

# Assessing the Causality Factors in the Association between (Abdominal) Obesity and Physical Activity among the Newfoundland Population—A Mendelian Randomization Analysis

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**ABSTRACT:** A total of 1,263 adults from Newfoundland and Labrador were studied in the research. Body mass index (BMI) and percent trunk fat (PTF) were analyzed as biomarkers for obesity. The Mendelian randomization (MR) approach with two single-nucleotide polymorphisms in the fat-mass and obesity (FTO) gene as instruments was employed to assess the causal effect. In both genders, increasing physical activity significantly reduced BMI and PTF when adjusted for age and the FTO gene. The effect of physical activity was stronger on PTF than BMI. Direct observational analyses showed significant increase in BMI/PTF when physical activity decreased. A similar association in MR analyses was not significant. The association between physical activity and BMI/PTF could be due to reversed causality or common confounding factors. Our study provides insights into the causal contributions of obesity to physical activity in adults. Health intervention strategies to increase physical activity among adults should include some other plans such as improving diet for reducing obesity.

**KEYWORDS:** central obesity, physical activity, FTO, trunk fat percentage, Mendelian randomization, instrumental variable, confounding

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## Introduction

There is worldwide acceptance among medical authorities that physical activity is an important element of healthy living.<sup>1–3</sup> Conversely, physical inactivity is increasingly being viewed as one of the most serious causal factors for public health problems in developed countries today.<sup>4</sup> The World Health Organization (WHO) estimates that 3.3 million people die around the world each year due to physical inactivity, making it the fourth leading underlying cause of mortality.<sup>5</sup> It is confirmed that seven chronic diseases have been consistently associated with physical inactivity: coronary heart disease, hypertension, stroke, colon cancer, breast cancer, type 2 diabetes, and osteoporosis.<sup>6–9</sup>

“A 10% reduction in the prevalence of physical inactivity” in Newfoundland and Labrador (NL) “can potentially reduce \$150 million annually in direct health care costs. In 2009, 55% of females and 56% of males in Eastern Health Region reported being physically inactive during leisure time. There has been increase in inactivity for both genders between 2003 and 2011” (Health Promotion Strategic Framework 2012–17; Eastern Health).

Katzmarzyk and Janssen in 2004 reported physical inactivity and obesity as the two of the greatest threats to public health in Canada.<sup>9</sup> Data from the 2012 Canadian Census show that 61.9% of Canadian adults are overweight or obese,

which is of great concern. Moreover, NL has had the highest percentage of overweight/obese residents in Canada since 2008 with 63.2% adults reported being overweight or obese that year. In 2013, 30% of residents of NL were obese, showing a significant increase since 2003 (Newfoundland and Labrador Centre for Health Information, September 2014). According to a recent study,<sup>10</sup> by 2019 an estimated 71% of the adult population in NL will be overweight or obese.

Physically inactive people are also more likely to be obese (BMI  $\geq 30$  kg/m<sup>2</sup>), which is itself an important risk factor for many chronic diseases including coronary artery disease, stroke, hypertension, diabetes, and cancer.<sup>11</sup> A large number of epidemiological studies have focused on examining the relationship between physical activity levels and obesity. Several studies suggest that high physical activity is beneficial in reducing central (abdominal) obesity.<sup>12–15</sup> However, there is no current study, which confirms that levels of physical activity are a *causal effect* of obesity in this province. The purpose of this study was to find answers to the following causality questions:

- 1) Does (abdominal) obesity causally influence inactivity in NL?
- 2) Is the well-known association between physical activity and obesity a “reversed causality” or due to common “confounding factors?”



Findings from this study will be informative for those of us targeting obesity as one of the efforts to increase levels of physical activity in NL, or promote other solutions, such as maintenance of diet.

## Materials and Methods

**Subjects.** All data used were taken from the CODING (Complex Diseases in the Newfoundland Population: Environment and Genetics) study. Eligibility of participants for the CODING study was based upon the following inclusion criteria: 19 years of age or older; at least a third-generation Newfoundlander; and healthy, without any serious metabolic, cardiovascular, or endocrine diseases. The primary method of subject recruitment for the CODING study was the use

of posters and handouts, which were distributed throughout public facilities in the city of St. John's, NL. Each individual completed a number of questionnaires to provide information regarding lifestyle and physical activity. The baseline descriptive characteristics of the individuals included in this study are presented in Table 1. This study was approved by the Health Research Ethics Authority of the Faculty of Medicine of Memorial University, St. John's, Newfoundland, Canada. Informed assent and consent were obtained from all of the volunteers.

