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The association of dietary carbohydrate with *FTO* gene expression in visceral and subcutaneous adipose tissue of adults without diabetes

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ABSTRACT

Objectives: The aim of the present study was to investigate the association of dietary carbohydrates with fat mass and obesity-associated gene (*FTO*) expression in visceral and subcutaneous adipose tissue.
Methods: In this cross-sectional study, visceral and subcutaneous adipose tissues were gathered from 58 obese (body mass index ≥30 kg/m^2) and 44 non-obese (body mass index ≤18 to<30 kg/m^2) participants, aged ≥20 y, who had undergone elective abdominal surgery with minimal effect on dietary intake. Dietary intake was collected using a valid and reliable food frequency questionnaire, and daily intake of total carbohydrates, total sugar, sucrose, glucose, fructose, lactose, and maltose were calculated. The mRNA expression of the *FTO* gene in visceral and subcutaneous adipose tissues was measured by real-time quantitative polymerase chain reaction.
Results: No significant difference was observed for *FTO* gene expression in subcutaneous and visceral fat mass between non-obese and obese participants. After adjusting for age and sex, total carbohydrate intake was inversely associated with *FTO* gene expression in subcutaneous ($\beta = -0.403$; $P = 0.003$) adipose tissues among obese participants. Furthermore, higher intake of total sugars, sucrose, glucose, and lactose was inversely and higher intake fructose was directly associated with *FTO* mRNA expression in subcutaneous adipose tissue among participants with obesity.
Conclusion: Dietary intake of total sugars, sucrose, glucose, and lactose in obese participants only was inversely and dietary fructose was positively associated with *FTO* gene expression from the subcutaneous adipose tissue.

Introduction

Obesity and obesity-related diseases have increased dramatically in recent decades and have become a crucial problem in public health. The prevalence of obesity (body mass index [BMI] >30 kg/m^2) among US adults is >30%; a major concern in the early 21st century was that one in every three children develop diabetes with a consequent reduction in life expectancy [1]. Obesity and type 2 diabetes are heterogeneous disorders caused by the interaction of both non-genetic and genetic components [2]. Because unhealthy lifestyles, including sedentary and low-quality dietary patterns, are the prime suspects for developing obesity, lifestyle changes have been suggested as strategies to prevent and treat obesity. Genome-wide association studies (GWAS) demonstrated that the fat mass and obesity-associated gene (*FTO*) is the strongest common genetic predictor of obesity, adiposity development, and type 2 diabetes in humans identified so far [3–5].

The *FTO* gene, located in the chromosome region 16 q12.2, is highly expressed in the hypothalamus, which is suggested to play a potential role in the central control of energy homeostasis by modification of the appetite [6]. In addition, protection from obesity in rodents with inactivated *FTO* gene indicated the important role of *FTO* in peripheral energy homeostasis, mitochondrial coupling, and substrate cycling [7]. Thus, adipose tissue remains the main source of *FTO* gene expression, and research focusing on the regulation of *FTO* in adipocytes is warranted.

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Adipose tissue metabolism may be modified by environmental factors, particularly physical activity, and dietary composition. Results from studies suggest that a number of dietary compounds might affect gene expression in visceral and subcutaneous adipose tissue [8,9]. Some recent observational studies revealed that there is an interaction between *FTO* genetic variations and dietary intake [10,11]. Notably, no significant differences were reported between *FTO* expression levels in adipose tissue among individuals with various *FTO* genotypes [12–14].

Dietary carbohydrates, as a main source of energy in a regular diet, have a profound effect on several aspects of body weight status, endocrinology, and appetite [15,16]. Adipose tissues are affected by carbohydrates directly via the process of glycolysis or triacylglycerol (TG) synthesis (lipogenesis) and indirectly throughout subsequent hormonal changes after carbohydrate consumption. To our knowledge, the effects of carbohydrates and its subtypes on *FTO* gene expression in adipose tissue have never been investigated. To understand whether intake of carbohydrates and its subtypes are associated with *FTO* mRNA regulation, we conducted a cross-sectional study in humans to investigate the association of dietary carbohydrate and its subtypes (sucrose, glucose, lactose, maltose, and fructose) with *FTO* gene expression in visceral and subcutaneous of adipose tissue among obese and non-obese adults.

