attention as an important risk factor for obesity and related diseases. It is possible that *FTO* interacts with TSS to affect BMI, and/or that *FTO*'s putative effect on BMI is mediated through TSS.

SUBJECTS/METHODS: We tested these hypotheses in two cohorts of the Framingham Heart Study (FHS) (Offspring: $n = 3430$ and Third Generation: $n = 3888$), and attempted to replicate our results in the Women's Health Initiative (WHI; $n = 4756$). Specifically, we examined whether an association exists between *FTO* and self-reported TSS, and whether an interaction exists between *FTO* and TSS on BMI, while adjusting for several important covariates such as physical activity.

RESULTS: In FHS, we find a significant positive association between the BMI-increasing *FTO* allele and TSS. We find a similar trend in WHI. Mediation analyses suggest that the effect of *FTO* on BMI is mediated through TSS. In FHS, we find a significant interaction of *FTO* and TSS on BMI, whereby the association of TSS with BMI is greatest among those with more *FTO* risk alleles. In WHI, we also find a significant interaction, although the direction is opposite to that in FHS. In a meta-analysis of the two data sets, there is no net interaction of *FTO* with TSS on BMI.

CONCLUSIONS: Our study suggests that *FTO* exerts its effect on BMI, at least partly, through energy expenditure mechanisms such as TSS. Further research into the intersection of genetics, sedentary behavior and obesity-related outcomes is warranted.

International Journal of Obesity (2016) **40,** 411–416; doi:10.1038/ijo.2015.190

INTRODUCTION

Obesity, a major worldwide health problem, arises due to both genetic and environmental risk factors.[1] A variant in the fat mass and obesity associated (*FTO*) gene is the most well-established genetic risk factor for obesity.[2,3] The exact mechanism through which this variant is linked to obesity is still unknown, although there is some indication that it involves the hypothalamus, and thus potentially operates through energy expenditure and/or food intake.[4–8]

Interaction analyses of *FTO* and physical activity (PA) have suggested that the putative effect of the *FTO* variant is diminished among individuals who are more physically active.[9,10] Although PA is an important lifestyle factor, another important factor that has received only recent attention is physical inactivity, such as time spent sitting (TSS).[11,12] Prolonged and sustained sitting behavior has been identified as an independent risk factor for metabolic and cardiovascular disease,[13] possibly by deactivating large postural muscles of the back and legs,[14] leading to decreased lipoprotein lipase activity.[15,16]

Despite the growing recognition of physical inactivity as an important risk factor for obesity and related diseases, little is known regarding how it may be linked to *FTO* as either (1) a potential mechanism connecting *FTO* to obesity, or (2) a modifier of the putative effect of *FTO* variation as has been discovered in the case of PA. Several studies have examined the interaction of body mass index (BMI)-associated genetic variants with sedentary behavior. Qi *et al.*[17] examined the interaction of overall genetic risk to obesity and television watching, and found that the association of genetic risk with obesity was accentuated among those who spent more time watching television. Graff *et al.*[18] found two BMI-associated single nucleotide polymorphisms (SNPs) that interacted with screen time behavior, such that the association of these SNPs with BMI was accentuated among those who engaged in more screen time.

Although television watching is often used as a proxy for sedentary behaviors, this variable does not fully capture total daily sitting time. TSS is an important and comprehensive lifestyle risk factor which has not yet been assessed directly in a genetic interaction study. Furthermore, the extent to which *FTO* may be associated with TSS, and thus potentially mediate the *FTO*-BMI association, has not been examined. Here, we tested the association of *FTO* with TSS, and the interaction of *FTO* with TSS on BMI in two studies, and in multiple ethnic/racial groups. We also use mediation analysis to determine the extent to which TSS mediates the association between *FTO* and BMI.