### Anthropometric and body composition measurements.

Anthropometric, body composition, and biochemical measurements were performed following a 12-hour fasting period. Height (nearest 0.1 cm) and weight (nearest 0.1 kg)

**Table 1.** Baseline descriptive characteristics of the study population stratified by gender.

VARIABLE	CATEGORIES	TOTAL (n = 1263)			MALE (n = 280)			FEMALE (n = 983)		
		MEAN	SD	%	MEAN	SD	%	MEAN	SD	%
BMI (kg/m <sup>2</sup> )	Overall	26.5	4.9	–	27.4	4.3	–	26.2	4.5	–
	Underweight (<18.5 kg/m <sup>2</sup> )	17.7	0.6	1.2	17.7	–	0.4	17.7	0.7	1.4
	Normal weight (18.5–24.9 kg/m <sup>2</sup> )	22.5	1.5	43.2	22.9	1.6	30.3	22.6	1.6	46.9
	Overweight (25–29.9 kg/m <sup>2</sup> )	27.0	1.4	35.5	27.2	1.3	43.6	27.1	1.5	33.2
	Obese (≥30 kg/m <sup>2</sup> )	34.0	3.8	20.1	33.1	3.0	25.7	34.4	4.0	18.5
Maternal BMI (kg/m <sup>2</sup> )	Overall	26.9	5.5	–	26.4	5.1	–	27.0	5.6	–
	Underweight (<18.5 kg/m <sup>2</sup> )	17.1	1.1	2.9	17.1	1.1	4.3	17.1	1.1	2.4
	Normal weight (18.5–24.9 kg/m <sup>2</sup> )	22.7	1.6	37.0	22.5	1.7	40.4	22.7	1.6	36.0
	Overweight (25–29.9 kg/m <sup>2</sup> )	27.3	1.4	36.3	27.4	1.4	32.5	27.3	1.4	37.6
	Obese (≥30 kg/m <sup>2</sup> )	34.1	5.6	23.8	33.5	3.5	22.8	34.2	6.0	24.0
PTF (%)	–	36.3	9.7	–	29.1	10.1	–	38.4	8.6	–
Age (years)	–	41.7	12.1	–	38.4	14.2	–	42.7	11.3	–
Physical activity score	–	6.2	1.3	–	6.4	1.4	–	6.1	1.3	–
Carbohydrate (g)	–	287.4	148.9	–	337.4	189.8	–	273.1	131.7	–
Calories	–	1995.1	1009.2	–	2423.3	1322.9	–	1873.1	862.8	–
Trans-fat (g)	–	0.1	0.1	–	0.1	0.1	–	0.1	0.1	–
Food energy (kJ)	–	8343.1	4220.8	–	10134.3	5533.7	–	7833.0	3608.2	–
Starch (g)	–	37.8	23.3	–	46.6	26.7	–	35.3	21.7	–
Sugars (g)	–	120.6	81.3	–	140.3	108.2	–	115.0	70.9	–
Saturated fat (g)	–	17.7	17.6	–	21.5	15.5	–	16.6	18.0	–
Cholesterol (mg)	–	211.7	161.0	–	285.3	227.5	–	190.8	129.0	–
Medical status	Taking prescribed medication	–	–	54.1	–	–	33.2	–	–	58.3
	Not taking medication	–	–	45.9	–	–	66.8	–	–	41.7
Smoking status	Non smokers	–	–	89.2	–	–	88.2	–	–	89.4
	Smokers	–	–	10.8	–	–	11.8	–	–	10.6
Drinking status	None	–	–	19.0	–	–	13.9	–	–	20.4
	Casual	–	–	67.1	–	–	62.5	–	–	68.5
	Moderate	–	–	12.3	–	–	20.0	–	–	10.1
	Heavy	–	–	1.6	–	–	3.6	–	–	1.0
Diabetic status	Not diabetic	–	–	97.4	–	–	97.9	–	–	97.3
	Diabetic	–	–	2.6	–	–	2.1	–	–	2.7



measurements were collected and BMI was calculated. BMI was defined as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). Obesity status has been grouped as normal weight (BMI 18.50–24.99), overweight (BMI 25.00–29.99), and obese (BMI  $\geq 30 \text{ kg}/\text{m}^2$ ) as recommended by the WHO. Percent trunk fat (PTF) was measured utilizing dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Medical Systems, Madison, WI, USA). DXA produces a measurement of adipose tissue in the body with a low margin of error. The enCORE software package (version 12.2, GE Medical Systems, Madison, WI, USA) was used for DXA data acquisition.

