The interaction between rs 3807992 genotypes with the dietary inflammatory index on Leptin, leptin resistance, and Galectin 3 in obese and overweight women

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Research Article

Keywords: Leptin, Leptin resistance, Galectin 3, Caveolin-1, dietary inflammatory index, interaction

Posted Date: April 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1507858/v1

Abstract

Objective: Obesity is related to increasing leptin and some inflammatory factors that are related to low-grade inflammation. Several studies have shown Caveolin-1 (CAV1) genetic variations may correlate with dietary intake. The current study aimed to evaluate the interaction of CAV1 rs3807992 with types of the energy-adjusted dietary inflammatory index (EDII) in leptin, leptin resistance, and Galectin 3 as inflammatory factors.

Methods: This cross-sectional study was carried out on 363 overweight and obese females. Dietary intake and DII were obtained from a 147-item food frequency questionnaire (FFQ). The CAV-1 genotype was measured using the PCR-RFLP method. Anthropometric values and serum levels of leptin and Galectin 3 were measured by standard methods.

Results: Increased adherence to EDII in the interaction with CAV1 genotypes containing risk alleles (AA+AG) leads to an increase in leptin level 79.15 ng/ml (β= 79.15, CI= -1.23,163.94, P= 0.04) in model 3 after controlling for further potential confounders. By contrast, adherence to EDII in the interaction with that genotype include risk alleles showed no significant interaction even after adjustment in model 3 (β=0.55, CI= -0.99, 2.09, P=0.48). Although marginal positive significant interaction was found between EDII and CAV1 genotypes on Galectin 3 after adjustment in model 3 (β=31.35, CI=0.13, 77.13, P= 0.05).

Conclusions: A positive interaction between EDII and CAV1 genotypes on leptin and Galectin3, while no interaction was found on leptin resistance

Introduction

Obesity affects a large number of adults across the world, as hits epidemic proportions [1], it is causing a growing economic and health strain, with an extraordinarily high incidence rate among women of childbearing age. There was a significant difference between the prevalence of obesity between females (29.8) and males (15.3%) [2]. Overweight and obesity are caused by the combination of various genetic, occupational, physiological, and eating habits, which result in inflammation [3]. Inflammation is affected by a variety of influences, including lifestyle, nutrition, and physical exercise, as well as genetic [4]. Adiposity is linked to a higher incidence of non-communicable diseases (NCDs) [5, 6]. This systemic and adipose tissue inflammation, which causes increases in the production of leptin and pro-inflammatory cytokines, is one of the pathways that could illustrate the connection between obesity and the progression of NCDs, with the cause of chronic low-grade inflammation [7–9]. The overabundance of leptin released by adipocytes to the amount of body fat [10] has a key role in homeostatic regulations of feeding and energy balance thus body weight management and also insulin sensitivity [11–13]. One of the related factors to leptin and obesity is Galectin-3 that is belongs to a family of animal lectins that bind beta-galactosides and is distinguished from others by the inclusion of tandem repeats in its N-terminal region. Galectin 3, like the other members, lacks a conventional signal chain, but it is secreted by a nonclassical secretory pathway and can act in an autocrine or paracrine manner extracellularly that released in a constitutive or inducible manner by nearly all immune and inflammatory cell types. The role of Galectin 3 in the modulation of these cell functions has been shown in a wide body of work especially inflammation such that Galectin-3 deficient mice have added to the evidence that this protein plays a significant role in the inflammatory response [14, 15].

Another factor that could affect inflammation is the genotype, which seems there is a relation between leptin and rs 3807992 genotypes (Caveolin 1 (CAV1)), such that leptin upregulates CAV1 expression [16]. CAV1 is a transmembrane scaffolding protein that controls essential cell functions such as proliferation, apoptosis, cell division, and transcytosis via a variety of signaling pathways and the progression of atherosclerosis and obesity. On the other hand, according to recent research CAV1 expression is increased in human obesity, suggesting that leptin can play a crucial role [17]. CAV1 indeed works in the same way as the suppressor of the cytokine signaling family of proteins, which are components of the classic negative feedback circuit [6]. They are upregulated by cytokines, and as a result, they block cytokine-induced signaling pathways in the cell. Also, some studies have shown that genetic variations in CAV1 can interfere with other risk factors, such as dietary intake [18, 19].