Materials and methods

Participants

The current cross-sectional study recruited 58 obese individuals with BMI $\geq$ 30 kg/m^2 and 44 non-obese individuals with a BMI $\leq$18 to <30 kg/m^2, $\geq$20 y of age, who had undergone elective abdominal surgery with minimal effects on dietary intake at the Mostafa Khomeini and Khatam Al-Anbia hospitals, Tehran, Iran. In each patient, samples were obtained by the same specialist. The eligibility criteria considered to recruit participants were hospital length of stay <3 d, free of diagnosed diabetes mellitus or cancer, not using any lipid-lowering or antiobesity medications, not pregnant or lactating, and not on any special diets. Before surgery, blood samples and data on anthropometrics, demographic characteristics, and dietary intake were obtained. During the surgery, ~100 mg of subcutaneous and visceral adipose tissues was collected.

Ethical approval was obtained from the ethics committee of the Research Institute for Endocrine Sciences (RIES) of the Shahid Beheshti University of Medical Sciences, and the study was conducted in accordance with the Declaration of Helsinki and RIES institutional guidelines. Written informed consent was obtained from all participants.

Dietary measurements

Regular dietary intake of each participant was assessed by an expert interviewer using a valid and reliable semiquantitative food frequency questionnaire (FFQ) [17,18]. Because the Iranian food composition table (FCT) is incomplete, we used the US Department of Agriculture (USDA) FCT to analyze food and beverages. However, the Iranian FCT was used for some traditional food and beverages not listed in the USDA FCT; for the present study, we considered carbohydrates and its subtypes, including fiber, sucrose, glucose, fructose, lactose, and maltose.

The reliability and validity of the FFQ, evaluated in a previous study against twelve 24-h dietary recalls and biomarkers, indicated that it provides reasonably valid measures of the average long-term dietary carbohydrate intake [17,18].

Quantitative real-time polymerase chain reaction analysis of gene expression

Total RNA was extracted from visceral and subcutaneous fat tissues according to the manufacturer's protocol. The tissue samples were incised and weighed (30–50 mg tissue) and added to 1 mL RNX to plus solution (Cinnagen, Iran) along with 250 μL phosphate-buffered saline. The mixture was homogenized using a homogenizer (Qiagen, Germany). Then, 200 μL chloroform was added to the mixture. Proteins, lipids, carbohydrates, and cell debris were eliminated through extraction of the aqueous. The quality of the extracted RNA was evaluated by Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the ratio of absorption (260:280 and 260:230 nm) of all preparations was within an acceptable range. Total RNA was treated with DNase I to remove traces of genomic DNA before complementary DNA (cDNA) synthesis. The cDNA synthesis kit (Thermo Fisher Scientific) was used according to manufacturer's recommendations. The product was stored at −20°C for further investigations.

Primers based on the sequences of the National Center for Biotechnology Information (NCBI) GenBank database were checked by Genrunner Software (version 3.05). The *GAPDH* gene was used as the reference gene for normalization across samples. Primer sequences of *FTO* and *GAPDH* were as follows: *FTO* Forward: 5′- TCT GAC CCC CAA AGA TGA TG-3′; *FTO* Reverse: 5′- CTC GGA GAA TTA GTT TAG GAT ATT TCA-3′; *GAPDH* Forward: 5′-CTG CTC CTC CTG TTC GAC AGT-3′; and *GAPDH* Reverse: 5′-CCG TTG ACT CCG ACC TTC AC-3′. To evaluate the efficiency of primers, both *FTO* and *GAPDH* were obtained as 0.9.

The real-time quantitative polymerase chain reaction (qPCR), carried out using a real-time PCR instrument (Rotor-Gene 6000, Sydney, Australia), was performed in 25 μL volumes containing 12.5 μL 2 X SYBR Green Master mix (Thermo Fisher Scientific), 0.3 μL forward primers, 0.3 μL reverse primers, 8.9 μL RNase-free water, and 3 μL of the cDNA. For each gene, samples were run in duplicate for interassay control along with *GAPDH* (housekeeping) and the non-template control (NTC); qPCR amplification was performed with the following thermal cycling conditions: 5 min at 95°C for denaturation, followed by 45 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s for annealing, amplification, and quantification. The relative expression of *FTO* in each sample was calculated based on its threshold cycle (Ct), normalized to the Ct of the reference gene. All qPCR laboratory procedures were performed according to the MIQE guidelines [19].

