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Habitual dietary intake of fatty acids are associated with leptin gene expression in subcutaneous and visceral adipose tissue of patients without diabetes

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ABSTRACT

The purpose of the study was to investigate the association of leptin gene expression in visceral and subcutaneous adipose tissues with habitual fatty acid intake and its subtypes in adults.

Visceral and subcutaneous adipose tissues were gathered from 97 participants aged ≥ 20, who had undergone elective abdominal surgery. Dietary fatty acid intakes including total fatty acids (TFA), saturated fatty acid (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3, n-6, and n-9 fatty acids were collected using a valid and reliable food-frequency questionnaire (FFQ). The leptin gene expression in visceral and subcutaneous adipose tissues was measured by Real-Time PCR.

After controlling for body mass index (BMI) and insulin, energy-adjusted dietary intake of SFA was positively and MUFA and n-3 fatty acids were negatively associated with subcutaneous and visceral adipose tissues leptin gene expression. Besides, a significant negative association of PUFA, n-6, and n-9 fatty acids with leptin mRNA from visceral adipose tissue were observed. In order to better interpretations of the results, the participants were allocated two groups including non-obese (BMI < 30 kg/m^2) and obese subjects (BMI ≥ 30 kg/m^2). Among non-obese participants, the SFA had positive and PUFA had negative association with leptin gene expression in both adipose tissues. Furthermore, in obese participants, n-3, n-6, and n-9 fatty acids had a negative association with visceral leptin gene expression.

Habitual intake of SFA, MUFA, and n-3 fatty acids were associated with leptin gene expression in visceral and subcutaneous adipose tissues, suggesting an important role of quality and quantity of fatty acids intake in adipose tissue to regulate leptin expression.

1. Introduction

The adipose tissue is not only known as a passive energy storage but also considered as an active endocrine organ producing a variety of factors in the term of the adipokines [1]. Excess accumulation of fat alters adipose tissue metabolic and endocrine functions which contributes to the adipokine levels, hence influence the insulin sensitivity and the regulation of whole body energy homeostasis [1–3].

Among the adipokines, leptin is one of the hormones directly connects to body fat and obesity. Leptin consists of a 167-amino acid peptide which is produced by the human obese (OB) gene. It is a hormone that is mainly expressed and secreted in adipose tissues and has a number of important effects on regulation of body weight, energy expenditure, appetite, thermogenesis, and eating behavior [4]. Leptin also leads to increase oxidation of fatty acids, decrease synthesis of triglyceride, alleviate lipogenic action of insulin, and increases insulin sensitivity; thus, it has the favorable effect on glucose homeostasis [4]. One of the known factors affecting the regulation of leptin expression and concentration is meal composition and intake of nutrients. Most dietary-related factors that have potential effects on leptin levels are

Abbreviations: BMI, Body mass index; TFA, Dietary total fatty acids; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; FFQ, Food frequency questionnaire; SBP, Systolic blood pressure; DBP, Diastolic blood pressure

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energy-supplying nutrients such as fatty acids, carbohydrates, and proteins. Investigating on the impacts of dietary intakes of fatty acids on adipose tissue metabolism have emerged as a fascinating area for researchers which they reported that dietary intakes of fatty acids can affect and regulate gene activity in adipose tissues both directly and indirectly, with positive or negative effects [5,6]. The effect of fatty acids intake on leptin gene expression was limited to animal studies; however, several human investigations assessed the relation of fatty acids consumption on only leptin concentrations. In a study showed that higher dietary intake of fat was positively correlated with plasma leptin concentrations [7]. In addition, decreased intake of saturated fatty acids (SFA) and increased intake of polyunsaturated fatty acids (PUFA) over one year can reduce leptin concentration [8]. Therefore, it seems that the type of fat in the habitual diet has also been found to influence the plasma leptin concentration [9].

To the best of our knowledge, evidence specifically examining the association of habitual dietary intake of fatty acids and its subtypes with leptin gene expression in adipose tissue is rare. Using the nutrigenomics in order to illustrate how dietary factors in particular fatty acid intakes influence gene expression and subsequently impact on adipose tissue metabolism. A common approach is the examination of individual mRNA levels relative to an intake of certain dietary nutrients. Therefore, we investigated the association of dietary fatty acids quality and quantity with leptin gene expression in visceral and subcutaneous adipose tissues.