METHODS

Studies

We used data from 7318 European-American participants from the Offspring (Exam 4; $n = 3430$) and Third Generation (exam 1; $n = 3888$) cohorts of the Framingham Heart Study (FHS), which is a prospective cohort study to examine the causes of heart disease.[19] The Offspring cohort initiated in 1971 includes the offspring of the Original cohort participants as well as the spouses of the offspring.[20] Third Generation participants includes children of the Offspring cohort participants as well as their spouses.[21] Our replication data set consisted of 4756 participants in the Women's Health Initiative (WHI) study.[22] Of these, 1542 are self-identified Hispanic-Americans (HA) and 3214 are self-identified African-Americans (AA). Based on the assumptions of our model described below, we have over 80% statistical power to detect an effect of FTO on a

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Received 9 April 2015; revised 17 August 2015; accepted 30 August 2015; accepted article preview online 22 September 2015; advance online publication, 13 October 2015

phenotype with an r^2 as low as 0.35%, with a total sample size of 4000, at an alpha of 0.05. An r^2 of 0.35% corresponds to the proportion of BMI variation explained by FTO among individuals of European descent.[3] We have no *a priori* expectation regarding the proportion of variation in TSS that variation in FTO might explain. Approval for this study was obtained from the University of Arizona Institutional Review Board. Data were obtained from the database of Genotypes and Phenotypes.

Phenotypic and lifestyle measurements

BMI was measured at baseline as weight (kg) divided by squared height (m). TSS in FHS was measured through the following question: 'Number of hours typically sitting in a typical day?' In WHI, TSS was measured through the following question: 'During a usual day and night, about how many hours do you spend sitting? Include the time you spend sitting at work, sitting at the table eating, driving or riding in a car or bus, and sitting up watching TV or talking.' In WHI, TSS was recorded as one of eight categories ranging from <4 h to >16 h, with the rest being in 1-h increments. We chose the mean for each time-interval category for the purpose of our analyses. PA in FHS was measured using a questionnaire assessing the number of hours spent in slight, moderate and heavy PA on a typical day. As previously described,[23] the slight, moderate and heavy activity were further multiplied by factors of 1.5, 2.4 and 5, respectively, to account for the contribution of these levels of PA in enhancing cardiovascular disease-free life expectancy in adults aged >50 years. These quantities were subsequently summed to create an overall measure of PA in FHS. In WHI, PA was derived by summing metabolic equivalent-hours per week of energy expenditure in mild, moderate and hard exercise activity. Examples of each of these types of activities were provided in the questionnaire. Metabolic equivalent-hours per week were calculated as the summed product of the frequency, duration and intensity of reported activities.[24,25] The intensity of the activity was assigned according to a

Table 1. Descriptive characteristics of the FHS and WHI samples

	FHS (n = *7318*) *European-Americans*	*WHI* (n = *4756*)	
		African-Americans (n = *3214*)	*Hispanic-Americans* (n = *1542*)
Age	45.35 ± 10.91	61.38 ± 7.01	60.23 ± 6.84
Sex (% female)	52	100	100
BMI (kg m^{-2})	26.86 ± 5.25	30.09 ± 6.40	28.13 ± 5.63
Sitting time (h per day)[a]	6.63 ± 3.27	6.80 ± 3.72	5.86 ± 3.36
Smoking (% smokers)	20.5	49.5	35.5
rs9939609 (% A allele)	41	47	31

Abbreviations: FHS, Framingham Heart Study; WHI, Women's Health Initiative. [a]Inferred from categorical variable in WHI sample (see Methods section). Mean and s.d. are shown.

Table 2. Association of FTO (rs9939609) with BMI (top half) and association of FTO with sitting time (bottom half)

