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The Role of FTO Genotype in the Association Between FTO Gene Expression and Anthropometric Measures in Obese and Overweight Adolescent Boys

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Abstract

The role of FTO genotype in the effect of FTO gene expression level on change in body mass index and body composition has not been studied. This study aimed to investigate the role of FTO genotype in the association between change in the expression level of the FTO gene with changes in anthropometric measurements in obese and overweight adolescent boys. Eighty-four boys aged 12 to 16 years participated in this longitudinal study. A bioimpedance analyzer (BIA) was used to estimate percentage of body fat (%body fat) and percentage of skeletal muscle (%skeletal muscle). The FTO gene expression level in peripheral blood mononuclear cells (PBMCs) was assessed using quantitative Real Time PCR (qPCR). The DNA samples were genotyped for the FTO gene polymorphisms by DNA sequencing. All measurements were performed at baseline and after intervention. A significant association was observed between the level of gene expression and %skeletal muscle. The gene expression fold change was significantly associated with change in %skeletal muscle in AA or AG genotype carriers ($\beta = 0.34$, $p = .02$). No significant association was detected between the change in FTO gene expression with change in anthropometric indices in GG genotype carriers. In conclusion, the association between FTO gene expression and body composition can be influenced by FTO genotype. Future studies are required to assess the interactions between FTO genotype, FTO gene expression in different tissues, and body composition.

Keywords

FTO, gene expression, skeletal muscle, body fat, body mass index, obesity

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What is already known about this subject?

- FTO gene polymorphisms are associated with BMI
- FTO gene polymorphisms are associated with body composition

What does this study add?

- This study is the first human study to examine the relationship between the changes of FTO gene expression in PBMCs with changes in anthropometric measurements.

The fat mass and obesity-associated (FTO) locus is one of the most important genetic risk factors for obesity across different populations (Frayling et al., 2007; Goodarzi, 2017). There is no general agreement about the mechanism of the effects of the FTO locus on obesity. FTO rs9939506 GG genotype is related to higher body mass index (BMI) compared to the people with AA and AG genotype (Wrzosek, Zakrzewska, Ruczko, Jabłonowska-Lietz, & Nowicka, 2008). FTO rs9930506 is reported to be significantly

associated with higher BMI in a G allele dose-dependent manner (BMI + 1.4 kg/m^2 per G allele; Wrzosek et al., 2016). In other studies, a relationship between the levels of FTO gene expression in the hypothalamus and adipose tissue with body composition was reported (Fischer et al., 2009; Klöting et al., 2008). Suppression of the FTO gene in rats resulted in reduction of the ratio of white adipose tissue to brown adipose tissue (WAT/BAT; Tews, Fischer-Posovszky, & Wabitsch, 2010).

The FTO gene is expressed ubiquitously (Sebert et al., 2016), and the role of the FTO gene expression in other cells is not yet clear. For example, it is not clear whether the relationship between FTO gene expression and body weight and composition exists in all tissues or is exclusive to some tissues such as adipose and hypothalamus. Recent studies demonstrated a critical role of the FTO gene on cellular metabolic pathways associated with growth and proliferation. Overexpression of the FTO gene increases PI3K/AKT/mTOR pathway activity, which is important in regulating the cell cycle and consequently, reduces apoptosis and allows proliferation (Akbari, Gholamalizadeh, Doaei, & Mirsafa, 2017). The FTO gene might have a different role in cellular metabolism apart from its effect in adipocytes and the hypothalamus. For example, some recent human studies reported association of FTO gene expression level in peripheral blood mononuclear cells (PBMCs) with different metabolic pathways that affect BMI (Berulava & Horsthemke, 2010), ghrelin production (Karra et al., 2011), and the risk of type 2 diabetes (Shen et al., 2015).

Today, identification of factors affecting the expression of the FTO gene is especially interesting (McMurray et al., 2015). It's reported that knock-down of FTO expression with siRNAs in preadipocyte resulted in a decrease of differentiation into mature adipocytes (Zhang et al., 2015). Recently, attention has been paid to the use of new drugs to selectively inhibit FTO activity. The effect of FTO genotype on the association between FTO expression and anthropometric indices is not still recognized. Identifying differences in the relationship between FTO gene expression and anthropometric indices in individuals with different FTO genotypes may be helpful in determining those who would benefit from drug therapies.