**Physical activity.** Levels of physical activity were measured using the Ability of the Atherosclerosis Risk in Communities (ARIC) questionnaire,<sup>16</sup> which consists of a Work Index, Sports Index, and Leisure Time Activity Index. A variable excluding workplace activity was used in the data analysis.

**Genotyping.** Genotyping was completed using a blood sample drawn from each individual. Genotyping of 11 SNPs—rs9939609, rs1421085, rs1121980, rs7193144, rs8050136, rs9939973, rs16945088, rs17817449, rs3751812, rs9935401, and rs9941349 from the fat-mass and obesity-associated (FTO) gene—was performed using the high-throughput MassARRAY<sup>®</sup> platform (Sequenom Inc, San Diego, CA, USA). Genotypes were assessed using MassARRAY<sup>®</sup> Typer Analyzer version 4.0. SNP genotyping success rate was over 99%.

**Measuring (central) abdominal obesity.** In this study, we considered PTF as a measurement for central (abdominal) obesity and BMI as a measurement for general obesity. BMI as the standard way of measuring overweight and obesity reflects total body fat without considering the distribution of fat. PTF, which for this study was measured by DXA, is more closely associated with obesity-related detrimental effects on health.<sup>17,18</sup>

**Causality.** When compared to randomized experiments, concluding causal effects under observational studies for the issue of confounding factors are considered to be inadequate. Causal inference from observational data revolves around the crucial assumption that some component of the observational study happened by chance or that the predictor of treatment referred to as an instrumental variable was randomly assigned.<sup>19</sup>

Using variation in genes as instrumental variables, Mendelian randomization provides an alternative way of dealing with the problems of confounding factors in observational studies;<sup>20</sup> these factors are believed to be present but cannot be controlled for because they are not fully understood.<sup>21</sup> Mendelian randomization can be used to assess whether central obesity causally affects physical activity.

**Statistical analysis.** For the basic characteristics of the CODING study participants, the mean and standard deviation of all continuous variables as well as the proportion of individuals within each categorical variable were calculated. Data were analyzed using R version 3.0.0 GUI.

For all model assessments, the raw (unstandardized) variables were used, as both F-statistic and *P*-values are insensitive

to standardizing variables. However, for comparing the magnitude of the effects (BMI versus PTF, or the two SNPs of the FTO gene), variables were standardized.

Direct association studies between the obesity-related measurements (PTF/BMI) and physical activity were conducted using multiple linear regression analysis adjusted for age. Analysis of variance technique was applied for further analysis to adjust for potentially confounding factors between obesity and physical activity. Among the factors associated with obesity in adults as found in some studies,<sup>22–24</sup> the following factors were available in the CODING study: gender, age, smoking status, physical activity score, diabetic status, maternal BMI, drinking status, (prescribed) medical status and eight dietary intake variables.

The Mendelian randomization technique requires that the factors that may potentially confound the association between abdominal obesity and physical activity be independent of the instrumental variable (FTO gene). This assumption was therefore examined by analyzing the association between each of these factors and the FTO genotyping. To assess the associations between every two categorical variables (eg, FTO and smoking) a chi-squared test was applied.

Many studies have shown an association between the *FTO* gene (fat mass- and obesity-associated) and obesity-related traits in populations of different ethnic backgrounds. More specifically, a single-nucleotide polymorphism (SNP) in the *FTO* gene, namely, rs9939609, was previously found to have association with obesity.<sup>25–29</sup> Out of 11 SNPs studied for this research, rs9939609 and rs1121980 (with the largest effect size in the meta-analysis of genome-wide associations for BMI)<sup>30</sup> and the association studies for PTF<sup>15</sup> were considered for further analysis. The basic genetic information about these two SNPs can be found in Table 2.

Payne et al<sup>15</sup> recorded that the *FTO* SNP rs9939609 within the NL population is in high linkage disequilibrium (nonrandom association of alleles at different loci) with rs1121980 ( $r^2 > 0.80$ ). Therefore, the entire research was replicated using the two SNPs as instrumental variables. Moreover, based on the results from their study, different genetic models were investigated and additive models were selected for both SNPs.

