Effect of FTO Gene and Physical Activity Interaction on Trunk Fat Percentage Among the Newfoundland Population

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ABSTRACT

OBJECTIVE: To explore the effect of FTO gene and physical activity interaction on trunk fat percentage.

DESIGN AND METHODS: Subjects are 3,004 individuals from Newfoundland and Labrador whose trunk fat percentage and physical activity were recorded, and who were genotyped for 11 single-nucleotide polymorphisms (SNPs) in the FTO gene. Subjects were stratified by gender. Multiple tests and multiple regressions were used to analyze the effects of physical activity, variants of FTO, age, and their interactions on trunk fat percentage. Dietary information and other environmental factors were not considered.

RESULTS: Higher levels of physical activity tend to reduce trunk fat percentage in all individuals. Furthermore, in males, rs9939609 and rs1421085 were significant (α = 0.05) in explaining central body fat, but no SNPs were significant in females. For highly active males, trunk fat percentage varied significantly between variants of rs9939609 and rs1421085, but there is no significant effect among individuals with low activity. The other SNPs examined were not significant in explaining trunk fat percentage.

CONCLUSIONS: Homozygous male carriers of non-obesity risk alleles at rs9939609 and rs1421085 will have significant reduction in central body fat from physical activity in contrast to homozygous males of the obesity-risk alleles. The additive effect of these SNPs is found in males with high physical activity only.

KEYWORDS: FTO, rs9939609, rs1421085, physical activity, Newfoundland population

Introduction

Overweight and obesity have become a major epidemic problem in Newfoundland and Labrador (NL) and throughout Canada. These conditions can lead to high risks of contracting heart diseases, musculoskeletal disorders, and some cancers that can be detrimental to health and even fatal. Data from the 2012 Canadian census show that 61.9% of Canadian adults are overweight or obese, which is of great concern. NL has had the highest percentage of self-reported overweight/obese residents in Canada since 2008 with 63.2% of adults reporting being overweight or obese that year. It is of interest to explore potential causes of these escalating rates of obesity in NL in order to educate individuals and stratify patients into risk classes to improve the treatment of obesity.

Although many recent studies (including genome-wide association studies) confirm significant genetic associations with obesity-related traits such as body mass index (BMI) and waist circumference, the variability of the traits attributable to genetic factors is still small. Heritability estimates for obesity-related traits indicate that a considerable percentage of

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phenotypic variation still needs to be explained. It is suggested that the interaction between certain genes and environmental factors may contribute to the variability in those traits, since most studies have not taken gene–environment interactions into consideration.

BMI is commonly used when examining obesity due to its ease and low cost of measurement. Trunk fat percentage is a more precise measurement of central body fat not commonly explored in obesity studies. Trunk fat percentage, which for this study has been measured by dual-energy X-ray absorptiometry (DXA), is more closely associated with obesity-related detrimental effects on health.10–12 DXA is superior to BMI in the measurement of body fat, and BMI has been shown to significantly misclassify obesity compared to DXA measurement,11 with one study referring specifically to the NL population.12

Many studies have shown association between the FTO gene (fat mass and obesity associated) and obesity-related traits in populations of different ethnic backgrounds.13–15 More specifically, two single-nucleotide polymorphisms (SNPs) in the FTO gene, namely rs9939609 and rs1421085, have been previously found to have association with obesity.16–17 These studies have all contributed to the worldwide attribution of the A and C alleles, respectively, in these SNPs to an increase in BMI. Other SNPs in the FTO gene have been explored less often; however, nine other SNPs will be considered in this study.