The result of previous studies suggested that the role of FTO gene can be varied at different ages. Associations between BMI and the FTO gene are age dependent and the effect of the FTO genotype on BMI becomes evident only after age 7 (Hubacek, Pitha, Adamkova, Lanska, & Poledne, 2009). Another study reported that the effect of FTO single nucleotide polymorphisms (SNPs) may be, in some populations at least, restricted to males and postmenopausal females (Rzehak et al., 2010). Boys have a higher prevalence of obesity than girls in Iran (Kelishadi, Haghdoost, Sadeghirad, & Khajehkazemi, 2014). So, the aim of the present study was to investigate the role of FTO genotype in the relationship between changes in the FTO gene expression level in PBMCs with changes in body weight, BMI, and body composition in obese and overweight adolescent boys.

Methodology

This study was an ancillary study within a randomized, controlled, school-based trial that carried out a comprehensive weight-reduction program. Personalized diet and physical activity intervention was implemented for each participant. In addition, parents were provided an educational session on healthy diet and physical activity to creating a supportive environment at home. The personalized diet was adopted with free snacks offered in school days by researchers. Furthermore, a high-intensity interval training was carried out for improving the physical activity at the schools. In this method, students warmed up for 10 min and they were involved in high-intensity exercise for a minimum of 30 min. The details of the interventions were published previously (Kalantari et al., 2017). The study involved students in two high schools (7th, 8th, and

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9th grade students) of a district of Tehran city that was chosen randomly (district 5). The original study had an intervention group ($n = 44$) and a control group ($n = 40$). Data from the control and intervention groups were combined for purposes of the present study ($n = 84$).

Study Population

In total, 96 students expressed interest in participating in the ancillary study. Twelve were excluded due to failure to collect blood samples and 84 students enrolled and provided both baseline and week 18 blood samples. The inclusion criteria were age between 12 and 16 years and BMI percentile reported as ⩾85th percentile for age and sex. Non-inclusion criteria included diagnosed weight-related diseases (including hypothyroidism, insulin resistance, and Cushing's syndrome) and use of weight-related medications (including drugs for diabetes, such as insulin, thiazolidinediones, and sulfonylureas; antipsychotic drugs such as haloperidol, clozapine, and lithium; and antidepressant drugs like amitriptyline, paroxetine, and sertraline) determined by self-report by the participants.

The aims, methods, and the other details of the project were explained to the participants and their parents, and written consent forms from both parents and students were obtained. All measures were taken between morning and noon at the baseline and after 18 weeks of the study.

Anthropometric Measures

The measures of baseline and week 18 were obtained by trained personnel. The height of students was measured with a calibrated tape line fastened to a wall. A bioimpedance analysis scale (Omron BF511, Kyoto, Japan) was used to measure weight, BMI, %body fat, and %skeletal muscle after entering their age, gender, and height. This device is a digital, mobile, and non-invasive device that has eight electrodes that sends an extremely weak electrical current of 50 kHz and less than 500 πA through the body to determine the amount of fat tissue. The validity of this device has been confirmed in a previous study (Bosy-Westphal et al., 2008). All data were classified according to the *z*-score guidelines defined by WHO recommendations (for weight and BMI; Cole, Flegal, Nicholls, & Jackson, 2007) and recently published papers (for %body fat and %skeletal muscle; McCarthy, Samani-Radia, Jebb, & Prentice, 2014).

Gene Expression

At baseline and week 18, blood samples (5 ml) were collected from all students who participated in the study, and then transferred to EDTA tubes and stored at −80 °C. The PBMCs were isolated from anticoagulated peripheral blood by density centrifugation, and total RNA was subsequently isolated using the GeneAll RNA extraction kit (GeneAll, South Korea), cDNA synthesis was performed using the cDNA synthesis kit (Gene All, South Korea), and mRNA expression levels were determined using the Opticon real-time PCR detection system (Bio-Rad Laboratories, California). Reactions were carried out in duplicate using the SYBR Green Gene Expression Master Mix (Cat. no. 638317; Takara). The Hypoxanthine Phosphoribosyltransferase (HPRT) gene was chosen as a reference gene for its stable expression in PBMCs. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method and expressed as fold change (Pfaffl, Horgan, & Dempfle, 2002).