Pleiotropy (the influence of one gene on multiple unrelated phenotypic traits) and population stratification (systematic difference in allele frequencies between subpopulations of a population), which are the most known limitations of Mendelian Randomization, were then assessed. NL has been found to have unique genetic architecture based on homogeneity, isolation, and extended linkage disequilibrium.<sup>15,31</sup> However, the instrumental variable assumption that there should not be any direct effect of any genotype on the outcome (as implication for pleiotropy) is not violated, since the genetic variants used as instruments were related to the exposure of interest (BMI/PTF) and not the outcome (physical activity).<sup>32</sup> Major population stratification is unlikely, since this study benefits from a large sample of unrelated individuals from the NL population.

**Table 2.** Basic genotyping information from the CODING study by gender.

SNP	GENOTYPE	MALE		FEMALE		TOTAL	
		FREQUENCY	%	FREQUENCY	%	FREQUENCY	%
rs9939609 (FTOA)	AA	58	20.7	132	13.5	190	15.1
	TA	126	45.0	477	48.5	603	47.7
	TT	96	34.3	374	38.0	470	37.2
rs1121980 (FTOC)	CC	60	21.4	138	14.1	198	15.7
	CT	133	47.5	489	49.7	622	49.2
	TT	87	31.1	356	36.2	443	35.1

The two-stage least squares (TSLS) approach was then performed to estimate the magnitude of the causal effect of each phenotype on the levels of physical activity. More specifically, in the first stage, PTF/BMI was modeled based on the FTO SNPs and age for each gender. In the second stage, the fitted values of PTF/BMI from the first stage were plugged in into the model for physical activity based on PTF/BMI, age, and confounding factors. In order to reduce the potential bias of a weak instrumental variable, F-statistics from the first-stage regression between genotype and BMI/PTF were examined. The Durbin-Wu-Hausman (DWH) test was applied to compare effect estimates from TSLS analysis and direct observational analysis.

## Results

**Baseline characteristics.** Samples from 1,263 independent, unrelated individuals were considered for the analysis in order to avoid biased results due to genetic resemblance of related individuals. Out of the total subjects, about 22% (280 subjects) were males and about 78% (983 subjects) were females. The average age for the males was 38.4 years (SD = 14.2 years) and that of the females was 42.7 years (SD = 11.3 years).

The overall BMI was 27.4 kg/m<sup>2</sup> among the males and 26.2 kg/m<sup>2</sup> among the females. 55.6% of the entire subjects were overweight and obese. This proportion was 51.7 and 69.3

for females and males, respectively. Moreover, males reported lower average PTF (29.1%, SD = 10.1%) than females (38.4%, SD = 8.6%). Overall PTF was 36.3% (SD = 9.7%) across the entire subjects used in this study (Table 1). Furthermore, PTF and BMI were moderately correlated (Pearson's correlation coefficient = 0.63).

Findings from the outcome variable of this study show physical activity score averages at 6.2 units (SD = 1.3 units) for all the subjects. Among males, the physical activity score stood an average of 6.4 units (SD = 1.4 units) and at an average of 6.1 units (SD = 1.3 units) among females.

Based on a *t*-test comparing the trunk fat percentage and BMI for both the genders, and differences in minor allele frequencies, all the following models were stratified by gender.

**Association studies.** Payne et al<sup>15</sup> showed that in males, rs9939609 and rs1421085 were significant in explaining PTF, but none of the two SNPs was significant in females. They also did not find an association between the two SNPs and BMI in the NL population for either gender.

Many studies show that higher levels of physical activity can reduce the likelihood of being overweight or obese.<sup>12,13,33</sup> Our study on the NL population confirms these results for both genders. More specifically, the results from Table 3 (first-stage least squares, modeling PTF/BMI based on the FTO SNPs and age), show that every score increase in physical activity is highly associated with 1.82 and 2.02 percent

**Table 3.** Association study between PTF/BMI and physical activity by gender (adjusted for age and FTO SNP), first stage.

SNP	RESPONSE	GENDER	SAMPLE SIZE	Z-SCORE COEFFICIENT*	COEFFICIENT*	95% C.I.	P-VALUE
FTOA_ADD	PTF	Male	280	-0.253	-1.818	(-2.591, -1.044)	0.000
		Female	983	-0.300	-2.018	(-2.409, -1.627)	0.000
	BMI	Male	280	-0.129	-0.400	(-0.769, -0.031)	0.033
		Female	983	-0.178	-0.694	(-0.934, -0.454)	0.000
FTOC_ADD	PTF	Male	280	-0.252	-1.817	(-2.592, -1.042)	0.000
		Female	983	-0.300	-2.018	(-2.410, -1.626)	0.000
	BMI	Male	280	-0.128	-0.396	(-0.76, -0.0267)	0.036
		Female	983	-0.178	-0.695	(-0.935, -0.454)	0.000