Diet, especially dietary patterns, plays a significant role in influencing obesity and circulating inflammatory markers in adults [8]. The Dietary Inflammatory Index (DII), developed by Shivappa et al. calculates the consumption of nutrient and non-nutrient components of the food was recently introduced to measure the inflammatory properties of the diet, thus consider as an overall picture of the inflammatory properties of the diet [20–22].

To our knowledge, there is only one report from a cross-sectional study conducted in females, which found an interaction between the DII score and CAV1 on leptin and Galectin 3. The DII must be evaluated in different demographic environments because dietary habits differ throughout societies and can have an effect on the DII quality. Furthermore, since other influences such as climate, lifestyle, and genetic history vary around population settings, the association between the DII score and inflammation status, and interaction with genotype can be influenced. Therefore, this study aimed to evaluate the variants in CAV1 (rs 3807992 genotypes) that could interact with the DII index for serum Leptin, leptin resistance, and Galectin-3 Levels in an obese and overweight Iranian population.

Method

Research design and Study population

To perform a multicenter unregulated cross-sectional study, this observational study used a multistage cluster random sampling approach. The participants in this sample were 363 healthy obese and overweight women between the ages of 18 and 48 who had a body mass index (BMI) of 25–40 at Tehran University of Medical Sciences, who were referred to urban health centers. Those with a medical history, opioid, or nicotine usage or alcohol consumption, thyroid disorder, diabetes mellitus, cardiovascular diseases (CVDs), malignancies, hepatic or renal conditions, menopause, lactation, breastfeeding, acute or chronic infections, people with weight loss diets and specific nutritional therapies, such as insulin, and cardiovascular diets, as well as weight fluctuations in recent months, and people the past three months, have been taking dietary supplements, or estimated energy intakes of more than 4200 kcal/d or less than 800 kcal/d were excluded [23]. This study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (ID number: IR.TUMS.VCR.REC 1398.142). Before the start of the clinical screening tests, all patients were required to have written and informed consent. Grant ID: 97-03-161-41017).

Anthropometric measurements

The InBody 770 scanner, a multi-frequency bioelectrical impedance analyzer, has been used to determine body composition, comprising weight, BMI, fat mass, and fat-free mass (FFM) (Inbody Co., Seoul, Korea). This electrical impedance analyzer measures the resistance of body tissue to the passage of an electrical signal emitted by both hands and feet. If the current passes more quickly through certain parts of the body, the amount and ratio of body fat-free mass and fat mass can be calculated, according to the manufacturer's instructions. Height was measured on a Seca scale stadiometer with an accuracy of 0.5 cm following standards. Waist circumference (WC) was measured in the narrowest part of the waist using a non-elastic tape with an accuracy of 0.5 cm while people were at the end of a normal exhalation. The largest part of the hip circumference (HC) was measured with an accuracy of 0.5 cm. The waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). The waist-to-height ratio (WHtR) was measured by dividing the waist circumference (cm) by the height (cm). A trained dietician places a measuring tape across the neck, beginning one inch from the point where the neck and shoulders meet, which may be the lower portion of Adam's apple, to determine neck diameter (NC). leptin resistance is measured by the following formula: leptin (ng/ml)/BMI (kg/m^2), This index assesses leptin levels when accounting for the influence of BMI [24].