	FHS			*WHI*			*Meta-analysis*
	Offspring	*Third generation*[a]	*Combined*	*AA*[a]	*HA*[a]	*Combined*[a]	
BMI (kg m^{-2})							
FTO	0.43 (5.48E-4)	0.47 (8.45E-5)	0.45 (2.07E-7)	0.01 (0.95)	0.38 (8.79E-3)	0.12 (0.16)	0.29 (0.08)
Age	0.04 (4.91E-7)	0.10 (< 2E-16)	0.07 (< 2E-16)	−0.11 (< 2E-16)	−0.05 (2.48E-3)	−0.09 (< 2E-16)	
PA	−0.03 (4.46E-4)	−0.05 (4.89E-11)	−0.03 (4.73E-9)	−0.06 (1.25E-15)	−0.06 (1.98E-10)	−0.06 (< 2E-16)	
Smoking	−0.47 (0.01)	−0.23 (0.32)	−0.15 (0.30)	−0.02 (0.89)	0.33 (0.10)	0.11 (0.36)	
Gender	−1.55 (< 2E-16)	−1.98 (< 2E-16)	−1.79 (< 2E-16)	NA	NA	NA	
Educ.	NA	−0.22 (< 2E-16)	NA	−0.22 (< 2E-16)	−0.12 (2.62E-6)	−0.18 (< 2E-16)	
Employed	NA	−0.05 (0.48)	NA	0.69 (4.69E-5)	−0.43 (0.05)	−0.58 (2.1E-5)	
Diet	NA	NA	NA	9.00E-4 (< 2E-16)	7.89E-4 (4.18E-14)	8.61E-4 (< 2E-16)	
Race	NA	NA	NA	NA	NA	−2.62 (< 2E-16)	
TSS (h per day)							
FTO	0.12 (8.7E-3)	0.09 (0.03)	0.10 (9.94E-4)	0.10 (0.25)	0.19 (0.14)	0.13 (0.08)	0.11 (2.3E-4)
Age	−0.01 (7.2E-4)	−0.01 (5.78E-4)	−0.01 (1.53E-5)	−0.05 (6.54E-7)	−0.00 (0.64)	−0.03 (6.24E-5)	
BMI	0.01 (0.02)	0.02 (4.78E-6)	0.026 (4.88E-9)	0.05 (1.85E-6)	0.010 (0.512)	0.04 (2.47E-7)	
PA	−0.25 (< 2E-16)	−0.25 (< 2E-16)	−0.254 (< 2E-16)	−0.01 (0.02)	−0.02 (0.13)	−0.01 (4.3E-3)	
Smoking	0.10 (0.17)	0.08 (0.31)	−0.11 (0.05)	0.36 (6.91E-3)	0.29 (0.09)	0.33 (1.87E-3)	
Gender	−0.46 (9.23E-13)	−1.00 (< 2E-16)	−0.79 (< 2E-16)	NA	NA	NA	
Educ.	NA	−0.00 (0.52)	NA	0.08 (2.08E-6)	0.13 (7.52E-11)	0.10 (1.65E-14)	
Employed	NA	0.66 (2.78E-11)	NA	0.68 (8.16E-6)	1.13 (2.00-11)	0.91 (7.77E-14)	
Diet	NA	NA	NA	2.62E-4 (2.79E-4)	3.05E-4 (1.1E-13)	2.67E-4(3.32E-6)	
Race	NA	NA	NA	NA	NA	−0.59 (1.02E-6)	

Abbreviations: AA, African-Americans; BMI, body mass index; Educ., education; FHS, Framingham Heart Study; HA, Hispanic-Americans; NA, not applicable; PA, physical activity; TSS, time spent sitting; WHI, Women's Health Initiative. [a]Additionally adjusted for cohort (FHS) or race (WHI). **Additionally adjusted for total dietary intake, employment, and education, cigarette smoking. Coefficients followed by *P*-values in parentheses are shown for each of the covariates included in the model. Analyses were performed separately in each cohort of FHS, and in the combined FHS (offspring+third generation), and separately in AA and HA of WHI in the combined WHI (AA+HA). Meta-analyses of the association of FTO with BMI and TSS were performed on the estimates of FHS-combined and WHI-combined.

standardized classification.[26] Dietary intake was assessed in WHI through estimation of the amount of total dietary energy consumed (Kcal per day) on a normal day. Employment was dichotomized in both FHS (Third Generation) and WHI to reflect the current employment status of each individual (0 if not employed, 1 if employed). Education was recoded into years of education in FHS (Third Generation) and WHI. Cigarette smoking in both data sets was dichotomized to reflect any current or past smoking. Individuals with any missing values for the above variables were excluded from the analyses.

FTO genotype

We used a common variant, rs9939609 (A/T) repeatedly identified in many BMI genome-wide association studies in European-American and AA.[27–29] Given the lack of association of the rs9939609 SNP in some studies of AA,[30] we also considered other SNPs identified in fine-mapping efforts in AA: rs1421085, rs56137030, rs17817964 and rs8050136.[31,32] Genotyping was originally performed using the Affymetrix 500 SNP Array in FHS, and the Affymetrix 6.0 SNP Array in WHI (Affymetrix Inc., Santa Clara, CA, USA). In WHI, the DNA was extracted from blood samples collected at enrollment, and genotyping QC included concordance rates for blinded and unblended duplicates.[33] To test multiple FTO SNPs in WHI, we used imputed genotypes that were available in the database of Genotypes and Phenotypes. In brief, genotypes were imputed using BEAGLE software,[34] and 1000 Genomes data[35] as reference. Individuals with missing values of FTO genotypes were excluded from all analyses.