**Note:** \*Refers to physical activity.

**Table 4.** Direct observational analysis (reversed association study) between PTF/BMI and physical activity by gender.

VARIABLE	GENDER	Z-SCORE COEFFICIENT*	COEFFICIENT*	95% C.I.	P-VALUE	F-STATISTIC (DF)
<b>MODEL ONE**</b>						
PTF	Male	-0.292	-0.040	(-0.057, -0.024)	0.000	24.22 (2, 277)
	Female	-0.314	-0.047	(-0.056, -0.038)	0.000	56.91 (2, 980)
BMI	Male	-0.132	-0.042	(-0.079, -0.005)	0.026	14.54 (2, 277)
	Female	-0.179	-0.045	(-0.061, -0.029)	0.000	21.73 (2, 980)
<b>MODEL TWO***</b>						
PTF	Male	-0.269	-0.037	(-0.054, -0.021)	0.000	21.08 (3, 276)
	Female	-0.303	-0.045	(-0.054, -0.036)	0.000	32.94 (4, 978)
BMI	Male	-0.100	-0.032	(-0.069, -0.005)	0.087	14.57 (3, 276)
	Female	-0.171	-0.044	(-0.059, -0.028)	0.000	14.56 (4, 978)

**Notes:** \*Refers to PTF/BMI. \*\*Adjusted for age. \*\*\*Adjusted for confounding factors.

decrease in PTF for males and females, respectively. For BMI, an increase in every unit of physical activity is highly associated with 0.4 and 0.69 kg/m<sup>2</sup> decline in BMI for males and females, respectively. Moreover, physical activity in the presence of the SNPs and age explains an additional 8.8% and 5.6% of the variability reduction in PTF in females and males,

respectively. The additional variability in BMI, however, is negligible (less than 2% in both genders; Coefficients of Partial Determination from the output of R, data not shown).

Our results from Model One (reversed association formulated as PTF = physical activity + age + FTO + error) in Table 4 show that for males, every percent increase in PTF

**Table 5.** Association study between the potential confounding factors and physical activity by gender.

FACTOR	MALES				FEMALES			
	INTERCEPT	COEFFICIENT	95% C.I.	P-VALUE	INTERCEPT	COEFFICIENT	95% C.I.	P-VALUE
<b>Smoking status</b>								
Non Smoker	6.5	–	(6.3, 6.7)	0.000	6.2	–	(6.1, 6.3)	0.000
Smoker	6.5	-1.000	(-1.5, -0.5)	<b>0.000</b>	6.2	-0.300	(-0.6, -0.07)	<b>0.012</b>
<b>Drinking status</b>								
None	6.1	–	(5.6, 6.4)	0.000	5.9	–	(5.7, 6.1)	0.000
Casual drinkers	6.1	0.300	(-0.1, 0.8)	0.164	5.9	0.268	(0.07, 0.5)	<b>0.009</b>
Moderate drinkers	6.1	0.700	(0.1, 1.2)	<b>0.013</b>	5.9	0.298	(-0.009, 0.6)	0.057
Heavy drinkers	6.1	0.000	(-1.00, 0.90)	0.947	5.9	0.233	(-0.6, 1.0)	0.572
Food energy (kj)	6.1	0.000	(0.000, 0.000)	<b>0.026</b>	5.9	0.000	(0.000, 0.000)	<b>0.002</b>
Carbohydrates (g)	6.0	0.001	(0.000, 0.002)	<b>0.011</b>	5.9	0.001	(-0.000, 0.007)	<b>0.004</b>
Starch (g)	6.4	0.000	(-0.006, 0.006)	0.928	6.3	-0.003	(-0.007, 0.001)	0.116
Sugars (g)	6.2	0.001	(0.000, 0.003)	0.061	6.0	0.001	(-0.000, 0.002)	0.081
Saturated fat (g)	6.5	-0.003	(-0.013, 0.007)	0.566	6.2	0.000	(-0.004, 0.004)	0.860
Trans fat (g)	6.4	-0.252	(-1.80, 1.30)	0.745	6.2	-1.200	(-2.3, -0.1)	<b>0.025</b>
Cholesterol (mg)	6.2	0.000	(0.000, 0.001)	<b>0.046</b>	6.1	0.000	(-0.000, 40.0)	0.316
<b>Medical status</b>								
Not taking medication	6.5	–	(6.30, 6.70)	0.000	6.3	–	(6.20, 6.40)	0.000
Taking medication	6.5	-0.402	(-0.7, -0.05)	<b>0.024</b>	6.3	-0.269	(-0.4, -0.1)	<b>0.001</b>
Maternal BMI	5.7	0.027	(-0.005, 0.06)	0.101	6.4	-0.010	(-0.02, 0.004)	0.155
<b>Diabetic status</b>								
No	6.4	–	(6.3, 6.6)	0.000	6.2	–	(6.0, 6.2)	0.000
Yes	6.4	-0.681	(-1.8, 0.4)	0.204	6.2	-0.257	(-0.7, 0.2)	0.302