Dietary Measurement*s* and EDII calculation

To measure dietary consumption in the 12 months before the report, a 147-item semi-quantitative food FFQ was administered on a regular, weekly, annual, or yearly basis, a qualified questioner interviewed participants and collected their food intake number and frequency. A 147-item semi-quantitative FFQ validity and reliability has been confirmed by previous studies [25, 26]. The servings and portion sizes reported by study subjects were converted to grams per day. Using household proportions, the portion sizes of the eaten items are translated to grams [27]. The Nutritionist IV program was then used to do a diet study (version 7; N-Squared Computing, Salem, OR, USA). EDII scores were calculated for all participants using FFQ-derived dietary data. The dietary data after energy adjustment were connected to a globally representative database that included food intake from eleven populations around the world, yielding a reliable mean and standard deviation estimation for each parameter. [21]. The "standard global mean" was subtracted from the real dietary intake level, and the result was separated by the standard deviation to get z-scores. These z-scores were then translated into percentiles, with each percentile score being doubled and then subtracted by one to reduce the impact of ‘right skewing. 'To achieve a food parameter-specific DII score for an individual, the based percentile score for each food parameter for each individual was compounded by the corresponding food parameter impact score. [21]. After that, the average DII score was calculated by adding all of the food parameter-specific DII scores together. The higher the DII score, the more pro-inflammatory the diet, the lower the score, the more anti-inflammatory the diet. [21]. The DII was determined using 29 food parameters from the FFQ (energy, starch, protein, total

fat, monounsaturated fat, polyunsaturated fat, saturated fat, omega-3, omega-6 fatty acids, cholesterol, fiber, thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, vitamin A, C, D, E and tea, onion, caffeine, iron, magnesium, selenium, zinc, and beta carotene).

Biochemistry Analysis

The Nutrition and Biochemistry Laboratory of the School of Nutritional and Dietetics at Tehran University of Medical Sciences was referred to participants in this project. After a 10–12 hour overnight quick, venous blood samples were taken. Centrifuged for 15 minutes at 3000 rpm to isolate the EDTA anticoagulant plasma and serum samples, and the remaining blood was washed three times with 0.9 g/l NaCl solution. After serum isolation, the samples were instantly frozen at -80°C for laboratory testing. Pars Azmoon laboratory kits were used to measure triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), fasting blood pressure (FBS), and insulin levels in the blood (Pars Inc, Tehran, Iran). The active form of Galectin 3 was also measured using the ELISA-Quantikine kit's enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Industrial enzyme-linked immunosorbent assay kits were used to assess serum leptin concentrations (ng/mL) (Mediagnost, Reutlingen, Germany). The homeostatic model assessment insulin resistance (HOMA-IR) was used to measure insulin resistance (mIU/ml), with the following equation: [fasting plasma glucose (mmol/l) and insulin (IU/l)] /22.5) [28]. After 15 minutes of rest, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times with a mercury sphygmomanometer.

Genotyping

Genotyping is the process of determining an individual's DNA was isolated from whole blood using a Mini Columns package to genotype the CAV1 polymorphisms (Type G; Genall; Exgene). CAV1 polymorphisms (rs3807992) in gene fragments were investigated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (major allele G and minor allele A). The following primers were used for PCR: F:3'AGTATTGACCTGATTTGCCATG5' R:5'GTCTTCTGGAAAAAGCACATGA-3' In a DNA thermocycler, PCR reactions were carried out in a volume of 20 µl, comprising 1 l isolated DNA, 1 µl Forward primers, 1 µl Reverse primers, 7 µl purified water, and 10 µl Taq DNA Polymerase Master Mix. The DNA templates were denatured for 3 minutes at 94 degrees Celsius, followed by 40 cycles of denaturation at 94 degrees Celsius, annealing at 42–50 degrees Celsius, and elongation at 72 degrees Celsius for 2 minutes. Amplified DNA was digested overnight at 37°C with Hin1II (NlaIII) restriction enzyme, then isolated on an agarose gel by electrophoresis (2%). Uncut homozygous AA (213bp), cut heterozygous GA (3 bands: 118, 95, and 213 bp), and cut homozygous GG genotypes of the CAV1 rs3807992 variant were identified (2 bands: 118 & 95 bp).