Anthropometric and laboratory measurements

With participants wearing light clothing, weight was measured to the precision of 0.1 kg on a SECA digital weighing scale (Seca 707; Seca Corporation, Hanover, MD, USA; range 0.1–200 kg), and height was measured without shoes to the nearest 0.1 cm. BMI was calculated as weight (kg) divided by square of height (m^2). Physical activity during interviews was assessed using the long forms of the reliable and validated Persian version of the International Physical Activity Questionnaire [20]. To measure energy expenditure, the concept of metabolic equivalents (MET) was used. Physical activity levels were classified as low ($\geq$600 min/wk), moderate (<600 to <3000 min/wk), and vigorous activity (>3000 min/wk).

Arterial blood pressure (BP) was measured by a mercury sphygmomanometer for each participant in the sitting position. Systolic blood pressure was determined by the onset of the tapping Korotkoff sound, and diastolic blood pressure was determined as the disappearance of this sound. BP was measured twice, and the average was considered as the participant's BP.

Blood samples were collected before surgery from all participants who had fasted for 10 to 12 h overnight. Fasting plasma glucose (FPG) was measured using an enzymatic colorimetric method with glucose oxidase. Inter- and intraassay coefficients of variation (CV) were both 1% for FPG. Triacylglycerol (TG) levels were determined using the enzymatic colorimetric method with glycerol phosphate oxidase. Inter and intraassay CV for TGs were 0.4 and 2.1%, respectively. Measurements of FPG and TGs were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran). Insulin was measured using the enzyme-linked immunosorbent assay (ELISA) with Mercodia kits (Uppsala, Sweden). Inter- and intraassay CVs of insulin were 1.7 and 2.3, respectively.

Statistical analysis

Normality of the distribution of variables was assessed by histogram and the Kolmogorov–Smirnov test. Continuous variables were described as mean ± SD. Because plasma TGs and insulin were skewed, we reported as median and interquartile range and log transformation was used. G*Power was used to estimate the power of the sample sizes. When effect size was considered 0.3 in Pearson correlation, the sample power was ~0.84 for non-obese participants and ~0.89 for those who were obese, indicating that the sample size of groups was sufficient for detecting a relationship between dietary carbohydrate intake and *FTO* gene expression.

Energy-adjusted values for carbohydrates and its subtypes were calculated by adding the residuals of regressing the nutrient on total energy intake to the median intake of that nutrient [21]. The t and χ^2 tests were used to compare demographic, anthropometrical, dietary intake, and serum biochemical parameters between obese and non-obese participants. Linear regression was performed to determine the association of total carbohydrates and its subtypes with *FTO* mRNA expression in subcutaneous and visceral adipose tissues, and standardized β (STZβ) was reported after adjusting for age and sex.

In sensitivity analyses, we excluded individuals who developed insulin resistance according to the following definition:

$$\text{HOMA}-\text{IR} = [\text{fasting insulin}(\mu\text{U/mL}) \times \text{fasting glucose (mmol/l)}]/22$$

where HOMA-IR stands for homeostatic model assessment of insulin resistance.

Participants with HOMA-IR >3.2 were considered to be insulin resistant. We also performed further analysis after adjusting for insulin concentration, family history of diabetes, physical activity, the percent of energy from protein, and dietary fiber. All data were analyzed using SPSS version 15 (SPSS Inc, Chicago IL, USA), and $P < 0.05$ was considered statistically significant.

Table 1
Demographic, anthropometric, dietary intake, and serum biochemical parameters*