2. Materials and methods

2.1. Participants

In the current cross-sectional study, we selected 97 participants, who aged ≥ 20 and underwent minor abdominal surgery with minimal impact on dietary intakes at the Mostafa Khomeini Hospital and Khatam Al-Anbia Hospital, Tehran, Iran. All participants were hospitalized less than 3 days. The eligibility criteria were participants who were free of diagnosed diabetes mellitus or cancer, not using any lipid lowering or anti-obesity medications, not pregnant or lactating, and not on special diets. During the surgery, approximately 100 mg of subcutaneous and visceral adipose tissues were collected. Before surgery, blood samples, anthropometrics, demographics, and dietary intakes were obtained.

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 conducted in accordance with the Declaration of Helsinki as well as our institutional guidelines. Written informed consent was obtained from all participants.

2.2. 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) [10,11]. 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 dietary total fatty acids (TFA) and its subtypes that included saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), n-3 fatty acids, n-6 fatty acids, and n-9 fatty acids.

The reliability and validity of the FFQ evaluated in a previous study against twelve 24-h dietary recalls and biomarkers and indicated that the FFQ provides reasonably valid measures of the average long-term dietary fatty acids intake [12–14].

2.3. Quantitative real-time polymerase chain reaction analysis of gene expression

We extracted total RNA from both adipose tissues using the RNX-plus solution kit (Cinnagen, Iran) according to the manufacturer's protocol. The quality of the extracted RNA was assessed by NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and the ratio of absorption (260/280 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. For cDNA synthesis, Fermentas kit (Thermo Scientific, USA) according to the manufacturer's recommendations was used. The product was stored at −20 °C for further analysis.

Primers based on the sequences of the National Center for Biotechnology Information (NCBI) GenBank database were checked by Genrunner Software (version 3.05). GAPDH was used as a reference gene for normalization across samples. The primer sequences of leptin and GAPDH were as following: leptin Forward: 5′- CATTTCACA CACGCAGTCAGTC-3′;

leptin Reverse: 5′- CAGTGTCTGGTCCATCTTGGATA-3′;

GAPDH Forward: 5′-CTGCTCCTCCTGTTCGACAGT-3′;

GAPDH Reverse: 5′-CCGTTGACTCCGACCTTCAC-3′

To evaluate the efficiency of primers, both leptin and GAPDH, obtained as 0.9.

The Real-Time quantitative PCR (qPCR) was carried out in a Real-Time PCR instrument (Rotor-Gene 6000, Sydney, Australia). The qPCR was performed in 25 μL volumes containing 12.5 μL 2X SYBR Green Master mix (Thermo Scientific, USA), 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 inter assay 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 amount of leptin mRNA expression in each sample was calculated based on its threshold cycle (Ct) normalized to the Ct of the reference gene (GAPDH). All qPCR laboratory procedures were performed according to the MIQE guidelines [15].

2.4. 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).

The physical activity was assessed by long forms of the Persian version of International Physical Activity Questionnaire (IPAQ) which has been developed by WHO during an interview. In order to measure energy expenditure, the concept of metabolic equivalents (MET) was used. MET is the ratio of a person's working metabolic rate relative to the resting metabolic rate [16]. Reliability and validity of the Persian version of the long form IPAQ were acceptable [17]. Physical activity level was classified as 'low' (MET ≥ 600), 'moderate active' (600 < MET < 3000) and 'vigorous activity' (MET > 3000).

Arterial blood pressure (BP) was measured by mercury sphygmomanometer for each subject in the seat 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 the Korotkoff sound. Blood pressure was measured twice and the average was considered as the participant's BP.

Blood samples were collected from all subjects in potassium EDTA-containing tubes before the surgery who have an overnight fast of 10–12 h. The samples were then centrifuged at 3000*g* for 15 min according to the standard protocols and plasma was collected. 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. The triglyceride 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. Total cholesterol (TC) was assayed with the cholesterol esterase and cholesterol oxidizes method which inter- and intra-assay CV were 0.5 and 1.7, respectively. All measurements 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 CV of insulin were 1.7 and 2.3, respectively.