Other Covariates Assessment

Participants' physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), and its validity and reliability were verified. This questionnaire contains seven questions, each of which has two sections (number of exercises per week and length) that indicate the participants' level of physical activity [29]. Other demographic characteristics such as age, educational level, marital status, and income were collected using standard questionnaires were completed by interview.

Statistical Analysis

The Kolmogorov-Smirnov method was conducted to test the data's normality ($p > 0.05$). The Hardy-Weinberg Equilibrium deviation among CAV1, G32124A allele frequencies were determined using Pearson's chi-square test. The discrepancies between the two groups of the median of EDII and genotype according to risk allele were assessed using an independent sample t-test, Chi-square test expressed as mean and standard error (SE) and for categorical variables as numbers and percentages respectively. Confounding removing results were assessed using an analysis of covariance (ANCOVA) test. Linear regression has been used for the association between leptin, leptin resistance, and Galectin 3 with EDII and genotypes that present by B and 95% confidence interval (CI). Generalized linear model (GLM) applied for an estimate of interaction between EDII and Genotype on leptin, leptin resistance, and Galectin 3. SPSS v.25 program (SPSS Inc., IL, USA) was used for statistical analysis, and the significance level was set at a P-value of 0.05 and P-value 0.05, 0.06, and 0.07 consider as marginally significant.

Result

Study Population Characteristics

General characteristics of participants, such as body composition, biochemical assessment, and others **among** lower vs higher than the median of EDII and genotypes, are presented in **Table 1**. A total of 363 women with BMI mean and SD 30.9 (3.90) kg/m^2 were divided into two groups, based on EDII median (0.07) lower (n=172) and upper than (n=191) median. The range of EDII was -3.83 to 3.19. About 70.8% of the study population were married. The level of leptin in individual's serum had 27.7 (11.8) ng/mL, and 4.02 (7.26) ng/mL of Galectin3 (**Table1**).

Association between population characteristics across rs 3807992 genotypes and median EDII score

Association between population characteristics across rs 3807992 genotypes and median EDII score have shown in **Table1**. Age of starting obesity and history of losing weight was higher in upper than median group of EDII, after controlling potential confounder including age, BMI, energy intake, and physical activity, there was a marginally significant mean difference among median of EDII (P=0.05). in the crude model significant mean differences were found for physical activity, starting obesity age (P<0.05), in body composition and biochemical variables in terms of fat-free mass (FFM), skeletal muscle mass (SMM), soft lean mass (SLM), fat-free mass index (FFMI), and HDL, there have shown significant mean difference (P<0.05). Categorical variables such as economic, and education status were significant differences across the median of EDII (P<0.05), moreover all the mentioned factors had a higher mean in the lower median of EDII. Also, EDII associated with body fat mass (BFM) (P=0.05) and BFM (%) (P=0.01), bone mineral content (BMC) (P=0.04), trunk fat (P=0.01), visceral fat (P=0.03), fat mass index (FMI) (P=0.01), and marginal significant for TG (P=0.06) after adjustment in top of EDII median was higher mean of all the mentioned variables.

Association between population characteristics among genotype category

Additionally, subjects were divided into two groups according to risk alleles of CAV1 genotypes: GG (n=75) without risk alleles and AA+AG with risk alleles (n=198). A marginal significant means difference was found in visceral fat (P=0.05) and FMI (P=0.07) and HC (P=0.06) among CAV1 genotypes category in the crude model. There was a significant difference was found for TG (P=0.04) after adjustment among genotype category groups. Also, a significant mean difference for HDL remained stable at its substantial differences (P=0.04) (**Table1**).

