

RESEARCH PAPER



Interactions between macro-nutrients' intake, FTO and IRX3 gene expression, and FTO genotype in obese and overweight male adolescents

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ABSTRACT

This study is the first to identify the effects of FTO genotype on the interactions between the level of macro-nutrients intake and the expression level of fat mass and obesity associated (FTO) and homeobox transcription factor iriquois-3 (IRX3) genes. This longitudinal study was carried out on 84 overweight and obese adolescent boys in Tehran, Iran. The rs9930506 SNP in FTO was genotyped at baseline and the level of FTO and IRX3 expression in PBMCs and macro-nutrients' intake were assessed at baseline and after 18 weeks of the intervention. The results identified that the higher carbohydrates intake significantly up-regulated the FTO gene ($P = 0.001$) and down-regulated the IRX3 gene ($P = 0.01$). Protein intake up-regulated the FTO gene ($P = 0.001$). In carriers of GG genotype of FTO gene, the amount of dietary carbohydrate had a positive association with FTO gene expression ($p = 0.001$, and $p = 0.04$, respectively). In AA/AG carriers, dietary protein was positively associated with FTO gene expression ($p = 0.001$) and dietary carbohydrate was negatively associated with IRX3 gene expression ($P = 0.04$). Therefore, dietary carbohydrate seems to be associated with FTO and IRX3 genes expression. These associations are influenced by FTO genotype.

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Introduction

Obesity in childhood, especially during the second decade of life, has been proposed as a strong predictor of adult obesity [1,2] and is associated with many chronic diseases including diabetes, cardiovascular disease, cancer, psychological conditions [3]. It is a multifactorial disorder caused by both environmental and genetic factors [4,5] and recent studies have reported that genes contribute to 25% to 40% of childhood obesity [5].

Some genes such as the fat mass and obesity-associated (FTO) gene are reported to be strongly associated with obesity and overweight [4,5] after the age of 7 y [6]. The FTO gene, located in the chromosome region 16q12.2, is expressed in many tissues, especially in brain and hypothalamus [4]. It is indicated that FTO genotype had a strong association with body composition and body weight. FTO gene has been supposed to have an important role in food intake regulation and basal metabolic rate [7]. Results from recent studies identified that there is

a mutual interaction between FTO gene and the intake of dietary macronutrients [8,9]. Some studies indicated that dietary macronutrients including fats, carbohydrates and proteins might affect FTO gene expression [8]. On the other hand, the effects of dietary macronutrients on body weight, hormone secretion, and appetite are influenced by FTO genotype [9,10].

Recent studies have tried to explore the interaction of macronutrient composition with FTO gene expression [11,12]. Dietary composition especially dietary carbohydrates, as the main source of calorie in a regular diet, have an important role in regulation energy balance and body weight [12]. Also, the level of protein intake could modify the association between FTO genotype and BMI [13]. Moreover, recent studies reported that some of the effects of the FTO gene on body weight may be due to the effects of FTO gene on the expression level of Iroquois-related homeobox 3 (IRX3) [14]. The IRX3 gene is a member of Iroquois homeobox gene family, known to play multiple roles in the early steps of neural development. It is reported

that the *IRX3* gene expression is controlled by sequence of intron 1 in the *FTO* gene [15]. The expression level of this gene in hypothalamus is associated with calorie intake and body composition [16].

In order to understand whether the intake of macronutrients is associated with *FTO* and *IRX3* genes, we conducted a field trial study to investigate the interactions between changes in the amount of dietary carbohydrate, protein and fat, with the expression level of *FTO* and *IRX3* genes and *FTO* genotype in obese and overweight male adolescents.

Methodology

This study was an ancillary study within a randomized, controlled, school-based trial that carried out a comprehensive weight-reduction program. 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 ($n = 84$). A personalized diet was implemented for each participant. In addition, parents were provided an educational session on healthy diet to creating a supportive environment at home. 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).

Research context and subject recruitment

The following details are presented in accordance with the CONSORT reporting guidelines for randomized trials of non-pharmacologic treatment (sup. 1). This study was a field trial and details of the trial have been published elsewhere [17]. In brief, participants were adolescent boys, overweight or obese ($BMI \geq +1$ z-scores), aged 12–16 years. The specific exclusion criteria included: uncontrolled chronic diseases affecting weight such as thyroid dysfunction or psychiatric diseases and taking medications affecting weight. Five hundred and forty students in two boys' high schools (including grades 7–9) of a randomly chosen district of Tehran city (District 5) attended an information session and 246 were eligible to participate in the parent trial. Of these, 96 expressed interest in participating in the ancillary study, 84 enrolled and consented to the blood sampling at baseline and week 18, and 62 provided both baseline and week 18 blood samples. Thus, 62 participants were included in the analysis. The inclusion criteria were age between 12 and 16 years and BMI percentile reported as ≥ 85 th 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. All measures were taken between morning and noon at baseline and after 18 weeks of intervention.

