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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