Galectin-3, leptin, and leptin resistance across rs 3807992 genotypes and median EDII score

Although leptin was a significant mean difference among the EDII median (P=0.03), after further controlling with economic status and education, starting obesity age, there was no significant mean difference for leptin resistance (P=0.21). Moreover, a marginal significant mean difference was found for Galectin 3 (P=0.06) (**Table 2**). The other variables were not any significant mean difference among median of EDII (P>0.05). Food group intake of study population among EDII category have shown in **Figure 2**.

The association between EDII score and with the leptin, leptin resistance and Galectin3

The association between EDII score and rs 3807992 genotypes with the leptin, leptin resistance, and Galactin3 were presented in **Table 3**. Increased adherence to EDII leads to an increase of 16.73 ng/mL in leptin level (β= 16.73, 95% CI= 1.56, 39.3, P= 0.04) and 0.55 in leptin resistance (β=0.55, 95% CI=0.00, 1.30, P= 0.06) in model 2 which was adjusted for, economic status, education level, age of starting obesity, losing weight history. By contrast, increased adherence to EDII in the association with that genotype showed no significant association in Galectin3 in model 2 adjustment (β= 0.91, 95% CI= -0.64, 2.48, P=0.24) (**Table 3**).

The association between rs 3807992 genotypes with the leptin, leptin resistance and Galectin3

After adjustment, there were not any significant association between CAV1 genotypes with a risk allele in the association with leptin (β=2.53, 95%CI= -3.42,8.48, P= 0.39), leptin resistance (β=0.08, 95%CI= 0.00,0.28, P=0.08) and Galectin 3 (β=1.08, 95%CI= -3.40,5.58, P=0.62) **(Table 3).**

The interactions between adherence of EDII across rs 3807992 genotypes on the leptin, leptin resistance, and Galectin3

The interactions between adherence of EDII across rs 3807992 genotypes on leptin, leptin resistance, and Galectin 3 were presented in **Table 4**. A marginal positive interaction was observed between EDII and risk alleles group (AA+AG) genotype in model 2 further adjustment by removing, economic status, education level more as confounders on leptin level (β=18.84, CI=3.25,65.33 , P=0.06)

(**Figure 1**). Increased adherence to EDII in the interaction with CAV1 genotypes containing risk alleles (AA+AG) leads to an increase in leptin level 79.15 ng/ml (β= 79.15, CI= -1.23,163.94, P= 0.04) (**Figure 1**) in model 3 after controlling for further potential confounders (age, BMI, total energy intake, and physical activity, economic status, education level, age of starting obesity, history of losing weight). By contrast, adherence to EDII in the interaction with that genotype which includes risk alleles (AA+AG) showed no significant interaction on leptin resistance not in the crude model (β=0.57, CI=-0.36,1.51, P=0.22), and even after adjustment in model 3 (β=0.55, CI= -0.99,2.09, P=0.48). Although no significant interaction was found between EDII and CAV1 genotypes on Galectin 3 in the crude model, there was a marginal positive interaction after adjustment in model 3 (β=31.35, CI=.013,77.13, P= 0.05) (**Figure 1**) (**Table 3**).

Discussion

To the best of our knowledge, the present study is the first cross-sectional study to investigate the interaction of EDII and CAV1 genotypes on leptin, leptin resistance, and Galectin 3 as outcomes. We found that after taking into account confounding variables increased adherence to EDII in the association with leptin level and leptin resistance leads to an increase in both of them. Also, our results suggest that EDII interacts positively with CAV1 genotypes including risk alleles (AA + AG) on leptin level and Galectin 3.