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 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 $500 \mu A$ through the body to determine the amount of fat tissue. The validity of this device has been confirmed in a previous study [18]. All data were classified according to the z-score guidelines defined by WHO recommendations [19] and recently published papers (for %body fat and %skeletal muscle [20]).

Quantitative real-time PCR

At baseline and week 18, blood samples (5 ml) were collected of all students who participated in the study, transferred to EDTA tubes and stored at $-80^{\circ}C$. Total RNA from peripheral blood mononuclear cells (PBMCs) was subsequently isolated using the GeneAll RNA extraction kit (GeneAll, South Korea), cDNA synthesis was performed using the GeneAll cDNA synthesis kit (GeneAll, 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, Japan). The *HPRT* gene was used as the reference gene for normalization, chosen because of its stable expression in blood cells. Quantification of transcripts of interest relative to the internal house-keeping control gene *HPRT* was performed using the $2^{-\Delta\Delta C_t}$ method and expressed as fold change. Changing *FTO* and *IRX3* expression was evaluated using the REST (Relative Expression Software Tool) software.

Data on changes of gene expression were transferred to SPSS software in order to analyse their relationships with dietary intake and *FTO* genotype.

Genotyping

The DNA extraction kit manufactured by GeneAll was used to extract and purify DNA samples. The NanoDrop device (Thermo Scientific, Wilmington, DE, USA) was used to quantify DNA concentration. The optical density (OD) of the samples was measured at a wavelength of 260–280 nm. 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 rs9930506 SNP in *FTO* was genotyped in all subjects and the quality and average length of the sequence library for each sample was assessed using the Chromas software (version 2.33, <http://www.technelysium.com.au/chromas.html>).

Macronutrients' intake

Usual Macronutrients' intakes of participants were examined by a validated 168-item semi-quantitative FFQ [10]. 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. Macronutrients' consumption frequencies were converted to grams per day by using household measures. Daily intakes of energy were measured for each person by using the modified US Department of Agriculture food consumption database, which was modified for Iranian foods.

Statistical analysis

We expected that changes in macronutrients' intake in the subjects are significantly associated with the level of genes expression over a period of 18 weeks. We aimed to test the hypothesis that there is an association between changes of the level of *FTO* and *IRX3* genes expression with

macronutrients' intake over a time period regardless of the effect of physical activity. All associations were evaluated under dominant models. We compared pre- and post-intervention values using the REST software and analysed means of two groups using independent t-test. Multiple linear regression was used to determine the relationship between changes in *FTO* gene expression with changes of macronutrients' intake of subjects with different *FTO* genotypes after adjusting for age, physical activity, and calorie 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 $P < 0.05$.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Reference Number: Ir.sbmunnfri.rec. 1394.22), Tehran, Iran. The schools that were involved in this study were asked permission to be part of this trial and consented for their students to participate. The details of the study were explained to students and their parents with an explanatory letter and written informed consent was obtained from both parents and students prior to joining the project.

Results

All measurement data were normally distributed ($P > 0.05$). At baseline, the obese participants had higher BMI, %body fat, and lower %skeletal muscle compared to overweight subjects ($p = 0.00$) (Table 1). No significant differences were found between two groups in dietary intake, physical activity, and *FTO* and *IRX3* gene expression at baseline.

The higher carbohydrates intake significantly up-regulated the *FTO* gene ($P = 0.001$) and down-regulated the *IRX3* gene ($P = 0.01$). Protein intake up-regulated the *FTO* gene ($P = 0.000$), with no significant effect on *IRX3* expression ($P = 0.57$). There was no significant association between fat intake and *IRX3* and *FTO* expression ($P = 0.65$, $P = 0.26$, respectively). (Table 2)

Table 1. Characteristics of study participants in obese and overweight adolescent boys at baseline.

	OverWeight (n = 30)	Obese (n = 32)	P
Age(year)	13.89 ± 0.914	13.90 ± .918	0.67
BMI(Kg/m ²)	23.5731 ± 1.62	29.1592 ± 2.94498	0.00
FM%	24.1214 ± 5.66	31.1673 ± 5.02230	0.00
SM%	36.4715 ± 2.39	33.6735 ± 2.09352	0.00
PA(MET-minute per week)	1744 ± 2355.46	1894 ± 2598.27318	0.31
Calorie(Kcal)	2168 ± 772.35	2192 ± 1134.02343	0.92
Protein intake(gr/day)	166.12 ± 280.18	88.29 ± 48.95	0.16
Carbohydrate intake(gr/day)	220.41 ± 86.16	248.49 ± 105.12	0.26
Fat intake(gr/day)	177.33 ± 89.89	170.36 ± 75.43	0.74
<i>FTO</i>	3.1764 ± 10.63104	1.7829 ± 2.03810	0.5
<i>IRX3</i>	11.2229 ± 4.57277	2.8662 ± 3.92054	0.38

Considering FTO genotype, in carriers of GG genotype of FTO gene, the amount of dietary protein and carbohydrate had significant associations with FTO gene expression ($p = 0.001$, and $p = 0.04$, respectively). In AA/AG carriers, dietary carbohydrate was significantly associated only with IRX3 gene expression ($P = 0.04$). (Table 3)

Discussion

This study is the first to identify the concurrent interactions of FTO genotype, FTO and IRX3 expression, and dietary macronutrients. The results indicate that increased amount of protein and carbohydrates intake significantly up-regulated the FTO gene. Increased carbohydrates intake also down-regulated the IRX3 gene. In AA/AG carriers, dietary protein was positively associated with FTO expression and carbohydrate was negatively associated with IRX3 gene expression. In carriers of GG genotype of FTO gene, the amount of dietary carbohydrate had a positive association only with FTO gene expression.