Variables	Total	Non-obese	Obese	*P*-value†
Age (y)	41.5 ± 14.6	45.7 ± 16.6	38.3 ± 12.1	0.015
Female (%)	77.5	65.9	86.2	0.018
Low physical activity (%)	45.1	47.7	43.2	0.667
Body mass index (kg/m^2)	35.2 ± 10.7	24.8 ± 3.2	43 ± 7.1	<0.001
Fasting plasma glucose (mg/dL)	87.2 ± 10.6	87.8 ± 10.6	86.7 ± 10.7	0.642
Insulin (μU/mL)	7.51 (4–10.1)	4.9 (2.8–10.1)	9.6 (5.5–11.2)	0.020
Insulin resistant (%)	13.7	9.1	17.2	0.264
Triacylglycerols (mg/dL)	72.5 (63–87.9)	69.5 (62.5–87.9)	80 (63–87.9)	0.082
Systolic blood pressure (mm Hg)	114.2 ± 12.1	112.3 ± 11.7	115.6 ± 12.3	0.178
Diastolic blood pressure (mm Hg)	72.9 ± 10.9	71.3 ± 7.5	75 ± 8.6	0.073
Total energy intake (kcal)	286 6 ± 844	2511 ± 686	3136 ± 858	<0.001
Total carbohydrate (% energy)	56.7 ± 7.2	58 ± 7.4	55.7 ± 6.9	0.058
Protein (% energy)	14.2 ± 2.7	13.9 ± 2	14.5 ± 3	0.294
Fat (% energy)	31.7 ± 6	30.4 ± 6.4	32.7 ± 5.6	0.066
Intake (g/d)				
Total carbohydrate	407 ± 60	398 ± 64	419 ± 53	0.076
Total sugars	147 ± 42	147 ± 37	147 ± 46	0.934
Sucrose	33 ± 18.8	33.7 ± 17.8	32.4 ± 16.6	0.732
Glucose	20 ± 10.8	20.7 ± 8.9	19.4 ± 12.1	0.536
Fructose	23.5 ± 12.2	23.9 ± 10.7	22.2 ± 13.3	0.479
Lactose	17.5 ± 11.6	15.7 ± 10.3	17.6 ± 12.5	0.332
Maltose	1.9 ± 0.8	2.1 ± 0.8	1.8 ± 0.9	0.115

*The residual model was used to adjust total energy intake for g/d intake of carbohydrate and its subtypes.
†The difference between non-obese and obese individuals analyzed by *t* test and χ^2 test.

Results

The study population comprised 102 participants without diabetes (44 non-obese and 58 obese) characterized by a mean age 41.5 ± 14.6 y (min 20/max 73 y), mean BMI 35.2 ± 10.7 kg/m^2 (min 18.7/max 58.6), and median insulin levels of 7.51 μU/mL. The mean of BMI was 28.8 ± 3.2 and 43 ± 7.1 kg/m^2, and the median insulin level was 4.9 and 9.6 μU/mL in non-obese and obese participants, respectively. Participants with obesity had higher total energy intakes than those who were not obese. There was no significant difference between non-obese and obese participants for carbohydrate and its subtypes (Table 1).

Although *FTO* mRNA levels in both visceral and subcutaneous tissues of obese participants were higher than their non-obese counterparts, this difference was not statistically significant (Fig. 1). Energy-adjusted total carbohydrate intake among non-obese participants was negatively correlated with visceral *FTO* mRNA expression ($r = -0.305$; $P = 0.044$); additionally, in obese participants, dietary carbohydrate intake had a significant negative correlation with both visceral ($r = -0.269$; $P = 0.041$) and subcutaneous ($r = -0.337$; $P = 0.010$) *FTO* gene expression (Fig. 2).

Linear associations of dietary carbohydrate and its subtypes with visceral and subcutaneous adipose tissues *FTO* mRNA expression are presented in Table 2. *FTO* gene expression in visceral and subcutaneous adipose tissue was inversely associated with a