Many studies show that higher levels of physical activity can reduce the likelihood of being overweight or obese.18–20 However, none of these studies consider the effect of genes as a modifier. Low physical activity has been thought to accentuate the effect of the FTO gene,21 and it has been shown that the effect of genetic susceptibility to obesity caused by FTO variants can actually be attenuated by increased physical activity.22 One study recently found that rs9939609 was associated with trunk obesity as early as in adolescence.23 However, the sample size was much smaller than in this study, and the adiposity measurements were made using a lipometer. A lipometer measures subcutaneous fat only and its accuracy may not be as dependable as the DXA measurements of trunk fat percentage used here.24

Our goal was to further explore the relationships between physical activity, FTO SNPs, and trunk fat percentage in an association study with a large sample of individuals from the NL population. NL has been found to have unique genetic architecture, and based on homogeneity, isolation, and extended linkage disequilibrium, it has been proposed that this population provides advantageous genetic mapping of complex diseases compared to many admixed populations.25 Due to the unique genetic population in NL and significant findings in this study, we believe that our results pertaining to trunk fat percentage variation add important and widely applicable findings to the literature.

Methods and Procedures

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 on 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. This literature was distributed throughout public facilities (offices and hospitals) in the city of St. John's, NL. Each individual completed a number of questionnaires to obtain information regarding lifestyle and physical activity. Anthropometric, body composition, and biochemical measurements were performed following a 12-hour fasting period. The basic characteristics of the individuals included in this study are presented in Table 1.

Anthropometric and body composition measurements. Height (nearest 0.1 cm) and weight (nearest 0.1 kg)
measurements were collected and BMI was calculated. BMI was defined as weight divided by height squared (kg/m²). Obesity status has been grouped as normal weight (BMI 18.50–24.99), overweight (BMI 25.00–29.99), and obese (≥30) as recommended by the World Health Organization (WHO).²⁶ Percent trunk fat was measured utilizing DXA (Lunar Prodigy, GE Medical Systems, Madison, WI, USA). DXA produces an accurate 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,²⁷ which consists of a Work Index, Sports Index, and Leisure Time Activity Index. A variable exclusive of workplace activity was used in the data analysis. This variable is continuous, however, three categories were constructed to organize individuals into low, moderate, and high physical activity, in order to generalize results and make them more accessible. Indices of 5 or less were classified as low, greater than 5 but less than 8 were classified as moderate, and indices of 8 and higher were classified as high activity.

Genotyping. Genotyping was completed using a drawn blood sample from each individual. Genotyping of SNPs rs9939609, rs1421085, rs1121980, rs7193144, rs8050136, rs9939973, rs16945088, rs17817449, rs3751812, rs9935401, and rs9941349 was performed using the high-throughput MassARRAY® platform (Sequenom Inc, San Diego, CA, USA). Amplification, Shrimp Alkaline Phosphatase (SAP) digestion, and extension were performed according to the manufacturer’s instructions. Unincorporated deoxyribonucleotides were SAP digested prior to MassARRAY® iPLEX Gold reagent kit. Extension products were desalted and dispensed onto a SpectroChip (Sequenom Inc, San Diego, CA, USA) using a MassARRAY® Nanodispenser prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometer analysis with a MassARRAY® Compact Analyzer mass spectrometer. Genotypes were assessed using MassARRAY® Typer Analyzer version 4.0. SNP genotyping success rate was over 99%. The genetic information of the individuals studied was presented in Table 2a.

Statistical analysis. All analyses were conducted using R version 3.0.1 and were repeated using both the complete sample of individuals, as well as a subsample of 1,843 independent, nonfamilial individuals, in order to verify that results were not biased due to genetic resemblance of related individuals. For all SNPs in this study aside from rs16945088, Chi-Squared tests were performed to determine whether or not the population followed Hardy–Weinberg proportions. For rs16945088, due to insufficient observations of individuals with GG genotype to warrant a Chi-Squared test, P values were calculated from binomial testing. The binomial tests compared the observed cases of these individuals with the expected probabilities under the assumption of Hardy–Weinberg equilibrium. Linkage disequilibrium was examined for each pair of SNPs based on R² values. Hardy-Weinberg equilibrium tests and linkage disequilibrium analyses were completed using the genetics package (version 1.3.8.1) in R.