2.5. Statistical analysis

The normality of the distribution of variables was assessed by histogram and the Kolmogorov–Smirnov tests. Continuous variables were described as mean ± standard deviation (SD). Because plasma TGs and insulin were skewed, log transformation was used. In order to adjust energy intake on fatty acid intakes, we also used the residual method [18]. One sample *t*-test was used to compare between leptin gene expression among obese and non-obese participants. Linear regression was performed to determine the association of TFA and its subtypes with leptin gene expression in subcutaneous and visceral adipose tissues, and standardized β was reported in three model including model 1 was crude, model 2 adjusted for age and BMI, and model 3 was additionally adjusted for insulin concentration. For better interpretation of our results, we conducted Linear regression after separating participants according to their obesity status, BMI < 30 kg/m^2 non-obese and BMI ≥ 30 kg/m^2 obese. 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.

3. Results

Characteristics of study participants are shown in Table 1. Participants presented a mean age of 41.6 years old and calculated the mean BMI of 35.4 kg/m^2. Among the participants, 79% of them were women, and 78% of them have low physical activity. The mean of FPG and insulin level were 87.2 mg/dL and 11.9 μU/mL, respectively. There were no significant differences between leptin gene expression in visceral and subcutaneous adipose tissue (P = 0.670; data not shown). Participants have 2912 kcal energy intake on average that consisted of 56.7% from carbohydrate, 14.2% from protein, and 31.7% from fatty acids. There was no significant difference between leptin gene expression in obese and non-obese subjects (Fig. 1).

Table 1
Characteristics of study participants.

	Mean ± standard deviation or percent
Age (years)	41.6 ± 10.5
Women (%)	79%
Low physical activity (%)	78%
Body mass index (kg/m^2)	35.4 ± 10.5
Fasting plasma glucose (mg/dl)	87.2 ± 11.5
Insulin (μU/mL)	11.9 ± 14.7
Triglycerides (mg/dl)	88.0 ± 54.8
Systolic blood pressure (mmHg)	113.7 ± 13.4
Diastolic blood pressure (mmHg)	72.7 ± 9.0
Dietary intakes	
Total energy (kcal)	2912.7 ± 837.5
Carbohydrate (% of energy)	56.7 ± 7.1
Protein (% of energy)	14.2 ± 2.7
Total fatty acids (% of energy)	31.7 ± 5.9
Saturated fatty acids (% of energy)	10.0 ± 2.7
Monounsaturated fatty acids (% of energy)	10.3 ± 2.2
Polyunsaturated fatty acids (% of energy)	6.34 ± 1.75
n-3 fatty acid (g/d)	2.45 ± 1.7
n-6 fatty acid(g/d)	18.7 ± 23.3
n-9 fatty acid(g/d)	30.0 ± 36.6

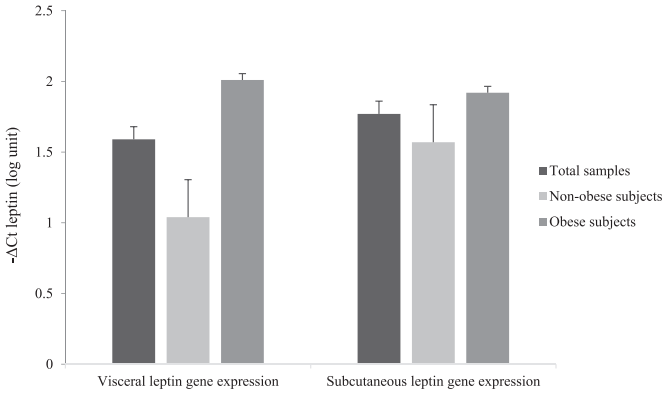

Fig. 1. One sample *t*-test was used to compare group means; Mean with standard error for leptin gene expression in adipose tissue.