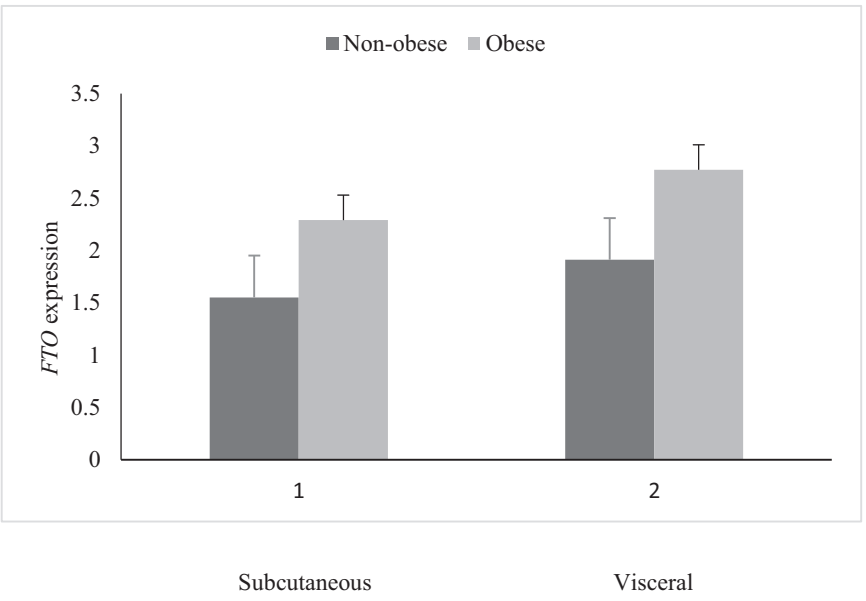

Fig. 1. *FTO* mRNA expression in visceral and subcutaneous adipose tissues in non-obese and morbidly obese participants. Results are expressed as mean ± SEM; mRNA levels were quantified with real-time quantitative polymerase chain reaction and normalized to the level of *GAPDH*.