A t-test comparing the trunk fat percentage for males and females was performed, resulting in stratification by gender. Analysis of association between trunk fat percentage and each SNP was performed using a general linear model, assuming an additive or dominant model for each SNP, depending on the results of Cochran–Armitage Trend Tests. For each SNP, three Cochran–Armitage Trend Tests (one assuming dominant to the first allele, one assuming dominant to the second allele, and one assuming an additive model) were performed, and the model associated with the lowest P value for each SNP was considered for further analysis. To compare the effect of genetic variants in each SNP on trunk fat percentage, t-tests were also performed to evaluate the difference in mean trunk fat percentage between each pair of allelic variants within each SNP. The tests performed were based on the genetic model resulting from the Cochran–Armitage Trend Test results. Multiple regression models were used to examine the association between physical activity and trunk fat percentage, and were adjusted for age and variants in each SNP. The interaction between the SNP variants and physical activity on trunk fat percentage was examined using analysis of variance tests and non-parametric Kruskall–Wallis tests. P values less than 0.05 were considered to be significant for all statistical analysis.

Results

Hardy–Weinberg proportions and linkage disequilibrium. Using Fisher’s exact test, and a binomial test for rs16945088, the null hypothesis that the population is in Hardy–Weinberg equilibrium was tested. Since the analysis of related individuals can lead to bias in Hardy–Weinberg proportion testing,²⁸ each test was also performed on the subsample of unrelated individuals. The P values for all SNPs were outside of the rejection region, thus validating the assumption of Hardy–Weinberg equilibrium in the population studied. The P values for all SNPs can be seen in Supplementary Tables 1 and 2. The minor allele frequencies observed for rs9939609 were 40.8% and 37.6%, respectively, for males and females. Similarly, the minor allele frequencies for rs1421085 were 43.1% and 39.0% for males and females, respectively. The difference in minor allele frequencies between males and females was significant for both SNPs mentioned. The minor allele frequencies of all other SNPs studied may be seen in Table 2b. Linkage disequilibrium between SNPs was examined using R² values. All R² values for the full sample may be viewed in Supplementary Table 3. Since all observed values are above 0.8 (aside from those associated with rs16945088)
and have high power to detect associated genetic effects at reasonable levels of heritability (as seen from Supplementary Fig. 1), it is assumed that no putative variant associated with the analyzed SNPs has been missed.

**Candidate SNPs association study.** Based on a $t$-test comparing the trunk fat percentage for both the genders ($P=0$) and differences in minor allele frequencies, all models were assessed separately by gender. For each SNP, an additive or dominant model was considered based on the smallest $P$ value from Cochran–Armitage Trend Tests. The results of these tests can be seen in Table 3. Multiple $t$-tests were performed for each gender comparing the mean trunk fat percentages of different variants in each SNP. The Bartlett test of homogeneity of variance confirmed the assumption of constant variance within variants of each SNP, thus pooled sample variance was used for each test. The results of the multiple $t$-tests and the $P$ value for each test can be seen in Table 4. Each $P$ value was compared to an adjusted $P$ value using the Benjamini–Hochberg procedure at level 0.05 to determine whether the result was significant, and these outcomes are also