Statistical analyses

For our main analysis, we used the rs9939609 *FTO* SNP. However, other genetic variants listed above were also tested and showed similar results. The rs9939609 was chosen to maintain consistency across studies. Linear multiple regression models were used to assess the association between (i) rs9939609 and BMI, adjusting for age, gender, smoking and PA. (ii) rs9939609 and TSS, adjusting for age, gender, BMI, smoking and PA. These models were also run in our replication data set of AA and HA in WHI, where we also adjusted for dietary intake. The distributions of all variables were visually examined to ensure no departure from normality. In WHI and the FHS Third Generation cohort, we also included employment and education as covariates. We performed a random-effects, inverse-variance weighted, meta-analysis of the association of rs9939609 with TSS, as well as for the interaction of rs9939609 with TSS, to combine estimates from FHS and WHI, using the metafor package in R.[36] We also examined the possible mediation effect of TSS and PA on the association between rs9939609 and BMI, by performing a causal mediation analysis using the 'mediation' package in R.[37] We included all covariates mentioned above in this analysis, and we included them additively, which is under the model assumption. Mediation analysis was carried out using a least squares regression to statistically test for the indirect effect or average causal mediation effect of TSS and PA (tested separately) on the association of rs9939609 with BMI, and of BMI on the association of rs9939609 with TSS. A non-parametric bootstrap (simulations = 1000) method was adopted to estimate parameter uncertainty. A sensitivity analysis was also conducted on the significant mediation results obtained in European-American, with age, sex, PA, smoking and cohort type as covariates. Lastly, we also tested the interaction of TSS and rs9939609 on BMI by including in the regression model the product of these two variables. All analyses were conducted using R software (version 3.0.2[38]) and two sided *P*-values < 0.05 were considered statistically significant.

RESULTS

Descriptive characteristics of the sample are shown in Table 1. The mean age of FHS participants is lower (mean≈45) than that of participants in WHI (mean≈61). Mean BMI is highest among AA in WHI (mean = 30.1 kg m^{-2}). The frequency of the rs9939609-A allele does not differ greatly across racial/ethnic groups (Table 1).

Table 3. Mediation effect of TSS on the association of *FTO* (rs9939609) with BMI

	Point estimate	*95% CI*	P-*value*
FHS offspring			
Indirect effect (FTO → TSS → BMI)	0.013	0.001, 0.033	0.02
Direct effect (FTO → BMI)	0.413	0.166, 0.658	< 0.01
Total effect	0.427	0.183, 0.671	< 0.01
Proportion of total effect by mediation	0.031	0.002, 0.113	0.02
FHS third generation			
Indirect effect (FTO → TSS → BMI)	0.021	0.003, 0.43	0.03
Direct effect (FTO → BMI)	0.454	0.211, 0.684	< 0.01
Total effect	0.475	0.224, 0.703	< 0.01
Proportion of total effect by mediation	0.044	0.008, 0.119	0.03
WHI AA			
Indirect effect	0.015	−0.015, 0.005	0.29
Direct effect	−0.112	−0.441, 0.200	0.48
Total effect	0.139	−0.400, 0.678	0.60
Proportion of total effect by mediation	−0.161	−1.625, 1.400	0.70
WHI HA			
Indirect effect	0.014	−0.006, 0.049	0.22
Direct effect	0.737	0.320, 1.186	< 0.01
Total effect	0.752	0.332, 1.193	< 0.01
Proportion of total effect by mediation	0.019	−0.008, 0.082	0.22

Abbreviations: AA, African-Americans; BMI, body mass index; CI, confidence interval; FHS, Framingham Heart Study; HA, Hispanic-Americans; TSS, time spent sitting; WHI, Women's Health Initiative. Indirect effect/ACME (FTO → TSS → BMI); direct effect (FTO → BMI).