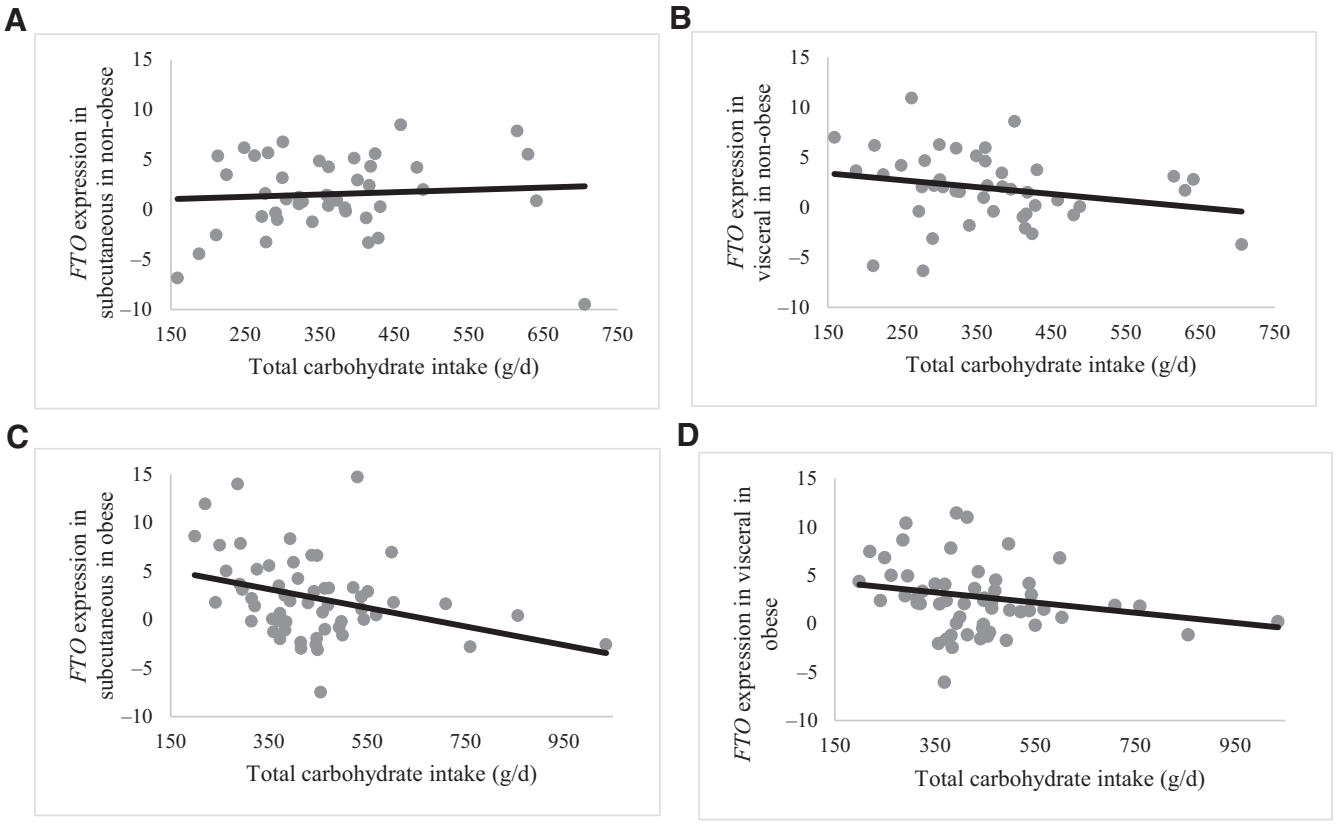

Fig. 2. Correlation between *FTO* gene expression in visceral and subcutaneous adipose tissues and dietary carbohydrate intake in non-obese and obese participants. Correlation of dietary total carbohydrate (**A**) with subcutaneous *FTO* mRNA expression ($r = 0.074$; $P = 0.635$), (**B**) with visceral *FTO* mRNA expression ($r = -0.305$; $P = 0.044$) in non-obese participants, (**C**) with subcutaneous *FTO* mRNA expression ($r = -0.337$; $P = 0.010$), and (**D**) with visceral *FTO* mRNA expression ($r = -0.269$; $P = 0.041$) obese participants.

1-SD increase in total carbohydrate intake among obese participants in models, adjusted for age and sex. Furthermore, after adjusting for confounders, *FTO* mRNA expression in subcutaneous adipose tissue was inversely associated with each SD higher intake of total sugars, sucrose, glucose, and lactose among participants with obesity. In addition, among obese participants, there was a significant association between dietary intake of fructose and *FTO* gene expression from the subcutaneous adipose tissue.

Table 2
Association of per 1 SD higher intake of total carbohydrate and its subtypes with *FTO* gene expression in adipose tissues

Exposures	Subcutaneous		Visceral	
	Standardized β	*P*-value	Standardized β	*P*-value
Non-obese				
Total carbohydrate (per 64.5 g/d)	−0.172	0.273	−0.272	0.072
Total sugars (per 37.3 g/d)	−0.124	0.430	−0.284	0.227
Sucrose (per 17.8 g/d)	0.120	0.440	−0.097	0.519
Glucose (per 8.9 g/d)	−0.240	0.123	−0.269	0.073
Fructose (per 10.7 g/d)	0.069	0.658	0.125	0.412
Lactose (per 10.3 g/d)	0.150	0.336	0.172	0.254
Maltose (per 0.8 g/d)	−0.110	0.417	−0.038	0.801
Obese				
Total carbohydrate (per 52.9 g/d)	−0.403	0.003	−0.052	0.701
Total sugars (per 46 g/d)	−0.441	0.001	−0.242	0.079
Sucrose (per 16.6 g/d)	−0.428	0.001	−0.252	0.066
Glucose (per 12.1 g/d)	−0.514	<0.001	−0.228	0.102
Fructose (per 13.3 g/d)	0.530	<0.001	0.089	0.490
Lactose (per 12.5 g/d)	−0.308	0.028	−0.209	0.139
Maltose (per 0.9 g/d)	−0.104	0.443	0.02	0.874

The residual model was used to adjust total energy intake for carbohydrate and its subtypes.
Adjustment for age and sex.

Sensitivity analyses

When the analyses were repeated after excluding participants who were insulin resistant, there was no notable change in the findings (Supplementary Table 1). There was no significant effect modification after additional adjusting for insulin concentration, family history of diabetes, the percentage of energy from protein, the percentage of energy from fat, and dietary fiber (data not shown).

Discussion

In the present study, we observed that after controlling for age and sex, dietary intake of total carbohydrate was inversely associated with *FTO* gene expression in the subcutaneous adipose tissue of obese participants; per 53 g/d increase in total carbohydrate intake, *FTO* gene expression in subcutaneous adipose tissue among obese participants decreased by 0.403 units. In addition, independent of potential confounding factors, habitual intake of total sugars, sucrose, glucose, and lactose had a negative association with subcutaneous *FTO* mRNA expression, and only fructose had a positive association.