| SNP          | FREQUENCY* | GENOTYPE | MALE FREQUENCY | MALE % | FEMALE FREQUENCY | FEMALE % |
|--------------|------------|----------|----------------|--------|------------------|----------|
| rs9939973    | 893        | GG       | 68             | 29.7   | 226              | 34.0     |
|              |            | AG       | 118            | 51.5   | 340              | 51.2     |
|              |            | AA       | 43             | 18.8   | 98               | 14.8     |
| rs1421085    | 2435       | CC       | 109            | 17.7   | 274              | 15.1     |
|              |            | CT       | 312            | 50.7   | 872              | 47.9     |
|              |            | TT       | 194            | 31.6   | 674              | 37.0     |
| rs1121980    | 892        | CC       | 67             | 29.6   | 228              | 34.2     |
|              |            | TC       | 116            | 51.3   | 339              | 50.9     |
|              |            | TT       | 43             | 19.1   | 99               | 14.9     |
| rs7193144    | 894        | CC       | 40             | 17.5   | 91               | 13.7     |
|              |            | TC       | 113            | 49.3   | 322              | 48.4     |
|              |            | TT       | 76             | 33.2   | 252              | 37.9     |
| rs16945088   | 638        | GG       | 0              | 0      | 4                | 0.8      |
|              |            | AG       | 26             | 3.2    | 53               | 10.7     |
|              |            | AA       | 122            | 27.6   | 437              | 88.5     |
| rs17817449   | 639        | GG       | 25             | 16.9   | 80               | 16.3     |
|              |            | GT       | 77             | 52.0   | 226              | 46.0     |
|              |            | TT       | 46             | 31.1   | 185              | 37.7     |
| rs8050136    | 894        | CC       | 76             | 33.5   | 249              | 37.3     |
|              |            | CA       | 113            | 49.8   | 326              | 48.9     |
|              |            | AA       | 38             | 16.7   | 92               | 13.8     |
| rs9935401    | 634        | GG       | 48             | 33.1   | 184              | 37.6     |
|              |            | AG       | 74             | 50.1   | 224              | 45.8     |
|              |            | AA       | 23             | 15.9   | 81               | 16.6     |
| rs3751812    | 640        | GG       | 46             | 31.1   | 183              | 37.2     |
|              |            | GT       | 77             | 52.0   | 227              | 46.1     |
|              |            | TT       | 25             | 16.9   | 82               | 16.7     |
| rs9939609    | 2428       | AA       | 100            | 43.9   | 260              | 14.3     |
|              |            | TA       | 300            | 48.9   | 845              | 46.6     |
|              |            | TT       | 213            | 34.8   | 710              | 39.1     |
| rs9941349    | 631        | CC       | 42             | 29.4   | 177              | 36.3     |
|              |            | CT       | 76             | 53.1   | 230              | 47.1     |
|              |            | TT       | 25             | 17.5   | 81               | 16.6     |

**Table 2a.** Basic genotyping information from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study by gender.

Notes: *Frequency refers to the total number of participants for which genetic information was available for each SNP.

A table showing the basic genetic information of the individuals considered in this study.
displayed in Table 4. There is a strong relationship between both rs9939609 and rs1421085, and trunk fat percentage for males, but a similar relationship is not significant for the other SNPs explored, or for females. Male carriers of rs9939609 risk genotype AA had mean trunk fat percentage that was 4.2 units higher than homozygotes of the common genotype TT (means were different with a \( P \) value of 0.0001). This difference was not significant for females. For rs1421085, homozygote males of the risk CC genotype had mean trunk fat percentage that was 3.8 units higher than homozygote TT males (\( P \) value of 0.0004), while the difference for females was again not significant. When environmental factors are ignored, genetic variants in these two SNPs have a greater effect on trunk fat percentage for males than for females (see Fig. 1). One study states that the rs17817449 SNP in the \( FTO \) gene was related to an increase in BMI,\(^3\) but as in this study, the relationship between the \( FTO \) gene and BMI existed only in males and postmenopausal females. This result may relate to our study since 77.3% of the females we considered were below the age of 52 years, the average age of menopause suggested by the Canadian Women’s Health Network.\(^3\)

**Physical activity.** The three categories of physical activity in this study were introduced as two dummy variables: moderate activity and high activity. Physical activity on its own is a significant explanatory variable for trunk fat percentage, for both males and females. Young females with high levels of physical activity appeared to have lower trunk fat percentage than young inactive females, and this effect seemed to decrease as age increased (although the effect was not statistically significant). The same relationship was not apparent for males (Fig. 2). For males studied, mean trunk fat percentages for individuals with low, moderate, and high activity were 34.6, 29.6, and 22.8, respectively. For females, these values were 42.2, 38.4, and 31.6, respectively. Trunk fat percentages for high and low activity were significantly different for both males and females (\( P = 0 \)). Although increased physical activity results in decreased trunk fat percentage, the following section describes how the magnitude of this effect may change with age and genotype of the SNPs explored in this study.