EDII was associated with leptin, although that seems it was not associated with leptin resistance. Similar to our study Freitag et al. showed that the DII score was positively associated with plasma leptin concentration [30] it is in line with a cross-sectional study in the USA that the DII score was not related to leptin [31]. Leptin is considered a cytokine that is created by adipocytes and it leads to induce inflammation. The pro-inflammatory properties of leptin have been proposed to be alike to those of immune cell-derived cytokines including interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [32, 33]. According to an observational study among children, exposure to a more pro-inflammatory diet in boys and girls is associated with an increase in leptin concentrations [34]. In a review study, it is illustrated that leptin resistance may depend on the variety of nutrients consumed from the diet, supporting the hypothesis of the vital role of an accurate distribution in the dietary pattern. Although recent studies are contributing to recognizing the mechanisms bringing to this impaired response to leptin, desensitization of leptin receptor, down-regulation of its intracellular signaling and inflammation seem as key routes involved [35]. The DII not only is included micronutrients and macronutrients, but also includes generally intake bioactive components containing flavonoids, spices, and tea. The whole score takes into account the complete diet, not just separate nutrients or foods [21]. Thus, inflammatory factors are affected by bioactive components [36].

The present study demonstrates that increased adherence to EDII in the association leptin and leptin resistance leads to an increase in leptin level and leptin resistance; however, there was not such a result for Galectin 3. CAV1 is considered a key factor with cellular functions including pinocytosis and regulation of cell signaling [37]. Singh et al. present a feedback loop of CAV1 mediated downregulation of leptin signaling [16]. The significant and novel findings pointed out that there is a strong association between leptin and CAV1 so it contributes to raising CAV1 expression that impairs the signaling of leptin. Also, the mechanisms bringing to these impacts need to be ascertained [38]. In line with our finding Schroeter et al. found that Caveolin deficiency was associated with the lack of leptin *in vivo* [39]. In this study we found no association between caveolin with leptin, leptin resistance, and galectin 3; by contrast, some studies demonstrate that galectin3 induces integrin function and Caveolin phosphorylation so it shows that Galectin 3 was related to Caveolin [40, 41].

We found that taking into account confounding variables increased adherence to EDII in the interaction with CAV1 genotype including risk alleles (AA + AG) leads to a positive interaction on leptin level and Galectin 3. Inflammatory stimuli can lead to raised Caveolin expression by inhibiting upstream regulators of antioxidant defense enzymes; Caveolin expression can further intensify the inflammatory reaction. Caveolae nutritional modulation may apply an opportunity to upset inflammatory signaling events. DII has strong anti-inflammatory effects and is accomplished by modifying the lipid raft microenvironment [42].

The major strength of this investigation is that it is the first study to evaluate the interaction of EDII and CAV1 genotypes on leptin, leptin resistance, and Galectin 3. These interactions remained significant for multiple testing, despite the high correlations among the outcomes of interest. Additionally, demographic characteristics were measured by the trained dietitian and FFQ was filled by them. Moreover, this knowledge may be applied to clinical practice and contribute to personalized therapies for the prevention and treatment of metabolic disorders.

Limitations of this investigation include the use of cross-sectional design. Longitudinal epidemiologic studies and biochemical experimentally based research are required to elucidate and strengthen the findings from this study. Furthermore, whether this interaction relates to changes in CAV1 genotypes has not been explored. Besides, the pathway linking dietary inflammatory index to

this CAV1 gene is unknown. Also, FFQ was used for recording the subject's food which is reliable to memory. Although the FFQ is typically used to examine long-term dietary consumptions, its closed structure with limited response options limits its ability to determine between-person variations so there is some misclassification. Moreover, the small sample size of the present study should be taken into consideration.

Conclusion

To sum up we found likely there are positive interaction between EDII and CAV1 genotypes on leptin and Galectin3, while no interaction was found in case of leptin resistance. Nevertheless, prospective study of the interaction between EDII and CAV1 genotypes should be a priority in further study of this interaction.