Table 4. Mediation effect of PA on the association of *FTO* (rs9939609) with BMI

	Point estimate	*95% CI*	P-*value*
FHS offspring			
Indirect effect (FTO → PA → BMI)	−0.001	0.009, 0.005	0.78
Direct effect (FTO → BMI)	0.413	0.167, 0.651	< 0.01
Total effect	0.412	0.168, 0.647	< 0.01
Proportion of total effect by mediation	−0.003	−0.029, 0.019	0.79
FHS third generation			
Indirect effect (FTO → PA → BMI)	−0.001	−0.007, 0.005	0.85
Direct effect (FTO → BMI)	0.454	0.228, 0.701	< 0.01
Total effect	0.453	0.228, 0.701	< 0.01
Proportion of total effect by mediation	−0.001	−0.018, 0.013	0.85
WHI AA			
Indirect effect	−0.012	−0.044,0.018	0.43
Direct effect	−0.112	−0.421, 0.191	0.52
Total effect	−0.125	−0.443, 0.191	0.49
Proportion of total effect by mediation	0.097	−0.807, 1.265	0.70
WHI HA			
Indirect effect	−0.055	−0.125, 0.005	0.07
Direct effect	0.737	0.344, 1.178	< 0.01
Total effect	0.682	0.287, 1.136	< 0.01
Proportion of total effect by mediation	−0.081	−0.303, 0.009	0.08

Abbreviations: AA, African-Americans; BMI, body mass index; CI, confidence interval; FHS, Framingham Heart Study; HA, Hispanic-Americans; WHI, Women's Health Initiative. Indirect Effect/ACME (FTO → PA → BMI); Direct Effect (FTO → BMI).

Association of rs9939609 with BMI and TSS

We first confirmed that the A allele of rs9939609 is associated with a higher BMI, after adjustment for age, sex and PA in the combined FHS ($P = 2.07 \times 10^{-7}$) and WHI HA ($P = 8.79 \times 10^{-3}$). Among AA, we do not observe a significant association of this allele with BMI ($P = 0.95$; Table 2). The regression coefficient of rs9939609 with BMI is slightly smaller upon including TSS in the model (0.43 and 0.48 before inclusion of TSS, and 0.41 and 0.45 after inclusion of TSS, in the FHS Offspring and FHS Third Generation, respectively). In this same latter model, we also find that TSS is positively associated with BMI ($P < 1 \times 10^{-6}$) in FHS. In WHI, the inclusion of TSS in the model with BMI as the outcome results in a decreased coefficient for rs9939609 in AA (0.01, before TSS inclusion vs −0.11, after TSS inclusion), but an increased coefficient in HA (0.39 vs 0.74). In the latter model, TSS is positively associated with BMI ($P < 1 \times 10^{-6}$) in WHI.

The A allele is also associated with greater TSS in the combined FHS ($P = 9.94 \times 10^{-4}$) after adjustment for multiple covariates, including BMI. In the combined WHI data set, we find a trend suggesting that the A allele is positively associated with TSS, although the association is not quite statistically significant ($P = 0.08$), adjusting for all covariates, including employment and education (Table 2). The meta-analyzed estimate of the rs9939609 association with TSS shows a significant positive association of rs9939609 with TSS ($\beta = 0.11$, $P = 2.3 \times 10^{-4}$), as shown in Table 2.

Mediation analysis revealed that the association of rs9939609 with BMI is partly mediated by TSS in FHS ($P = 0.02$ and $P = 0.03$, respectively in Offspring and Third Generation), but not by PA ($P = 0.78$ and $P = 0.85$, respectively in Offspring and Third Generation; Tables 3 and 4). In WHI, we find a similar trend, although the results are not statistically significant (Tables 3 and 4). Table 5 shows the results of the mediation by BMI of rs9939609 on TSS. BMI appears to partly mediate the association between rs9939609 and TSS, although there also appears to be a direct effect of rs9939609 on TSS. As above, the results are stronger in FHS than in WHI.