**Interaction of \( FTO \) variants with age and physical activity.** If the magnitude of genetic influence differs as a function of physical activity, it would suggest that gene–environment interaction is present for the outcome of interest, trunk fat percentage. The mean trunk fat percentages

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**Table 2b. Minor allele frequencies of individuals from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.**

| SNP            | FULL SAMPLE |            | INDEPENDENT SAMPLE |            |
|----------------|-------------|------------|-------------------|------------|
|                | OVERALL     | MALE       | FEMALE            | OVERALL    | MALE       | FEMALE    |
| rs9939973      | 41.3%       | 44.5%      | 40.4%             | 41.7%      | 45.6%      | 40.3%     |
| rs1421085      | 40.0%       | 43.1%      | 39.0%             | 40.0%      | 43.4%      | 39.0%     |
| rs1121980      | 41.3%       | 44.7%      | 40.3%             | 41.9%      | 45.5%      | 40.6%     |
| rs7193144      | 38.9%       | 42.1%      | 37.9%             | 40.3%      | 44.4%      | 38.8%     |
| rs16945088     | 6.7%        | 8.8%       | 6.2%              | 7.6%       | 12.1%      | 6.5%      |
| rs17817449     | 40.0%       | 42.9%      | 39.3%             | 47.5%      | 48.2%      | 46.5%     |
| rs8050136      | 39.0%       | 41.6%      | 38.2%             | 40.1%      | 43.7%      | 38.9%     |
| rs9935401      | 39.7%       | 41.4%      | 39.5%             | 47.9%      | 50.0%      | 47.4%     |
| rs3751812      | 40.3%       | 42.9%      | 39.7%             | 48.6%      | 51.7%      | 47.8%     |
| rs9939609      | 38.4%       | 40.8%      | 37.6%             | 38.6%      | 41.1%      | 37.8%     |
| rs9941349      | 40.9%       | 44.1%      | 40.2%             | 49.3%      | 53.8%      | 48.3%     |

A table showing the minor allele frequencies for each single-nucleotide polymorphism (SNP) considered in this study. For SNPs with a different minor allele in males and females, the overall minor allele is represented for both genders.

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**Table 3. Cochran–Armitage trend test results for testing dominance of single-nucleotide polymorphisms (SNPs) explored from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.**

| MODEL SELECTED | P VALUE FOR SELECTED MODEL/S |
|----------------|-----------------------------|
| rs9939973      | G dominant                   |
| rs1421085      | Additive                     |
| rs1121980      | C dominant                   |
| rs7193144      | Additive/T dominant          |
| rs16945088     | Additive/G dominant          |
| rs17817449     | T dominant                   |
| rs8050136      | C dominant                   |
| rs9935401      | G dominant                   |
| rs3751812      | G dominant                   |
| rs9939609      | Additive                     |
| rs9941349      | C dominant                   |

A table showing the results from three Cochran–Armitage trend tests for each SNP explored. These tests were used to determine appropriate dominance models. The model whose \( p \)-value was lowest for each SNP was selected.
for highly active males that are homozygotes of the obesity-risk alleles for both rs9939609 and rs1421085 were significantly higher than for highly active homozygote males of the non-obesity-risk alleles. When considering one-way ANOVA, Tukey’s test, and the Kruskall–Wallis test, this difference was significant only for rs9939609 and rs1421085, with a maximum *P* value of 0.020 for all tests on these two SNPs. The difference of mean trunk fat percentage between genetic variants was not, however, significant for those males with low or moderate activity.

Figure 3 shows that for those highly inactive individuals, genotype does not tend to affect trunk fat percentage. However, as physical activity increases, those individuals who are less susceptible to obesity tend to have lower trunk fat percentage than homozygotes of the obesity-risk alleles. To view this interaction effect by age, we modeled the interaction of the categorical physical activity variable with the rs9939609 SNP, adding age as a covariate. Figure 4 summarizes the result of this model, again supporting the previous claim. Genetic heritability creates the greatest variation in trunk fat percentage for active males, and has no significant effect for males with lower amounts of activity, or for females.

**Discussion**

In this study, we applied gene–environment interaction models to test whether or not the magnitude of genetics-associated effects on trunk fat percentage may be modified by physical activity. Our results showed the presence of interactions between genetic susceptibility of obesity caused by two SNPs in the *FTO* gene and physical activity. We found that for males only, physical activity accentuated the effect of rs9939609 and rs1421085 on obesity as indexed by trunk fat percentage. However, for females, the effect was not significant.