**Note:** Significant (level 0.05) factors are indicated with bold font.





**Table 6.** Association study between the potential confounding factors and the FTO gene among females.

FACTOR	FTOA			GENOYPE*			FTOC			P-VALUE
	GENOYPE*	INTERCEPT	COEFFICIENT	95% C.I.	GENOYPE*	INTERCEPT	COEFFICIENT	95% C.I.	P-VALUE	
Food energy	TA	7706.1	163.400	(-325.9, 652.6)	TC	7693.5	226.000	(-267.6, 719.5)	0.369	
	AA	7706.1	354.500	(-362.6, 1071.7)	CC	7693.5	192.800	(-517.6, 903.2)	0.594	
Carbohydrates	TA	270.5	1.503	(-16.3, 19.3)	TC	269.5	4.830	(-13.1, 22.8)	0.599	
	AA	270.5	13.721	(-12.4, 39.8)	CC	269.5	8.064	(-17.8, 34.0)	0.542	
Starch	TA	35.7	-1.484	(-4.4, 1.4)	TC	35.4	-0.800	(-3.7, 2.1)	0.596	
	AA	35.7	2.425	(-1.8, 6.7)	CC	35.4	2.049	(-2.2, 6.3)	0.346	
Sugars	TA	117.3	-4.091	(-13.7, 5.5)	TC	117.0	-2.604	(-12.3, 7.1)	0.598	
	AA	117.3	-2.283	(-16.3, 11.8)	CC	117.0	-5.268	(-19.2, 8.6)	0.459	
Saturated fat	TA	15.8	1.380	(-1.1, 3.8)	TC	15.8	1.620	(-0.8, 4.1)	0.197	
	AA	15.8	0.596	(-2.9, 4.1)	CC	15.8	0.073	(-3.4, 3.6)	0.968	
Trans fat	TA	0.1	0.000	(-0.01, 0.01)	TC	0.1	0.002	(-0.01, 0.01)	0.728	
	AA	0.1	0.005	(-0.01, 0.01)	CC	0.1	-0.002	(-0.01, 0.01)	0.777	
Cholesterol	TA	186.8	8.701	(-8.7, 26.1)	TC	186.8	9.249	(-8.3, 26.8)	0.304	
	AA	186.8	-1.895	(-27.5, 23.7)	CC	186.8	-4.642	(-30.0, 20.7)	0.720	
Maternal BMI	TA	26.9	0.221	(-0.5, 0.9)	TC	26.9	0.264	(-0.5, 1.03)	0.504	
	AA	26.9	0.505	(-0.6, 1.6)	CC	26.9	0.451	(-0.6, 1.5)	0.426	

**Note:** \*All coefficients should be interpreted as compared to the base genotype TT.



is associated with an average 0.04 score decrease in physical activity. This impact is slightly higher (lower z-score) in females. Moreover, every kilogram per meter square increase in BMI for females is associated with 0.045 unit decrease in physical activity. Comparing the z-score coefficients, adjusted R-squared and F-statistic values in Models One and Two (reversed association formulated as  $PTF = \text{physical activity} + \text{age} + \text{FTO} + \text{corresponding significant confounding factors} + \text{error}$ ) in the table, it can be seen that these associations were largely unchanged by adjusting for confounders. Moreover, for both genders in the two models, the magnitude of PTF versus BMI is higher for physical activity. For males, however, the association between BMI and physical activity (after adjusting for confounding factors) is no longer significant at level  $\alpha = 0.005$ .

The results of the investigation on potential confounding factors that may affect physical activity are presented in Table 5. Smoking and medical status for both genders significantly lower the physical activity score. Trans-fat intake has a negative impact on physical activity score only among females. These factors were employed as confounding factors for our Mendelian randomization study.