Genotyping

The DNA extraction kit manufactured by GeneAll was used to extract and purify the DNA samples. The NanoDrop device (Thermo Scientific, Wilmington, DE, USA) was used to quantify the DNA concentration. The optical density (OD) of the samples was obtained in the absorption rate of 260–280, and it was confirmed if the OD was from 1.8 to 2. Moreover, to check the quality of the extracted DNA, electrophoresis using the agarose gel technique was used. In brief, genomic DNA was amplified by PCR using the Taq DNA Pol 2X Master Mix Red (Cat. no. A180301; Ampliqon, Denmark). The PCR products were sent to GeneAll for DNA sequencing. The quality and average length of the sequence library for each sample were assessed using the Chromas software (version 2.33, http://www.technelysium.com.au/chromas.html).

Assessment of Other Variables

Usual dietary intakes of participants were examined by a validated 168-item semi-quantitative food frequency questionnaire (FFQ) (Esfahani, Asghari, Mirmiran, & Azizi, 2010). The FFQ consisted of 168 food items with standard portion sizes commonly consumed by Iranian people. A trained interviewer administered the FFQ through face-to-face interviews. All reported consumption frequencies were converted to grams per day by using household measures. Daily intakes of energy were measured for each person by using the U.S. Department of Agriculture food consumption database, which was modified for Iranian foods.

The International Physical Activity Questionnaire long form (IPAQ-LF) was used for measuring physical activity of participants through the face-to-face interview (Vasheghani-Farahani, Tahmasbi, Asheri, Ashraf, Nedjat, & Kordi, 2011). All results of the IPAQ were expressed as metabolic equivalents per minute (MET-minutes per week).

Table 1. Characteristics of Study Participants in Study of the Association FTO Gene Expression and Anthropometric Measures in Obese and Overweight Adolescent Boys ($N = 84$).

	Mean ± *SD*		
	AA or AG ($n = 57$)	GG ($n = 27$)	*p*
Age (years)	13.9 ± 0.9	13.9 ± 0.8	.85
Weight at baseline (kg)	74.9 ± 13.3	73.5 ± 13.2	.62
Height at baseline (cm)	167.3 ± 8.1	166.9 ± 9.3	.86
BMI at baseline (kg/m^2)	25.8 ± 3.6	27.8 ± 3.9	.02
%body fat at baseline	26.4 ± 6.4	30.5 ± 5.6	<.01
%skeletal muscle at baseline	35.6 ± 2.6	33.9 ± 2.4	<.01
Physical activity (MET-minutes per week)	1444 ± 501	2318 ± 427	.26
Calorie intake (kcal)	2237 ± 810	2118 ± 1120	.57
Δ Weight (kg)	−0.2 ± 4.8	−6.8 ± 8.6	.08
Δ BMI (kg/m^2)	−0.7 ± 3.7	−1.6 ± 5.3	.03
Δ %body fat	−0.8 ± 3.7	0.8 ± 2.7	.92
Δ %skeletal muscle	0.3 ± 1.9	0.6 ± 1	.4
Δ FTO expression ($2^{-\Delta\Delta Ct}$)	0.5 ± 1.1	0.1 ± 0.9	.17

Statistical Analysis

Given the age range of the participants, we expect weight and body composition changes in these individuals to be significant over a period of 18 weeks. We aimed to test the hypothesis that there is an association between changes of the level of FTO gene expression with anthropometric indices over a time period regardless of the effect of dietary intake and physical activity. All associations were evaluated under dominant models. Multiple linear regression was used to determine the relationship between changes in FTO gene expression with changes of anthropometric parameters of subjects with different FTO genotypes after adjusting for age, physical activity, and calorie, protein and carbohydrate intake. We confirmed that the assumptions of the linear regression model were met. Statistical analyses were performed using SPSS version 23.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). The results were considered statistically significant at $p < .05$.