Table 5. Mediation effect of BMI on the association of *FTO* (rs9939609) with TSS

	Point estimate	*95% CI*	P-*value*
FHS offspring			
Indirect effect (FTO → BMI → TSS)	0.007	0.001, 0.015	0.03
Direct effect (FTO → TSS)	0.124	0.024, 0.213	0.01
Total effect	0.129	0.035, 0.220	< 0.01
Proportion of total effect by mediation	0.049	0.004, 0.216	0.03
FHS third generation			
Indirect effect (FTO → BMI → TSS)	0.013	0.006, 0.024	< 0.01
Direct effect (FTO → TSS)	0.096	0.003, 0.188	0.05
Total effect	0.109	0.017, 0.202	0.02
Proportion of total effect by mediation	0.122	0.035, 0.558	0.02
WHI AA			
Indirect effect (FTO → BMI → TSS)	−0.005	−0.024, 0.012	0.59
Direct effect (FTO → TSS)	0.108	−0.069, 0.285	0.27
Total effect	0.103	−0.073, 0.281	0.28
Proportion of total effect by mediation	−0.053	−0.889, 0.674	0.71
WHI HA			
Indirect effect (FTO → BMI → TSS)	0.022	−0.003, 0.053	0.09
Direct effect (FTO → TSS)	0.196	−0.031, 0.455	0.09
Total effect	0.218	−0.008, 0.477	0.07
Proportion of total effect by mediation	0.098	−0.284, 0.670	0.14

Abbreviations: AA, African-Americans; BMI, body mass index; CI, confidence interval; FHS, Framingham Heart Study; HA, Hispanic-Americans; TSS, time spent sitting. Indirect Effect/ACME (FTO → BMI → TSS); Direct Effect (FTO → TSS).

Interaction of rs9939609 and TSS

As shown in Table 6, we find a significant interaction of rs9939609 and TSS on BMI in both FHS ($P = 3.4 \times 10^{-3}$) and WHI Hispanics ($P = 0.02$). In FHS, the association of TSS with BMI is strongest among those homozygous for the rs9939609 risk allele (Table 7). Conversely, the association of rs9939609 with BMI is strongest among those with high TSS, and weakest among those with low TSS. However, in WHI, we observed the opposite pattern of interaction whereby the association of TSS with BMI is strongest among those homozygous for the rs9939609 protective allele. As shown in Table 6, upon meta-analysis of the interaction estimate in WHI and in FHS, we find no significant interaction of rs9939609 with TSS on BMI ($\beta_{interaction} = -0.29 \times 10^{-4}$, $P_{interaction} = 0.99$).

DISCUSSION

In a large sample of European-Americans, we find that rs9939609 in *FTO* is associated with TSS, that TSS partly mediates the association of rs9939609 with BMI, and that the putative effect of sitting on BMI is greatest in those homozygous for the rs9939609 risk allele. In a replication sample of HA and AA, we find a similar, albeit not statistically significant, association of rs9939609 with TSS. In a meta-analysis of the two data sets we find a significant association of the rs9939609 with TSS that is independent of BMI, PA and other covariates. The weaker findings in WHI may be related to the smaller sample size in HA as well as our finding of no association of FTO variants with BMI in AA. As mentioned, we tried other *FTO* variants previously found to be associated with BMI in AA, but we did not find any association in this cohort with

Table 6. Interaction of rs9939609 with TSS (h per day) on BMI (kg m^{-2}), showing interaction coefficient and corresponding *P*-values in parentheses

	FHS β (P-*value*)			*WHI* β (P-*value*)			*Meta-analysis* β (P-*value*)
	Offspring	*Third generation*[a]	*Combined*[b]	*AA*[a]	*HA*[a]	*Combined*[c]	
*FTO**TSS	0.051 (0.21)	0.088 (0.01)	0.077 (3.4E-3)	−0.056 (0.19)	−0.140 (0.05)	−0.082 (0.02)	−0.29E-4 (0.99)

Abbreviations: AA, African-Americans; BMI, body mass index; FHS, Framingham Heart Study; HA, Hispanic-Americans; TSS, time spent sitting; WHI, Women's Health Initiative. [a]Additionally adjusted dietary intake, education and employment status. [b]Additionally adjusted for cohort effect. [c]Additionally adjusted for race.

