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PII: S0899-9007(18)30647-6
DOI: https://doi.org/10.1016/j.nut.2018.12.014
Reference: NUT 10428

To appear in: *Nutrition*

Received date: 1 July 2018
Revised date: 24 November 2018
Accepted date: 29 December 2018

Please cite this article as: Emad Yuzbashian , Golaleh Asghari , Mehdi Hedayati , Maryam Zarkesh , Parvin Mirmiran , Alireza Khalaj , The association of dietary carbohydrate with FTO gene expression in visceral and subcutaneous adipose tissue of adults without diabetes, *Nutrition* (2019), doi: https://doi.org/10.1016/j.nut.2018.12.014

Highlight

- There was no significant difference between non-obese and obese participants for FTO gene expression in subcutaneous and visceral fat mass.
- After adjusting for potential cofounders, total carbohydrate intake was inversely associated with FTO gene expression in subcutaneous adipose tissues among obese participants.
- Higher intake of total sugars, sucrose, glucose, and lactose was inversely associated with FTO mRNA expression in subcutaneous adipose tissue among participants with obesity
- Higher intake fructose was directly associated with FTO mRNA expression in subcutaneous adipose tissue among participants with obesity.

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

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Keywords: Nutrigenomic; gene expression; sucrose; simple sugar

Abstract

Aim: The aim of the current study was to investigate the association of dietary carbohydrates with FTO gene expression in visceral and subcutaneous adipose tissue.

Method: In this cross-sectional study, visceral and subcutaneous adipose tissues were gathered from 58 obese (BMI≥30 kg/m^2) and 44 non-obese (18≤BMI<30 kg/m^2) participants, aged ≥ 20 years, who had undergone elective abdominal surgery with minimal impact 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 FTO gene in visceral and subcutaneous adipose tissues was measured by real-time quantitative PCR.

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 (β=-0.403, *P* value=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: Only in obese participants, dietary intake of total sugars, sucrose, glucose, and lactose were 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 become a crucial problem in public health. The prevalence of obesity (body mass index (BMI) >30 kg/m^2) among US adults is over 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). Since unhealthy lifestyles including sedentary and low-quality dietary patterns are the prime suspect 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 16q12.2, 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). Besides, 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, the adipose tissue remains the main source of FTO gene expression and research focusing on the regulation of FTO in adipocytes is warranted.

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 intakes (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 impact 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 triglyceride 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. In order to understand whether intake of carbohydrate and its subtypes are associated with FTO mRNA regulation, we conducted a cross-sectional study in human 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

In the current cross-sectional study, we recruited 58 obese subjects with BMI≥30 kg/m^2 and 44 non-obese ones with 18≤BMI<30 kg/m^2, aged ≥20 years who had undergone elective abdominal surgery with minimal impact on dietary intakes at the Mostafa Khomeini and Khatam Al-Anbia hospitals, Tehran, Iran. In each patient undergoing, samples were obtained by the same specialist. The eligibility criteria considered to recruit participants were hospitalization less than 3 days, free of diagnosed diabetes mellitus or cancer, not using any lipid-lowering or anti-obesity medications, not pregnant or lactating, and not on any special diets. Before surgery, blood

samples, and data on anthropometrics, demographics and dietary intakes were obtained. During the surgery, approximately 100 mg of subcutaneous and visceral adipose tissues were collected.

Ethics approval was obtained from the ethics committee of the Research Institute for Endocrine Sciences (RIES) of the Shahid Beheshti University of Medical Sciences (NO: IR.SBMU.ENDOCRINE.REC.1395.171) 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 semi-quantitative food frequency questionnaire (FFQ) (17, 18). Because the Iranian food composition table (FCT) is incomplete, we used the United States 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 intakes (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 (totally 30-50 mg tissue) and added to 1 mL RNX-plus solution (Cinnagen, Iran) along with 250 microliter phosphate

buffered saline (PBS). The mixture homogenized using a homogenizer (QIAGEN, Germany). Then, 200 microliter chloroform had been added to the mixture. Proteins, lipids, carbohydrates, and cell debris were eliminated through extraction of the aqueous. Then, the quality of the extracted RNA was evaluated by Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and the ratio of absorption (260/280 and 260/230 nm) of all preparations was in acceptable range. Total RNA was treated with DNase I in order to remove traces of genomic DNA before complementary DNA (cDNA) synthesis. The cDNA synthesis kit (Thermo Scientific, USA) 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 following: 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′; GAPDH Reverse: 5′-CCG TTG ACT CCG ACC TTC AC-3′. To evaluate the efficiency of primers, both FTO and GAPDH, obtained as 0.9.