Linear associations of dietary fatty acids and their subtypes with visceral and subcutaneous adipose tissue leptin gene expression in total participants are presented in Table 2. After controlling for BMI, energy-adjusted dietary intake of SFA was positively associated with leptin gene expression from subcutaneous and visceral adipose tissues. This association remained significant after further controlling for insulin concentration. Energy-adjusted dietary MUFA had a negative association with visceral leptin mRNA expression (β = −0.130, *P* = 0.040) and marginally significant association with subcutaneous leptin mRNA expression (β = −0.138, *P* = 0.058). In addition, a significant negative association of n-3 fatty acids (β = −0.837, *P* = 0.006), n-6 fatty acids (β = −0.051, *P* = 0.020), and n-9 fatty acids (β = −0.033, *P* = 0.025) with leptin gene expression from the visceral adipose tissue after controlling for BMI and insulin were observed.

Linear associations of dietary fatty acids and their subtypes with visceral and subcutaneous adipose tissue leptin gene expression after separating participants in two groups including non-obese and obese are presented in Table 3. Among non-obese subjects with controlling for BMI and insulin, dietary intake of saturated fatty acid (β = 0.230, p = 0.015) were significantly and positively associated, whereas PUFA (β = −0.422, *P* = 0.021) was significantly but negatively associated with subcutaneous leptin mRNA expression. In addition, a significant positive association SFA intakes (β = 0.210, *P* = 0.004) and negative association of PUFA (β = −0.316, *P* = 0.029) and n-6 fatty acid (β = −0.243, *P* = 0.031) with visceral adipose tissue were observed. Among obese subjects after controlling for BMI and insulin, dietary intake of SFA (β = 0.198, *P* = 0.020), n-3 fatty acid (β = −0.769, *P* = 0.037), n-6 fatty acid (β = −0.053, *P* = 0.041), and n-9 fatty acids (β = −0.035, *P* = 0.050) was associated with subcutaneous adipose tissue leptin gene expression. Furthermore, a significant association of n-3 fatty acid (β = −0.959, *P* = 0.004), n-6 fatty acid (β = −0.061, *P* = 0.010), and n-9 fatty acids (β = −0.044, *P* = 0.006) with visceral adipose tissue leptin gene expression were also observed.

4. Discussion

In the current study, we observed that after controlling for insulin and BMI, SFA was positively associated with leptin gene expression in visceral and subcutaneous adipose tissue in total participants. In addition, habitual intake of MUFA and n-3 fatty acids had a negative association with subcutaneous, and MUFA, PUFA, n-3 fatty acids, n-6 fatty acids, and n-9 fatty acids had a negative association with visceral leptin gene expression.

Table 2
Standardized coefficients of total fatty acids and its subtype intakes with leptin gene expression in total population.[a]

	Subcutaneous adipose tissue		Visceral adipose tissue	
	β	P value	β	P value
Total fatty acid				
Adjusted for BMI	−0.005 (0.026)	0.845	−0.016 (0.023)	0.481
Adjusted for BMI and Insulin	−0.006 (0.026)	0.815	−0.017 (0.023)	0.454
Saturated fatty acid				
Adjusted for BMI	0.220 (0.058)	< 0.001	0.169 (0.052)	0.001
Adjusted for BMI and Insulin	0.214 (0.058)	< 0.001	0.164 (0.052)	0.002
Monounsaturated fatty acid				
Adjusted for BMI	−0.119 (0.071)	0.099	−0.113 (0.062)	0.073
Adjusted for BMI and Insulin	−0.138 (0.072)	0.058	−0.130(0.062)	0.040
Polyunsaturated fatty acid				
Adjusted for BMI	−0.160 (0.094)	0.093	−0.163 (0.082)	0.048
Adjusted for BMI and Insulin	−0.157 (0.094)	0.099	−0.160 (0.083)	0.052
n-3 fatty acid				
Adjusted for BMI	−0.418 (0.327)	0.205	−0.576 (0.297)	0.044
Adjusted for BMI and Insulin	−0.669(0.348)	0.058	−0.837 (0.297)	0.006
n-6 fatty acid				
Adjusted for BMI	−0.027 (0.023)	0.238	−0.032 (0.020)	0.116
Adjusted for BMI and Insulin	−0.047 (0.025)	0.062	−0.051(0.021)	0.020
n-9 fatty acid				
Adjusted for BMI	−0.013 (0.015)	0.399	−0.017 (0.013)	0.196
Adjusted for BMI and Insulin	−0.028 (0.017)	0.093	−0.033 (0.014)	0.025

[a] Energy-adjusted were calculated using the residual model for all exposure.