Table 7. Association of rs9939609 with BMI in three strata of TSS

	FHS[a] *β (SE)*	*WHI*[b] *β (SE)*
Low TSS	0.16 (0.12); $P=0.18$	0.31 (0.19); $P=0.11$
Moderate TSS	0.45 (0.17); $P=6.9E-3$	0.29 (0.23); $P=0.19$
High TSS	0.85 (0.18); $P=2.9E-6$	−0.38 (0.29); $P=0.20$

Abbreviations: BMI, body mass index; FHS, Framingham Heart Study; SE, standard error; TSS, time spent sitting; WHI, Women's Health Initiative. [a]Adjusted for cohort. [b]Adjusted for race, diet, income, education and employment status.

these SNPs. Mediation analyses provided support for a model in which TSS mediates the association of rs9939609 with BMI, but also for a model in which in which BMI mediates the association of rs9939609 with TSS.

The mechanism through which FTO increases BMI is still unclear, although there is evidence from knockout mice models suggesting *FTO* could be functionally involved in energy homeostasis via regulation of energy expenditure.[39] *FTO* is also found to be strongly expressed in satiety centers within the hypothalamus region,[40–42] where it could potentially influence increased energy intake,[8,43] dietary preferences for increased intake of dietary fats,[41,44] or protein,[7,45] increased appetite and reduced satiety,[46] as well as loss of control over eating.[47] However, the evidence that the *FTO* variant is associated with dietary intake is mixed, raising the alternative scenario in which *FTO* increases BMI through prolonged sedentary behavior. Previous studies of TSS and energy intake have shown that dramatic reductions in energy expenditure due to experimentally induced sitting do not lead to a reduction in appetite or a reduction in food intake.[48,49] Thus, it is possible that individuals with the higher-risk alleles are not modulating energy intake following long periods of reduced energy expenditures associated with high TSS.

Our results of interaction are in agreement with other studies suggesting that the effect of *FTO* is contingent on lifestyle factors such as PA.[50] The direction of the interaction in FHS is consistent with previous studies on PA interactions,[9,10] in that the putative effect of FTO is reduced among those who report low TSS. The inconsistent direction of the interaction in the FHS and WHI data sets makes it difficult to draw any firm conclusions. However, our results do suggest that further exploration of interactions of *FTO* and/or other genetic factors with sedentary behavior is warranted, especially in diverse populations.

The strengths of our study include the use of overall sitting behavior as opposed to only screen/television time, a replication data set, the use of mediation analysis, and the inclusion of many potential confounders as covariates in the statistical models. Limitations include the observational nature of the study, and the subjective and self-reported measurements of TSS, PA and dietary intake. Another limitation is that the replication data set is comprised of a different ethnic/racial group than the discovery data set, as genetic and other risk factors may differ across these groups.

Further efforts in other data sets with objectively measured sitting behavior are needed to confirm our findings and to contribute to our understanding of the physiological mechanisms underlying specific genetic variants, and how these interact with our lifestyle.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the participants and organizers of the FHS, and WHI studies. Data from these studies was obtained from database of Genotypes and Phenotypes through accession numbers: phs000007.v23.p8 and phs000200.v9.p3.c1. YCK was supported by NIH Grant K01DK095032. FHS: The FHS is conducted and supported by the National Heart, Lung and Blood Institute (NHLBI) in collaboration with Boston University (Contract No. N01-HC-25195). This manuscript was not prepared in collaboration with investigators of the FHS and does not necessarily reflect the opinions or views of the FHS, Boston University or NHLBI. Funding for SHARe Affymetrix genotyping was provided by NHLBI Contract N02-HL-64278. SHARe Illumina genotyping was provided under an agreement between Illumina and Boston University. WHI: The WHI program is funded by the National Heart, Lung and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through Contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32 and 44221. This manuscript was not prepared in collaboration with investigators of the WHI, has not been reviewed and/or approved by the WHI, and does not necessarily reflect the opinions of the WHI investigators or the NHLBI. SHARe: Funding for WHI SHARe genotyping was provided by NHLBI Contract N02-HL-64278.

AUTHOR CONTRIBUTIONS

YCK designed the study and wrote the manuscript. AA, AC and YCK performed data management and analysis. YCK, AA, AC, JZ and DR contributed to discussion, helped write the manuscript and reviewed/edited the manuscript.

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