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**Figure 1.** A graph of boxplots showing the distribution of trunk fat percentage for males and females for SNPs rs9939609 and rs1421085. Percent trunk fat is measured in percentage (%).
Table 4. T-tests for difference in mean trunk fat percentage between variants in single-nucleotide polymorphisms from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.

| SNP            | TEST          | MALE P VALUE | ADJUSTED P VALUE | FEMALE P VALUE | ADJUSTED P VALUE |
|----------------|---------------|--------------|------------------|----------------|------------------|
| rs9939973      | (GG, AG)–AA   | 0.177        | 0.0342           | 0.234          | 0.0214           |
|                | rs1421085     | 0.000448*    | 0.0526           | 0.363          | 0.0429           |
|                | CC–TT         | 0.00650*     | 0.0789           | 0.251          | 0.0286           |
|                | TC–TT         | 0.106        | 0.0289           | 0.331          | 0.0381           |
| rs1121980      | (CC, TC)–TT   | 0.199        | 0.0368           | 0.252          | 0.0310           |
| rs7193144      | (TC, TT)–CC   | 0.0951       | 0.0211           | 0.240          | 0.0262           |
|                | CC–TT         | 0.0310       | 0.0132           | 0.213          | 0.0167           |
|                | CC–TC         | 0.236        | 0.0447           | 0.288          | 0.0357           |
|                | TC–TT         | 0.0408       | 0.0184           | 0.383          | 0.0476           |
| rs16945088     | (GG, AG)–AA   | 0.0978       | 0.0237           | 0.226          | 0.0190           |
|                | GG–AA         | –            | –                | 0.381          | 0.0452           |
|                | GG–AG         | –            | –                | 0.467          | 0.0500           |
|                | AG–AA         | 0.0978       | 0.0263           | 0.240          | 0.0238           |
| rs17817449     | (GT, TT)–GG   | 0.201        | 0.0395           | 0.0330         | 0.00714          |
| rs8050136      | (CC, CA)–AA   | 0.129        | 0.0316           | 0.210          | 0.0143           |
| rs9935401      | (GG, AG)–AA   | 0.320        | 0.0474           | 0.0269         | 0.00476          |
| rs3751812      | (GG, GT)–TT   | 0.204        | 0.0421           | 0.0333         | 0.00952          |
| rs9939609      | AA–TT         | 0.000117*    | 0.00263          | 0.180          | 0.0119           |
|                | AA–TA         | 0.00780*     | 0.0105           | 0.257          | 0.0333           |
|                | TA–TT         | 0.0391       | 0.0158           | 0.352          | 0.0405           |
| rs9941349      | (CC, CT)–TT   | 0.320        | 0.0500           | 0.0218         | 0.00238          |

Notes: *Indicates significant when compared to its adjusted p-value, calculated from using a family confidence level of 0.05 over 19 simultaneous tests for males, and over 21 simultaneous tests for females.

A table showing the t-test results for difference in means when comparing mean trunk fat percentage between each pair of possible variants within single-nucleotide polymorphisms. For cases where two possible dominance models were selected from Cochran–Armitage trend tests, mean trunk fat percentages were compared using both plausible models.

Percentage. However, a similar result was not significant for the other nine SNPs examined. The novel finding of this study is that physical activity modified the predisposition on trunk fat percentage caused by the FTO gene, with a high level of physical activity increasing the additive genetic predisposition of high trunk fat percentage. These models, based on DXA measurements of trunk fat percentage, have not been explored before. We have also shown that high physical activity is significantly associated with a reduction of trunk fat percentage for both males and females, regardless of genetic variants of the SNPs explored. Other studies have yielded consistent results using waist circumference as a measure of obesity instead of trunk fat percentage.31,32