The Real-Time quantitative PCR (qPCR), carried out using a Real-Time PCR instrument (Rotor-Gene 6000, Sydney, Australia), was performed in 25µL volumes containing 12.5µL 2X SYBR Green Master mix (Thermo Scientific, USA), 0.3µL forward primers, 0.3µL reverse primers, 8.9µL RNase- free water, and 3µL of the cDNA. For each gene, samples were run in duplicate for inter assay control along with GAPDH (housekeeping) and the non-template control (NTC); qPCR amplification was performed with the following thermal cycling conditions: 5 minutes 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

Weight was measured in light clothing to the precision of 0.1 kg on a SECA digital weighing scale (Seca 707; Seca Corporation, Hanover, Maryland; range 0.1-200 kg) and height was measured without shoes to the nearest 0.1 cm. Body mass index (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 (IPAQ) (20). In order to measure energy expenditure, the concept of metabolic equivalents (MET) was used. Physical activity levels were classified as low (MET≥600 minutes per week), moderate (600<MET<3000 minutes per week) and vigorous activity (MET>3000 minutes per week).

Arterial blood pressure (BP) was measured by a mercury sphygmomanometer for each participant in the sitting position. Systolic blood pressure (SBP) was determined by the onset of the tapping Korotkoff sound while diastolic blood pressure (DBP) was determined as the disappearance of this sound. Blood pressure 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 a 10–12 h overnight. Fasting plasma glucose (FPG) was measured using an enzymatic colorimetric method with glucose oxidase. Inter- and intra-assay coefficients of variation (CV) were both 1.0% for FPG. Triglyceride (TG) levels were determined using the enzymatic colorimetric method with

glycerol phosphate oxidase. Inter- and intra-assay 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 intra-assay 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±standard deviation. Because plasma TGs and insulin were skewed, we reported as median and inter-quartile range and log transformation was used. G*Power was used to estimate the power of our sample sizes. When effect size was considered 0.3 in Pearson correlation, our sample power was ~ 0.84 for non-obese participants and ~0.89 for obese subjects, 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-test and chi-square test 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 carbohydrate and its subtypes with FTO mRNA expression in subcutaneous and visceral adipose tissues, and standardized β was reported after adjusting for age and sex.

In sensitivity analyses, we excluded individuals who developed insulin resistance according to the following definition: Homeostatic model assessment of insulin resistance (HOMA-IR) = [fasting insulin (μU/ml) × fasting glucose (mmol/l)]/22.5.

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 the Statistical Package for the Social Sciences program (SPSS) (version 15.0; SPSS Inc, Chicago IL) and *P*-values <0.05 were considered statistically significant.

Results

The study population comprised 102 non-diabetic participants (44 non-obese and 58 obese) characterized by a mean age 41.5±14.6 (min: 20 and max: 73 years) years, mean BMI 35.2±10.7 (min: 18.7 and max: 58.6) kg/m^2, and median insulin levels of 7.51 μU/mL. The mean of BMI was 28.8±3.2 and 43.0±7.1 kg/m^2, and median of 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 non-obese ones. 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 counterpart, this difference was not statistically significant (Figure 1). Energy-adjusted total carbohydrate intake, among non-obese participants, was negatively correlated with visceral FTO mRNA expression (r=-0.305, P=0.044); besides, 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 (Figure 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 tissues was inversely associated with a one standard deviation increase in total carbohydrates 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 standard deviation higher intake of total sugars, sucrose, glucose, and lactose among participants with obesity. Besides, among obese participants, there was a significant association between dietary intake of fructose and FTO gene expression from the subcutaneous adipose tissue.

Sensitivity analyses

When the analyses were repeated after excluding participants who were insulin resistant, there was no notable change in the findings (supplementary table). 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 0.403 unit.

Besides, habitual intake of total sugars, sucrose, glucose, and lactose had negative and only fructose had a positive association with subcutaneous FTO mRNA expression, independent of potential confounding factors.

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 with 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 to indicate the responses of FTO mRNA expression. Additionally, 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 as an endocrine organ is 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.