However, the results of previous experimental studies conducted on animals examining the effect of dietary intake on *FTO* gene expression are inconsistent and have reported decreased or increased *FTO* gene expression by manipulating dietary composition in the rodent. A high-carbohydrate diet, compared with a

high-fat diet in rats, showed a significant decrease in *FTO* gene expression of visceral and subcutaneous adipose tissues [22]. The findings of a recent study showed that *FTO* gene expression in mice fed a high-fat diet in comparison with those on the standard diet (normal carbohydrate) did not change [23]. Furthermore, a high-fat diet but not a high-protein diet led to up-regulation of *FTO* gene expression in both subcutaneous and visceral adipocytes of rats [24]. It seems that dietary composition might have a determinative role in indicating the responses of *FTO* mRNA expression. In addition, *FTO* gene expression in the hypothalamus of rats showed a significant up-regulation during 48 h of food deprivation [25]. Furthermore, hypothalamic *FTO* mRNA was reduced after a high-fat diet and up-regulated in the fasted state [26]. Most studies are focused on *FTO* gene expression in the hypothalamus because of its regulatory effect on the appetite [25,26]. Yet, very little attention has been given to the characterization of *FTO* regulation in fat depots. Nevertheless, adipose tissues are not only the primary site of storage for excess energy but also are capable of synthesizing a number of biologically active parameters, paracrine and endocrine, which regulate human's body metabolism. Furthermore, previous studies were all experimental; it seems that research focusing on the role of dietary intake, especially carbohydrates on the *FTO* gene expression in human white adipocytes, is required.

During the past decade it has been reported that *FTO* gene polymorphism has a significant association with obesity [27–29], and its relationship with type 2 diabetes was of lower magnitude [30,31]. However, the association of *FTO* gene expression in adipose tissue with obesity still remains a controversial issue in human. In the present study, no significant difference was seen between obese and non-obese participants regarding *FTO* mRNA expression in visceral and subcutaneous adipose tissues. Similar to our findings, some studies have revealed no significant difference in *FTO* mRNA expression between obese and lean participants [32,33]. In contrast, Klöting et al. indicated that *FTO* mRNA levels were reduced in participants with obesity depending on the adipose tissue depot [34]. These incompatible results suggest a role for *FTO* in adipose tissues and demand further investigation.

In the present study, carbohydrate intake was significantly associated with *FTO* mRNA expression in subcutaneous adipose tissue but not visceral fat, suggesting that in a given individual up- or down-regulation in one versus the other adipose tissue is controlled by nutritional signals other than the systemic hormonal, neuronal, or local signals. Presence of a strong association between *FTO* expression in subcutaneous adipose tissue and total carbohydrate intake in the present study, and the lack of difference between individuals who carry various *FTO* genotypes for *FTO* mRNA expression in fat depots, as previously reported [13], indicates dietary intake of carbohydrates to be a strong regulator of *FTO* gene expression in adipose tissue independent of age and sex and even insulin levels.

Interestingly, total carbohydrates, total sugars, sucrose, glucose, and lactose had a negative association with *FTO* gene expression, and only dietary fructose intake had a positive association with *FTO* expression. These findings might be explained by the effect of insulin in response to the insulin-stimulated carbohydrates. Unlike sucrose, glucose, and lactose, intake of fructose does not stimulate insulin secretion from pancreatic β-cells [35]; therefore, this might justify the inverse association of fructose intake and *FTO* gene expression. However, in the present study, we selected participants who were free of diabetes, and insulin concentration was adjusted in the sensitivity analysis. Other unknown factor and mechanism might exist to modulate distinct association of *FTO* gene expression in response to the different type of dietary carbohydrate.

Some limitations of this investigation need to be mentioned. Owing to the cross-sectional nature of the study design, causal inferences cannot be made. However, because it is less likely that *FTO* gene expression in fat depots influences carbohydrate intake, we consider our inference that carbohydrate intakes may have primary effects on *FTO* mRNA levels to be plausible. Because no complete Iranian FCT exists, we had to use the USDA FCT. Finally, because the findings of the present study were exploratory, the sample size in this study was not large enough to have a stratified analysis based on sex.

The strengths of the present study include this being the first study to provide data on habitual dietary intake and its association with the *FTO* gene expression. Also, the observational design of the present study reflected long-term habitual dietary intakes of carbohydrate and its subtypes on *FTO* gene expression.

Conclusion

The significant inverse association of total carbohydrate, sugar, lactose, and glucose intake and the positive association of fructose intake with the *FTO* gene expression in fat deposits might provide an initial step toward identifying a barrier to success of long-term weight loss and understanding nutrient effects on energy homeostatic pathways to consider future clinical approaches to dietary weight loss interventions.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2018.12.014.

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