Our study also shows that physically active males were significantly affected by the genetic variants of rs9939609 and rs1421085 in contrast to those with low amounts of activity. Although these reductions were similar for females, they were not statistically significant. This inconsistency may result from gender difference. Physically active male homozygotes of the common alleles showed significantly lower trunk fat percentages than physically active homozygotes for the obesity-risk alleles of the above SNPs. These results are similar to some previous studies, which have detected a genetic influence on weight changes for males with high levels of physical activity, but not females.33,34

This study benefits from a large sample of the NL population. NL’s generally isolated and homogeneous people provide a suitable population for generalized genetic results,25 and the large sample allowed validating results based on a relatively large subsample of independent (unrelated) individuals. Although there may be possible error or bias in self-reported physical activity level, Baecke’s method for assessment of physical activity has been shown to be highly effective.35 Trunk fat percentage in this study has been measured by DXA, which provides a more accurate measurement of trunk fat percentage compared with other methods of measurement. Gender and age, two major confounders, were considered and controlled as well.
In our study, age was found to be a significant moderator, as expected. In physical activity–age interaction models, our results (although not significant) suggest that the effect of high physical activity in males on the heritability of trunk fat percentage strengthens with increasing age. However, further studies are required to address the contribution of age groups.

There are some limitations in this study. A study with a larger sample size would provide higher power in detecting effects. Increased statistical power would be especially important in the gene–environment interaction analyses. In addition, our study has some limitations regarding the ability to generalize since it consists of volunteers rather than random individuals. Several dietary intake variables were considered in preliminary analysis for this study. However, their interactions with the FTO SNPs studied did not have any significant effects on trunk fat percentage for males or females. It is possible, however, that other dietary or environmental information not considered in this study may potentially affect the genetic heritability of high levels of trunk fat percentage. Furthermore, our study was based on cross-sectional models, and we were not able to determine whether high physical activity is effective in explaining trunk fat percentage over time. Aside from rs9939609 and rs1421085 which were selected due to previously studied associations, all other SNPs for this study were selected due to availability of data. The position of each SNP can be seen in Supplementary Table 4. There were far more individuals in our database with information recorded for rs9939609 and rs1421085 which were selected due to previously studied associations, all other SNPs for this study were selected due to availability of data. The position of each SNP can be seen in Supplementary Table 4. There were far more individuals in our database with information recorded for rs9939609 and rs1421085 compared with the other SNPs. It is possible that other genes and SNPs not studied may further, or even better, explain genetic predisposition to obesity. For example, a recent study suggests that the IRX3 gene expression may be effective in explaining body mass, and that obesity-associated regions of FTO may actually be regulated by IRX3 expression.

Our study suggests that higher physical activity is beneficial in reducing central obesity. These results are consistent with our findings.
with the basic concept that even moderate physical activity helps to relieve obesity at the population level. However, for people with specific genetic predisposition, particularly male FTO risk carriers, a higher level of physical activity is required to achieve a significant beneficial effect in reducing trunk fat percentage.

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Author Contributions

AP produced the original draft of this manuscript, consolidated future drafts, performed the majority of statistical analysis, and produced tables and figures. FC collected subject data, genotyped subjects, assembled the dataset, produced an updated manuscript draft, and was a primary consultant on the analysis, and are also thankful to Dr. Laurent Briollais for his generous time in reviewing this manuscript and for providing insight toward its improvement.

draft of the manuscript. All authors reviewed and approved of the final manuscript.

Supplementary Data

Supplementary table 1. P-values obtained from testing whether each SNP is in Hardy–Weinberg proportions under the null hypothesis that the SNP is in Hardy–Weinberg proportions for different subsamples of the full sample.

Supplementary table 2. P-values obtained from testing whether each SNP is in Hardy–Weinberg proportions under the null hypothesis that the SNP is in Hardy–Weinberg proportions for different subsamples of the sample of unrelated individuals.

Supplementary table 3. R$^2$ values for linkage disequilibrium analysis between each pair of SNPs analyzed.

Supplementary table 4. A table showing the position of each SNP studied.

Supplementary figure 1. A graph showing powers corresponding to certain levels of heritability and R$^2$ values obtained from linkage disequilibrium analysis between SNPs.

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