Results

At baseline, individuals with GG genotype of rs9930506 had higher BMI, %body fat, and lower %skeletal muscle compared to AA or AG genotype carriers ($p = .02$, .001, and .001, respectively) (Table 1). After 18 weeks, the participants with GG genotype experienced the following mean anthropometric parameter reductions: weight 6.8 kg, BMI 1.69 kg/m^2, and %body fat 0.78%. Moreover, mean of %skeletal muscle increased by 0.61%, and the FTO expression was 0.15-fold higher after 18 weeks than baseline. The participants with AA or AG genotype experienced the following mean anthropometric parameter reductions: weight 0.21 kg, BMI 0.69 kg/m^2, and %body fat 0.84%. Overall, mean of %skeletal muscle increased by 0.35%, and the FTO expression was 0.51-fold higher after 18 weeks than baseline. There were no outliers in the data, as assessed by box plotting.

Table 2. The Association Between the Level of FTO Gene Expression and Anthropometric Indices at Baseline Using Linear Regression* ($N = 84$).

	AA or AG ($n = 57$)		GG ($n = 27$)	
	β	*p* value	β	*p* value
Weight	0.5	.12	0.19	.56
BMI	0.43	.18	0.2	.55
%Body fat	0.82	.28	0.99	.34
%Skeletal muscle	0.76	.32	1.1	.24

Note. *Adjusted for physical activity, and calorie, carbohydrate, protein, and fat intake.

At baseline, there was no significant association between FTO expression level and anthropometric measurements (Table 2). Under the dominant model, we observed a significant association between change of the level of gene expression and change of %skeletal muscle. The gene expression fold change was significantly associated with change in %skeletal muscle in AA or AG genotype carriers ($\beta = 0.34$, $p = .02$). No significant association was detected between the change in FTO gene expression with change in anthropometric indices in GG genotype carriers (Table 3).

Table 3. The Association Between the FTO Gene Expression and Anthropometric Indices Changes Using Linear Regression Over a Period of 18 Weeks* ($N = 84$).

	FTO expression fold changes in AA or AG genotype ($n = 57$)		FTO expression fold changes in GG genotype ($n = 27$)	
	β	*p* value	β	*p* value
Δ Weight	0.1	.52	0.15	.3
Δ BMI	0.14	.37	0.28	.22
Δ %Body fat	0.24	.13	0.03	.89
Δ %Skeletal muscle	0.34	.02	0.23	.32

Note. *Adjusted for physical activity, and calorie, carbohydrate, protein and fat intake.

Discussion

The results identified an association between up-regulation of FTO in PBMCs with increase in %skeletal muscle at 18 weeks in individuals with AA or AG genotype of FTO rs9930506. However, there was no association between changes in FTO gene expression and weight, BMI, and %body fat, regardless of their genotype. The association between FTO gene expression and anthropometric measures has been reported in several studies. Berulava and Horsthemke reported that increased expression of FTO in PBMCs is associated with increased body mass (Berulava & Horsthemke, 2010). Most of the studies on the interactions between FTO gene expression and body composition have focused on the expression level of the FTO gene in the hypothalamus and adipose tissue due to its hypothetical role in the regulation of energy intake and adipocyte differentiation. For example, Church et al. (2010) identified that ubiquitous overexpression of FTO in the hypothalamus leads to a dose-dependent increase in body and fat mass. In the present study, no significant association was identified between the level of FTO gene expression in PBMCs and BMI and body fat which contrasts with the results of other studies on FTO gene expression and anthropometric parameters. The effect of FTO expression on anthropometric measurements may be tissue specific. It has been strongly suggested that the role of FTO in the tissues other than adipose tissue might be completely independent from its role in obesity (Akbari et al., 2017). Moreover, the relationship between FTO expression and anthropometric measurements may be age-specific. It's reported that the effects of rs9930506 variants on body size varied with age and the carriers of the risk allele develop an increase in body weight earlier in life (Sentinelli et al., 2012). The associations of FTO variant with BMI is identified to be more significant during childhood and adolescence, peaked in early adulthood, and then weakened in adult age (Hardy et al., 2009). It can be hypothesized that the effect of FTO on body composition may be more pronounced in adolescence and early adulthood when people have the most muscular mass in the entire lifetime.