However, that the results of recent studies on the effect of dietary macronutrients on FTO and IRX3 gene expression were inconsistent. In regards to dietary carbohydrate, Poritsano et al. reported that higher carbohydrate especially glucose intake up-regulated FTO gene expression [21]. On the other hand, Boender et al. found that increased sucrose administration reduced FTO gene expression [22]. Also, Olszewski et al. found no changes in hypothalamic FTO expression after a 48-h palatable sucrose feeding in mice. In regards to dietary protein, Olszewski et al. found that anorexigenic Leucine had reduced FTO gene expression in organotypic cultures of the hypothalamus at 48 h post intervention [23]. While Johansson et al. found that Leucine intake had increased

FTO gene expression at 48 h post intervention [24]. The exact mechanism of the changes of FTO gene expressions in response to the different type of dietary macronutrients remained unclear. FTO may play a key role in the relationship between amino acid levels and MTORC1 function [25]. Cheung et al. reported that FTO gene expression is downregulated by essential amino-acid deprivation [26]. The FTO gene is involved in amino acids pairing with MTORC1 signalling and in the absence of FTO, MTORC1 signalling is impaired [26].

Our study found no association between fat intake and gene expression. Zhong et al. found that a high-fat diet in comparison with a normal carbohydrate diet in mice did not change FTO gene expression [27,28]. Inconsistent with this study, Nowacka-Woszek et al. reported that a high-fat diet could lead to an increase FTO and IRX3 genes expression in white adipose cells [29]. Ronkainen et al. investigated the relationship between dietary fat, the FTO expression and the IRX3 expression in rats. The high-fat diet could influence on the level of the IRX3 expression and suppression of FTO increases IRX3 expression [30].

No study was found on the concurrent interactions of FTO genotype, FTO and IRX3 expression, and dietary macronutrients. Few studies are carried out on the effect of dietary macronutrients on the associations between the FTO genotype and FTO and IRX3 genes expression. Sonestedt et al. reported that the association between FTO genotype and obesity is restricted to the groups of individuals with high-fat and low-carbohydrate diets [13]. Interestingly, another study confirmed that the FTO rs9939609 polymorphism was not associated with BMI in adult with higher carbohydrate intakes [31]. Tanaka et al. found that the FTO polymorphism (rs1421085) was associated with BMI in the group with higher protein intake [27]. The present study was the first that indicated the effect of FTO genotype on the association between dietary macronutrients and FTO and IRX3 genes expression and found that higher protein intake can up-regulate FTO gene expression in carriers of AA/AG genotype, while higher carbohydrate intake can up-regulate FTO gene expression in GG genotype. Moreover, higher carbohydrate intake can down-regulate IRX3 expression in AA/AG genotypes, NOT in GG genotype.

Table 2. The association of changes of macronutrients' intake with changes in FTO and IRX3 gene expression over a period of 18 weeks.

	FTO		IRX3	
	Beta	P	Beta	P
Protein intake(gr/day)	0.65	0.000	-0.07	0.57
Carbohydrate intake(gr/day)	0.97	0.001	-1.07	0.01
Fat intake(gr/day)	-0.05	0.65	-1.16	0.26

Adjusted for age, Kcal, BMI, FM, SM, and PA.

Table 3. The association of macronutrients' intake with FTO and IRX3 gene expression based on different FTO genotypes.

	AA/AG				GG			
	FTO		IRX3		FTO		IRX3	
	Beta	P	Beta	P	Beta	P	Beta	P
Protein intake	0.66	0.000	-0.09	0.57	0.03	0.94	-0.01	0.98
Carbohydrate intake	0.49	0.21	-0.74	0.04	1.56	0.04	-0.7	0.43
Fat intake	-0.01	0.99	-0.19	0.49	-0.57	0.07	-0.67	0.29

Adjusted for age, Kcal, BMI, Fat mass, Skeletal Muscle, and Physical Activity.

Conclusion

FTO gene polymorphism is associated with dietary composition. The intake level of protein and carbohydrates significantly up-regulated the FTO gene and carbohydrates intake down-regulated the IRX3 gene. These findings highlighted the importance of FTO gene sequence on the possible link between dietary intake and gene expression and related functions. However, our study had some limitations including Small sample size, short period of follow-up, and genotyping for only one polymorphism. Further studies are needed to increase our understanding of the association between FTO and IRX3 gene and dietary intake.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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