

Up-regulation of FTO gene expression was associated with increase in skeletal muscle mass in overweight male adolescents

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Abstract

Introduction: The modifying effect of FTO gene expression level on change in body mass index and body composition has not been studied before. This study aimed to investigate the association between change in the expression level of the FTO gene and changes in anthropometric measurements in obese and overweight adolescent boys.

Material and methods: Eighty-four boys aged 12 to 16 years participated in this longitudinal study. A Bio Impedance Analyzer (BIA) was used to estimate percentage of body fat (%BF) and percentage of skeletal muscle (%SM). The FTO gene expression level in peripheral blood mononuclear cells was assessed using quantitative real-time PCR (qPCR). All measurements were performed at baseline and after 18 weeks.

Results: After 18 weeks, mean weight was reduced by 2.39 kg, body mass index by 0.09 kg/m², %BF by 0.82% and %SM increased by 0.44%. Moreover, the level of FTO gene expression increased 0.42-fold higher than baseline. The change in expression level of the FTO gene was positively associated with change in %SM ($\beta = 0.31$, $p = 0.02$).

Conclusions: FTO gene expression change was associated with change in %SM in male adolescents. Future studies are required to assess the interactions between FTO gene expression in different tissues and body composition.

Key words: FTO, gene expression, skeletal muscle, body fat, body mass index, obesity.

Introduction

Obesity in adolescents is the cause of many disorders in adulthood and has a genetic and environmental origin [1]. The FTO locus is one of the most important genetic risk factors for obesity in different populations [2]. There is no general agreement about the mechanism of the effects of the FTO locus on obesity. It is reported that the FTO polymorphisms play a key role in adjusting food and calorie intake; for example, people with AA and AT genotypes of the rs9939609 FTO polymorphism had 293 kcal/day higher calorie intake compared to the people with TT genotype [3]. Moreover, people with FTO risk alleles have reduced lipolysis in adipocytes, suggesting a possible role of FTO in adipose tissue metabolism [4]. In other studies, a relationship between the levels of FTO gene expression in the hypothalamus and adipose tissue with body mass index (BMI) was reported [5, 6]. Suppression of the FTO gene in rats resulted in reduction of the ratio of white adipose tissue to brown adipose tissue (WAT/BAT) [7]. However, the FTO gene is expressed ubiquitously [8], 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 in 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 [9]. Thus, 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 found an association of FTO gene expression level in peripheral blood mononuclear cells (PBMCs) with different metabolic pathways that affect BMI [10], ghrelin production [11], and the risk of type 2 diabetes [12].

Therefore, the aim of the present study was to investigate the relationship between changes in the FTO gene expression level in PBMCs with changes in body weight, BMI and body composition in adolescent boys after 18 weeks' intervention.

Material and methods

This study was an ancillary study within a randomized, controlled, school-based trial that carried out a comprehensive weight reduction program. This program consisted of personalized diet, physical activity and health education related to healthy nutrition and healthy weight for overweight and obese male adolescents. The details of

the interventions were published previously [13]. The study involved students in two high schools (7th, 8th, and 9th grade students) of a district of Tehran city that was chosen randomly (district 5). In the original study, we had an intervention group ($n = 44$) and a control group ($n = 40$). However, in the present paper, we combined all subjects into a single group ($n = 84$). All study procedures were reviewed and approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (reference number: Ir.sbm.u.nnftri.rec.1394.22).

Study population

Ninety-six students expressed interest in participating in the ancillary study, and 84 enrolled and provided both baseline and week 18 blood samples.

The inclusion criteria were age between 12–16 years and $BMI \geq +1$ z-scores. The specific exclusion criteria included: diagnosed weight-related diseases and use of weight-related medications.

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 Bio Impedance Analyzer scale (Omron BF511, Kyoto, Japan) was used to measure weight, BMI, percentage of body fat (%BF), and percentage of skeletal muscle (%SM) after entering their age, gender and height. This device is a digital, mobile and non-aggressive device that has eight electrodes that send an extremely weak electrical current of 50 kHz and less than 500 μ A through the body to determine the amount of fat tissue. The validity of this device has been confirmed in a previous study [14]. All data were classified according to the z-score guidelines defined by WHO recommendations (for weight and BMI) [15] and recently published papers (for %BF and %SM) [16].

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 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 SYBR Green Gene Expression Master Mix (Cat. no. 638317; Takara). The 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 [17].

Statistical analysis

Multiple linear regression was used to determine the relationship between baseline and changes in gene expression and baseline and change of anthropometric parameters after adjusting for age, physical activity, and calorie, protein and carbohydrate intake. 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 $p < 0.05$.

Results

At baseline, subjects had a mean BMI of 26.4 ± 3.7 kg/m² (Table I). After 18 weeks, subjects experienced the following mean anthropometric parameter reductions: weight 2.4 kg, BMI 0.09 kg/m², and %BF 0.82%. Moreover, mean %SM increased by 0.44%, and the FTO expression was 0.42-fold higher than baseline after 18 weeks.