**Mendelian randomization analysis (MRA).** We examined the required assumption for MRA that the confounding factors should be independent of the instrumental variable (FTO gene). Analyzing the association between these factors (listed in Statistical analysis) and the FTO SNPs, the assumption was confirmed. These findings are shown in Tables 6–9. This suggests that the aforementioned confounding factors may be used in MRA. The ANOVA test comparing the mean physical activity scores showed no significant difference among genotyping of the two FTO SNPs. These results (shown in Table 10) imply that the FTO SNPs do not directly affect levels of activities in either gender, which is required for MRA.

Although direct observational studies for males showed a significant association between PTF and physical activity, Mendelian randomization analyses did not show a similar and significant association (coefficients based on TSLS are reported in Table 11). The direct impact of PTF on the physical activity score was stronger in females as compared to males. For females only, the DWH test showed a significant difference between the two methods. This implies

that the direct association method is significantly different from the TSLS method, which means that the FTO SNP would work as an intermediate factor to make a significant contribution to the variability in PTF/BMI. However, as the study by Payne et al<sup>15</sup> showed no associations between the two SNPs and PTF in females, these SNPs were not suitable candidates for MRA in the first place. Therefore, the two SNPs of the FTO gene are not proper candidates for MRA on BMI, for either gender. The direct impact of BMI on physical activity score for females, however, is significant.

It suggests that for females, physical activity may be directly modeled based on BMI, age, and confounding factors, without the indirect influence of the FTO SNPs. These associations were largely unchanged when using FTOC instead of FTOA.

## Discussion

In this study, we assessed the causality of abdominal (central) obesity on physical activity among adults of the NL population. We also investigated bidirectional associations between physical activity and obesity in adults of NL. Our results show that for both genders, increase in physical activity significantly reduces BMI and PTF, when adjusted for age and the FTO gene. Our observational analyses showed that increase in BMI/PTF is significantly associated with decrease in physical activity when adjusted for age. However, similar association between PTF and physical activity in Mendelian randomization analyses was not significant.

While many studies on the causality effect of obesity on individuals' health use BMI as a biomarker for obesity,<sup>30,34,35</sup> in our research, we used PTF as a measure of fat-mass in addition to the standard measure of obesity (BMI). Our results show that the marginal effect of PTF on physical activity was stronger as compared to that of BMI. This was expected, as BMI does not differentiate between fat and lean mass, and physical activity positively correlates with lean mass.

We also inferred causality using the Mendelian randomization technique as outlined by other researchers.<sup>32,36,37</sup> This method uses variation in genes as instrumental variables to provide an alternative way of dealing with the problems of confounding factors in observational studies. Moreover, we used the FTO gene, which is reliably established in

**Table 7.** Association study between the potential confounding factors and the FTO gene among females.

FACTOR	FTOA			FTOC		
	CHI-SQUARE VALUE	DF	P-VALUE	CHI-SQUARE VALUE	DF	P-VALUE
Medical status	0.66	2	0.719	1.54	2	0.464
Smoking status	0.74	2	0.690	0.64	2	0.726
Diabetic status	0.87	2	0.649	1.05	2	0.591
Drinking status	2.83	6	0.830	3.92	6	0.688



**Table 8.** Association study between the potential confounding factors and the FTO gene among males.

FACTOR	FTOA				FTOC			
	GENOTYPE	INTERCEPT	COEFFICIENT	P-VALUE	GENOTYPE	INTERCEPT	COEFFICIENT	P-VALUE
Food energy	TA	10604.1	-757.600	0.316	TC	10707.1	-897.700	0.241
	AA	10604.1	-622.200	0.500	CC	10707.1	-683.200	0.463
Carbohydrates	TA	356.5	-31.880	0.217	TC	358.6	-33.790	0.198
	AA	356.5	-21.960	0.488	CC	358.6	-23.050	0.470
Starch	TA	48.1	-2.446	0.500	TC	46.7	-0.739	0.841
	AA	48.1	-1.994	0.654	CC	46.7	1.250	0.781
Sugars	TA	158.1	-25.260	0.085	TC	159.9	-26.560	0.075
	AA	158.1	-31.040	0.084	CC	159.9	-32.490	0.074
Saturated fat	TA	21.8	-0.525	0.804	TC	22.3	-1.401	0.515
	AA	21.8	-0.794	0.759	CC	22.3	-0.876	0.738
Trans fat	TA	0.1	-0.011	0.437	TC	0.1	-0.019	0.197
	AA	0.1	0.002	0.926	CC	0.1	-0.003	0.862
Cholesterol	TA	290.5	-21.560	0.485	TC	290.1	-19.620	0.533
	AA	290.5	21.800	0.565	CC	290.1	21.140	0.581
Maternal BMI	TA	26.4	-0.095	0.892	TC	26.4	-0.133	0.851
	AA	26.4	0.386	0.653	CC	26.4	0.166	0.848