Table 3
Standardized coefficients of total fatty acids and its subtype intakes with leptin gene expression in obese and non-obese participants after adjustment for body mass index and insulin.[a]

	Subcutaneous adipose tissue		Visceral adipose tissue	
	β	P value	β	P value
Non-obese subjects				
Total fatty acid	−0.021(0.049)	0.671	0.000 (0.039)	0.995
Saturated fatty acid	0.230 (0.090)	0.015	0.210 (0.068)	0.004
Monounsaturated fatty acid	−0.212 (0.131)	0.120	−0.112(0.110)	0.315
Polyunsaturated fatty acid	−0.422 (0.176)	0.021	−0.316(0.139)	0.029
n-3 fatty acid	−0.872(1.404)	0.538	−1.914 (1.067)	0.081
n-6 fatty acid	−0.160(0.144)	0.272	−0.243(0.108)	0.031
n-9 fatty acid	−0.202 (0.152)	0.192	−0.229(0.117)	0.058
Obese subjects				
Total fatty acid	−0.003(0.032)	0.918	−0.030(0.029)	0.308
Saturated fatty acid	0.198(0.082)	0.020	0.107 (0.079)	0.179
Monounsaturated fatty acid	−0.081(0.085)	0.330	−0.141 (0.077)	0.072
Polyunsaturated fatty acid	−0.051 (0.111)	0.645	−0.091(0.102)	0.374
n-3 fatty acid	−0.769(0.359)	0.037	−0.959 (0.319)	0.004
n-6 fatty acid	−0.053(0.025)	0.041	−0.061(0.023)	0.010
n-9 fatty acid	−0.035(0.017)	0.050	−0.044(0.015)	0.006

[a] Energy-adjusted were calculated using the residual model for all exposure.

Previous studies indicated that leptin gene expression pattern in visceral and subcutaneous adipose tissue are different [19,20]. In addition, the response of leptin gene expression to the stimulator might also depend on the region of fat depot [21]. However, in the current study leptin gene expression did not have significant difference between visceral and subcutaneous adipose tissues. While, there was a correlation between leptin gene expression in visceral and subcutaneous adipose tissue ($r = 0.655$ and $P < 0.001$).

It must be kept in mind that the current study was the first investigation that assessed the relationship between dietary fatty acids in the context of habitual intakes and leptin mRNA expression in adipose tissues. Nevertheless, our results could provide valuable insight from a prevention perspective. There were several studies investigating the association of fatty acids intake with leptin concentrations [22–24]. Similar to our findings, Murakami et al. found that dietary intake of TFA was not significantly associated with serum leptin concentration [22]. Other studies also reported no association between TFA intakes and leptin levels in 32 American men and women [23] and 114 Greek men and women [24]. In contrast, Cooling et al. revealed that plasma leptin concentrations were positively correlated with dietary intake of fatty acids; besides, participants who consume a high-fat diet compared with those on a low-fat diet had a higher concentration of leptin [7]. Furthermore, plasma leptin concentrations had a positive association with the TFA consumption among men with BMI < 25 kg/m^2 [25]. These discrepancies might be justified by the various populations under analysis, using various dietary assessments, and considering various number and type of variables as confounders. Furthermore, the composition of TFA which investigated on aforementioned studies might have a mediating role between fatty acids intakes and leptin responses. Further studies are needed in this poorly investigated field.

It seems that besides a number of fats, the type of fat in the habitual diet has the key roles on circulating leptin concentrations. Our results were in accordance with a previous cross-sectional study which found that among patients with type 1 diabetes mellitus the leptin serum

levels had positive correlation with consumption of linoleic acid and negative correlation with arachidonic acids among women [9]. In addition, Reseland et al. observed that by increasing intake of PUFA and decreasing intake of SFA over one year, leptin concentrations reduced independently of body fat mass in men [8]. In addition, Pieke et al. after intervention by two diets including high fat (total fat 39%, SFA 8%, MUFA 15%, n-3 PUFA 1.6%) and low fat diet (total fat 28%) for 3 weeks observed the leptin concentration of participants in high-fat diet group decreased [26].