At baseline, there was no significant association between FTO expression level and anthropometric measurements (Table II). The gene expression fold change was significantly associated with change in %SM ($\beta = 0.31$, $p = 0.02$). No significant association was detected between the change in FTO gene expression and change in other anthropometric indices (Table III).

Discussion

The results showed an association between up-regulation of FTO in PBMCs and increase in %SM at 18 weeks. The relationship 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 [10].

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. Church *et al.* reported that ubiquitous overexpression of FTO in the hypothalamus leads to a dose-dependent in-

crease in body and fat mass [18]. The underlying mechanisms for the effect of FTO gene expression on body weight and body composition are not understood [18]. However, the role of FTO in the

Table I. Characteristics of the subjects ($N = 84$)

Anthropometrics	Mean \pm SD
Age [years]	13.9 \pm 0.9
Weight at baseline [kg]	74.4 \pm 13.2
Height at baseline [cm]	167.1 \pm 8.5
BMI at baseline [kg/m ²]	26.4 \pm 3.7
BF at baseline (%)	27.7 \pm 6.4
SM at baseline (%)	35.0 \pm 2.6
Weight at 18 weeks [kg]	72.0 \pm 11.9
BMI at 18 weeks [kg/m ²]	26.3 \pm 3.67
BF at 18 weeks (%)	26.9 \pm 6.63
SM at 18 weeks (%)	35.48 \pm 2.66
Δ Weight [kg]	-2.4 \pm 4.1
Δ BMI [kg/m ²]	-0.1 \pm 4.4
Δ BF (%)	-0.8 \pm 3.4
Δ SM (%)	0.4 \pm 1.7
Δ FTO expression ($2^{-\Delta\Delta Ct}$)	0.4 \pm 2.8

Table II. The association between FTO gene expression and anthropometric indices at baseline using linear regression adjusted for physical activity, and calorie, carbohydrate, protein and fat intake ($N = 84$)

Parameter	Δ FTO expression at baseline	
	β	P-value
Weight	0.05	0.74
BMI	0.06	0.7
%BF	0.15	0.37
%SM	0.17	0.31

Table III. The association between FTO gene expression changes and changes in anthropometric indices using linear regression adjusted for physical activity, and calorie, carbohydrate, protein and fat intake ($N = 84$)

Parameter	FTO expression fold changes	
	β	P-value
Δ Weight	0.05	0.69
Δ BMI	0.01	0.92
Δ %BF	0.13	0.64
Δ %SM	0.31	0.02

hypothalamus is likely different from its role in other tissues [19].

Recent studies have shown 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 [9]. Moreover, more recent studies suggest that FTO acts as a mediator in the association between 5' AMP-activated protein kinase (AMPK) with the PI3K/AKT/mTOR pathway [20]. Such a critical role of the FTO gene may justify its ubiquitous expression.

However, it was observed that FTO overexpression reduces ghrelin mRNA and peptide levels and increases appetite [11]. Fischer *et al.* reported that the loss of function of the FTO gene in mice leads to a significant reduction in %BF and lean body mass. They suggested that the leanness of FTO-deficient mice is due to increased energy expenditure and systemic sympathetic activation [19]. Moreover, FTO gene polymorphisms can modify the expression level of FTO and other BMI-related genes such as IRX3 [21].

In the present study, we did not find any significant relationship between FTO gene expression in PBMCs and weight, BMI and %BF, which contrasts with the results of other studies on FTO gene expression and anthropometric parameters. FTO gene expression has been mainly assessed in obesity-related tissues such as the hypothalamus and adipose tissue. It has been strongly suggested that the role of FTO in the other tissues might be completely independent from its role in obesity [9]. The role of FTO gene expression in metabolic pathways related to cell growth and proliferation might be intensified in cells with a high metabolic rate (e.g., skeletal muscle cells). Moreover, it has been revealed that the relationship between FTO gene expression and %BF and BMI can be influenced by FTO polymorphisms [10, 22]. Our separate study on the same subjects showed that the FTO gene polymorphism rs9930506 had a risk allele frequency of 40% and was strongly associated with %BF and BMI [23]. Moreover, Hakanen *et al.* reported that the association between the FTO gene and BF and BMI could be altered at different ages [24, 25]. On the other hand, environmental factors such as lifestyle can 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 [26] and the effect of the FTO gene on obesity may be influenced by dietary components [27].

The present study had some limitations. This study was confined to male adolescents, and the results were reported regardless of the role of the FTO gene polymorphisms. It is possible that FTO gene ex-

pression is influenced by gender, age and genotype. Future studies in both sexes, a broader age range and consideration of the FTO genotype are needed to address these limitations. We did not adjust the significance level for the number of tests performed because we did not want to limit the power of the study to discover novel associations. Thus, our results should be reproduced in independent cohorts.

In conclusion, this study did not find any significant association between the FTO gene expression and weight, BMI and %BF. The up-regulation of the FTO gene expression in PBMCs that occurred during 18 weeks was positively associated with increase in %SM in male adolescents. 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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Conflict of interest

The authors declare no conflict of interest.

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