**Table 9.** Association study between the potential confounding factors and the FTO gene among males.

FACTOR	FTOA			FTOC		
	CHI-SQUARE VALUE	DF	P-VALUE	CHI-SQUARE VALUE	DF	P-VALUE
Medical status	4.40	2	0.111	3.49	2	0.175
Smoking status	0.94	2	0.375	0.89	2	0.726
Diabetic status	1.06	2	0.588	0.82	2	0.663
Drinking status	6.44	6	0.375	4.79	6	0.571



**Table 10.** ANOVA comparing the means of physical activity for different FTO genotyping.

INSTRUMENTAL VARIABLE	PHYSICAL ACTIVITY		
	F-VALUE	DF	P-VALUE
Males			
FTOA	2.228	1, 278	0.137
FTOC	2.691	1, 278	0.102
Females			
FTOA	2.409	1, 981	0.121
FTOC	2.684	1, 981	0.102

independent studies to be highly associated with the exposure variables (BMI and PTF).<sup>15,38,39</sup> Our study is on the NL population, which is generally isolated and homogenous (mostly Caucasians). It provides a suitable population for generalized genetic results.

Exploratory MR approach investigating the association between adiposity and activity levels may provide evidence for causality in this direction. However, it does not exclude the possibility that physical activity has a causal effect on obesity. A genetic instrument for physical activity is required to test the relationship in a bidirectional manner. Moreover, we were limited with few possibilities of finding all the factors that confound between BMI/PTF and physical activity, since our dataset did not include information on the social and economic status of the individuals.

Further work is required to determine a more accurate estimate of the causal effect in the reverse direction. This study still provides some insight into the causal contributions of (central) obesity to physical activity score in adults of NL. It means that the health intervention strategies at the provincial level that attempt to increase physical activity among adults in the province should perhaps include some other plans, such as improving diet, for reducing abdominal obesity.

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## Author Contributions

Analyzed the data: FB. Wrote the first draft of the manuscript: FB. Contributed to the writing of the manuscript: FB and TA. Agree with manuscript results and conclusions: TA. Jointly developed the structure and arguments for the paper: FB and TA. Made critical revisions and approved final version: TA. All authors reviewed and approved of the final manuscript.

**Table 11.** Comparing direct estimate of the effect of BMI/PTF vs. TSLS method on physical activity.

GENDER	RISK FACTOR	SNP	F-STATISTIC		DF	ADJUSTED R <sup>2</sup>		COEFFICIENT***		Z-SCORE COEFFICIENT***		DWH TEST P-VALUE
			DIRECT ESTIMATE**	TSLS*		DIRECT ESTIMATE	TSLS	DIRECT ESTIMATE	TSLS	DIRECT ESTIMATE	TSLS	
Males	PTF	FTOA_ADD	21.08	13.79	3, 276	0.178	0.121	-0.037	-0.062	-0.269	-0.193	0.5892
	BMI	FTOA_ADD	14.57	13.79	3, 276	0.127	0.121	-0.032	-0.229	-0.100	-0.180	~1
	PTF	FTOC_ADD	21.08	13.92	3, 276	0.178	0.122	-0.037	-0.076	-0.269	-0.276	0.5399
	BMI	FTOC_ADD	14.57	13.92	3, 276	0.127	0.122	-0.032	-0.238	-0.100	-0.189	~1
Females	PTF	FTOA_ADD	32.94	8.98	4, 978	0.115	0.031	-0.045	0.370	-0.303	0.618	<b>0.0380</b>
	BMI	FTOA_ADD	14.56	7.70	4, 978	0.052	0.027	-0.044	0.635	-0.171	0.389	0.0000
	PTF	FTOC_ADD	32.94	9.41	4, 978	0.115	0.033	-0.045	1.456	-0.303	-0.097	<b>0.0213</b>
	BMI	FTOC_ADD	14.56	8.18	4, 978	0.052	0.028	-0.044	1.456	-0.171	0.888	0.0000

**Notes:** \*TSLS Model is physical activity based on the estimated PTF from the first stage, adjusted for age and confounding factors. \*\*Direct Estimate model is physical activity based on PTF/BMI, adjusted for age and confounding factors. \*\*\*PTF/BMI. Significant (level 0.05) factors are indicated with bold font.



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