We found that one SD increase in SFA was associated with 0.22 and 0.16 log-unit increase in visceral and subcutaneous leptin mRNA expression. These findings were significant even after separating participants based on BMI status. Our finding might be close to the study which conducted among men and women with diabetes which showed the leptin levels was positively correlated with serum SFA levels ($r = 0.49$) [9]. However, among nutritionist Japanese girls, SFA intake was not significantly associated with leptin concentrations [22]. This inconsistency to our findings would be because the healthy lean Japanese girls had relatively healthy dietary habits and they have a narrower range of leptin levels.

We observed that n-3 fatty acid intakes were negatively associated with leptin gene expression in whole participants. In accordance with our findings, Supplementation with n-3 fatty acids along with a high-fat diet decreased leptin gene expression in adipose tissue [27]. In addition, Reseland et al. observed that higher intake of n-3 fatty acids among rats reduced both leptin concentration and adipose tissue gene expression [28]. Furthermore, a study conducted on patients with type 1 diabetes showed a negative correlation between serum n-3 fatty acids levels and leptin concentrations ($r = -0.48$) [9]. In addition, another study observed that participants who have a diet rich in fish had significantly lower plasma leptin levels than subjects that consumed no fish [29]. Notably, in the current study after separating subjects based on BMI status, the association between n-3 fatty acids intake and leptin gene expression remained significant only among obese subjects. Hence, it is interesting to further consider the effects of n-3 fatty acids on leptin regulation across different BMI groups. Although the available data are limited, it seems that supplementation with n-3 fatty acids in clinical trial studies more influenced leptin levels in obese subjects than lean individuals [30,31]. Further research in this area is warranted to investigate the effect of n-3 fatty acids on leptin gene expression and serum concentration in healthy individuals with differing BMI.

In the current study, we observed that n-6 and n-9 fatty acids intake was negatively associated with leptin gene expression in both adipose tissues of obese subjects. In contrast to our results, Cha et al. feed rats with different dietary fat sources including fish oil, safflower oil, and beef for 10 weeks, and they found that leptin concentrations in rats with a diet rich in PUFA and MUFA was higher than those fed a diet rich in SFA. Furthermore, this study indicated that dietary fatty acid composition was an important factor in diet-induced obesity leptin concentrations, independent of adipose tissue mass [32]. The diet composition, feeding pattern, and type of experimental species might influence the response of leptin regulation, giving inconclusive results. There may be both species differences between rodents and humans, and tissue differences in the regulation of leptin expression.

It has been suggested that the pattern of fatty acids might regulate transcription of leptin and several adipocyte-specific genes by changing the regulation pattern of the nuclear receptor PPARγ [33]. A study in rats fed with a fish oil-enriched diet found that the by increasing activation of PPARγ, the epididymal leptin mRNA levels decreased. In addition, a reduction in PPARγ expression in cultured cells treated with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was to approximately 70% of that in the control [28]. Besides, the n-3 fatty acids had an influence on leptin gene expression that was accompanied by a reduction in PPARγ and SREBP-1 mRNA levels [28]. Another regulatory mechanism that dietary fatty acids might affect leptin gene expression are epigenetic modifications. The different type of fatty acids can contribute to the cellular DNA by histone methylation leading to altered chromatin remodeling and gene expression [27]. The evidence suggested that n-3 fatty acids seemed to antagonize leptin promoter modifications induced by high-fat diet. Dietary n-3 fatty acids affected the methylation level of CpG sites at the leptin promoter in the adipose tissue of the high-fat diet mice [34].

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 adipokine influence fatty acids intake, we consider our inference plausible that fatty acids intake may have primary effects on leptin investigated in the present study. Secondly, as no complete Iranian FCT exists, we had to use the USDA FCT. Thirdly, as all subjects in the present study were Tehranian, the present findings may not apply directly to other races.

The strengths of the current study include this is the first study providing data on the habitual dietary intake and its association with the leptin gene expression. Also, the observational design of the current study reflected long-term habitual dietary intakes of fatty acids intakes on leptin in a broad population.

In conclusion, increased leptin gene expression in adipose tissue is connected to total dietary fatty acid intake and n-3 polyunsaturated fatty acids in both obese and non-obese individuals, suggesting an important role of quality and quantity of fatty acids intake in adipose tissue metabolism.

Support and financial disclosure declaration

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

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