FTO gene polymorphism, during the past decade, has been reported that had a significant association with obesity (27-29), and its relationship with type 2 diabetes was of lower magnitude (30, 31). However, the associations of FTO gene expression in adipose tissue with obesity still remain a controversial issue in human. In the current study, we observed that there was no significant difference between obese and non-obese participants regarding FTO mRNA expression in visceral and subcutaneous adipose tissues. Similar to our findings, studies 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, suggests that in a given individual up- or down-regulation in one vs the other adipose tissue is controlled by nutritional signals beside the systemic hormonal, neuronal and/or local signals. Presence of a strong association between FTO expression in subcutaneous adipose tissue and total carbohydrate intake in the current study, and the lack of difference between individuals who carry various FTO genotypes for FTO mRNA expression in fat depots, 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 current 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. Due to the cross-sectional nature of the study design, causal inferences cannot be made. However, as it is less likely that FTO gene expression in fat depots influences carbohydrate intakes, we consider our inference that carbohydrate intakes may have primary effects on FTO mRNA levels plausible. Secondly, as no complete Iranian FCT exists, we had to use the USDA FCT. Finally, finally, as the findings of the current study were exploratory, the sample size in our study was not enough to have a stratified analysis based on gender.

The strengths of the current 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 current study reflected long-term habitual dietary intakes of carbohydrate and its subtypes on FTO gene expression.

In conclusion, the significant inverse association of total carbohydrate, sugar, lactose, and glucose intake and positive association of fructose intake the FTO gene expression in fat depots, might provide an initial step towards determining 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.

Acknowledgment

We would like to acknowledge Ms. Niloofar Shiva for a critical edition of English grammar and syntax of the manuscript.

Support and Financial Disclosure Declaration

On behalf of all authors, the corresponding authors hereby declare that there is no conflict of interest.

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Table 1- Demographic, anthropometric, dietary intake, and serum biochemical parameters[1]

	Total	Non-obese	Obese	P value[2]
Age (years)	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	43.0±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(4.0-10.1)	4.9 (2.8-10.1)	9.6(5.5-11.2)	0.020
Insulin resistant (%)	13.7	9.1	17.2	0.264
Triglycerides (mg/dl)	72.5 (63.0-87.9)	69.5 (62.5-87.9)	80.0(63.0-87.9)	0.082
Systolic blood pressure (mmHg)	114.2±12.1	112.3±11.7	115.6±12.3	0.178
Diastolic blood pressure (mmHg)	72.9±10.9	71.3±7.5	75.0±8.6	0.073
Total energy intake (kcal)	2866±844	2511±686	3136±858	<0.001
Total carbohydrate (% of energy)	56.7±7.2	58.0±7.4	55.7±6.9	0.058
Protein (% of energy)	14.2±2.7	13.9±2.0	14.5±3.0	0.294
Fat (% of energy)	31.7±6.0	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	33.0±18.8	33.7±17.8	32.4±16.6	0.732
Glucose	20.0±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

[1] The residual model was used to adjust total energy intake for g/d intake of carbohydrate and its subtypes

[2] The difference between non-obese and obese subjects analyzed by t-test and chi-square test

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

	Subcutaneous		Visceral	
	β	P value	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 10.3 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 46.0 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 12.3 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

Legends to figures

Figure 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 qPCR and normalized to the level of GAPDH.

Figure 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.

Figure 1-

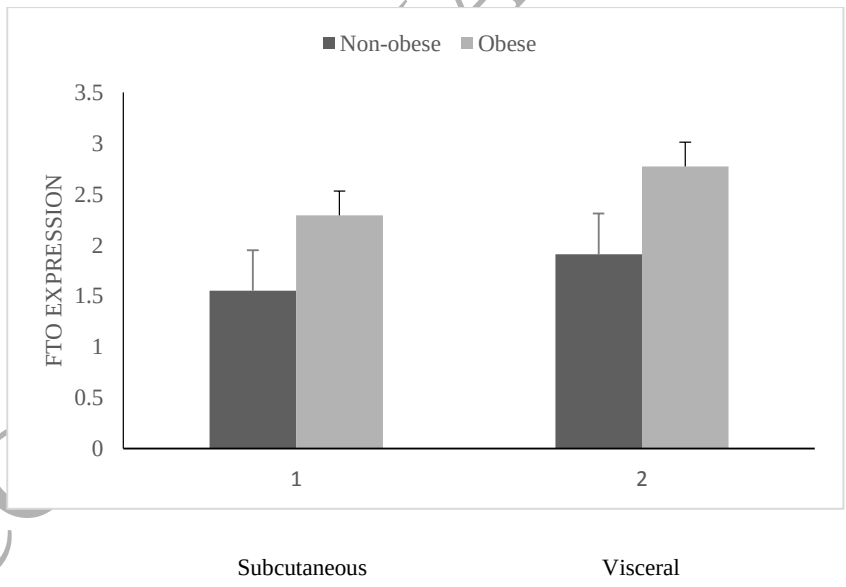

Figure 2 -