The underlying mechanisms for the effect of FTO gene expression on body weight and body composition are not recognized. However, the role of FTO in the hypothalamus is likely different from its role in other tissues (Fischer et al., 2009). Recent studies have reported that FTO may have an essential role in metabolic-related signaling in most cells. For example, it is suggested that the FTO gene regulates the proliferation and differentiation of cells via PI3K/Akt signaling, and FTO knockdown leads to suppression of cell proliferation (Akbari et al., 2017).

As the results of the present study indicated, FTO gene polymorphisms can modify the expression level of FTO and other BMI-related genes such as IRX3 and the regulation of the expression of FTO gene (e.g., by drugs or dietary supplements) can induce desired changes in individuals with desired genotype (Doaei, Gholamalizadeh, Jarrahi, Badakhanian, & Najafi, 2016). A separate study on the participants of the present study identified that the FTO gene polymorphism rs9930506 had a risk allele frequency of 40% and was strongly associated with higher %body fat and BMI (Kalantari et al., 2018). So, we should consider FTO genotyping in all studies focused on the association between FTO gene expression and its clinical manifestations and diseases including obesity.

However, some other factors can modify the effects of the level of FTO gene expression. Hakanen et al. (2009) reported that the association between the FTO gene and BF and BMI could be altered at different ages and FTO genotype is associated with BMI after the age of 7 years. In the present study, we investigated the changes in the expression of FTO gene in adolescents who are experiencing physical growth to identify the association between FTO genotype with changes in FTO expression and body composition. Environmental factors such as lifestyle can also play a key role in the interaction between FTO gene expression and anthropometric measures. For example, the level of FTO gene expression is influenced by dietary intake (Doaei, Kalantari, Mohammadi, Tabesh, & Gholamalizadeh, 2017) and the effect of the FTO gene expression on obesity may be influenced by dietary components (Kalantari, Doaei, Keshavarz-Mohammadi,

Gholamalizadeh, & Pazan, 2016). Although, we adjusted differences in dietary intake and physical activity. So, it can be inferred from the results of the present study that the level of FTO expression has a positive association with %skeletal muscle regardless of the effect of dietary intake and physical activity.

Overall, in the present study, we tried to identify differences between individuals with different FTO genotypes in the relationship between FTO gene expression and anthropometric indices. These results may be helpful in determining those who would benefit from drug therapies. However, it is also important to mention here that relationship between FTO gene expression and anthropometric indices might be tissue-specific and potential treatment related decisions may need to be made in the context of the tissues from which FTO expression is being assessed.

The present study had some limitations. This study was confined to male adolescents. Associations between BMI and the FTO gene may be age dependent (Rzehak et al., 2010) and more evident in males (Hubacek et al., 2009). Future studies in both sexes, a broader age range, and in a longer period of time are needed to address these limitations. The significance level was not adjusted for the number of tests performed in order to not limit the power of the study to discover novel associations. Thus, the results should be reproduced in independent cohorts.

Conclusion

The association between FTO gene expression and body composition can be influenced by FTO genotype. The upregulation of FTO gene expression was associated with increase in %skeletal muscle in male adolescents with AA or AG genotype of FTO rs9930506. This study did not identify any significant association between the FTO gene expression and anthropometric indices in carriers of the GG genotype of FTO rs9930506. Future studies are required to assess the interactions between FTO gene expression and anthropometric measurements and whether these associations can be observed in all types of cells or is exclusive to specific cells.

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Authors' Contributions

SD, NK, NKM, TS, MJ, ShR designed research. SD, MGH, GA, SR conducted the research. PI, HE, SD, MG analyzed the data, and SD, RJ, MS, and MG wrote the article. All authors read and approved the final manuscript.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no conflict of interest, financial or otherwise. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Ethics Approval

All study procedures were reviewed and approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (reference number: Ir.sbmu.nnftri.rec.1394.22).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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