Abbreviations

NCDs: non-communicable diseases; CAV1: caveolin1; DII: Dietary Inflammatory Index; CVDs: cardiovascular diseases; BMI: body mass index; FFM: fat-free mass; WC: Waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; NC: neck diameter; EDII energy-adjusted DII; FFQ: food frequency questionnaire; TG: triglyceride; TC: total cholesterol, HDL: high-density lipoprotein; LDL: low-density lipoprotein; FBS: fasting blood pressure; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; ELISA: enzyme-linked immunosorbent assay; HOMA: homeostatic model assessment; SBP: systolic blood pressure; and DBP: diastolic blood pressure; IPAQ: International Physical Activity Questionnaire; SD: standard deviation; ANCOVA: analysis of covariance; SMM: skeletal muscle mass, SLM: soft lean mass, ECW: extracellular water, ICW: intracellular water, FFMI: fat-free mass index; IL-6: interleukin 6; TNF-α: tumor necrosis factor-alpha.

Declarations

Sources of Support: Tehran University of Medical Sciences

Conflicts of Interest: None

Ethics approval and consent to participate

Ethics approval for the study protocol was confirmed by The Human Ethics Committee of Tehran University of Medical Sciences (Ethics Number: IR TUMS. VCR REC. 1398.142). Each participant was completely informed about the study protocol and provided a written and informed consent form before taking part in the study. This study was approved by the research council. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Each participant was completely informed about the study protocol and provided a written and informed consent form before taking part in the study.

Availability of data and materials

The data that support the findings of this study are available from correspond author but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of correspond author.

Competing interests

All authors declared that they have no competing interests

Funding

This study is funded by grants from the Tehran University of Medical Sciences (TUMS). (Grant ID: 97-03-161-41017).

Author's contributions

FSH, MF and NR wrote the paper, KhM had full access to all of the data in the study and took responsibility for the integrity and accuracy of the data. All authors read and approved the final manuscript.

Acknowledgments

We are grateful to all of the participants for their contribution to this research. This study was supported by grants from the Tehran University of Medical Sciences, Tehran, Iran.

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Tables

Table 1. Characteristics of the study population across rs 3807992 genotypes and median of EDII score in obese and overweight women (n=363)

Variables	EDII median		P-value	p-value*	rs 3807992 genotypes		p-value	p-value*
	<0.07	>=0.07			GG	AA+AG		
	N=172	N=191			N=75	N=198		
	Mean±SE				Mean±SE			
Age (years)	36.74±0.79	35.67±0.78	0.33	0.08	36.33±0.89	35.58±1.08	0.40	0.59
PA (MET-minutes/week)	1544.81±208.08	205.33±205.33	**0.007**	**0.04**	1062.62±193.41	947.60±234.38	0.64	0.70
Age of starting obesity	20.97±0.88	23.31±0.81	**0.10**	**0.05**	22.46±0.84	22.38±0.89	0.08	0.94
Anthropometric variables								
Weight (kg)	79.76±0.90	78.49±0.95	**0.05**	0.35	78.17±0.70	76.51±0.81	0.71	0.23
Height (cm)	161.84±0.84	160.60±0.93	0.68	0.35	161.41±6.27	160.12±5.97	0.24	0.56
BMI (kg/m^2)	30.60±0.57	30.00±0.63	0.43	0.49	29.61±0.39	30.51±0.48	0.11	0.12
Body composition								
WC (cm)	94.45±1.18	93.23±1.25	0.26	0.49	96.66±0.64	153.90±3.47	0.13	0.52
HC (cm)	112.08±0.66	113.51±0.70	0.60	0.15	104.56±0.20	103.97±0.24	0.35	**0.06**
NC (cm)	36.26±0.36	36.93±0.39	0.54	0.22	36.46±0.27	36.64±0.32	0.30	0.44
WHR	0.92±0.00	0.93±0.00	0.11	0.24	0.921±0.00	0.926±0.00	0.12	0.23
WHtR	0.58±0.00	0.58±0.00	0.14	0.78	0.59±0.00	0.60±0.00	0.04	0.16
BFM (kg)	31.91±0.38	33.06±0.40	0.27	**0.05**	31.70±0.31	31.84±0.36	0.10	0.64
BFM (%)	41.14±4.99	42.22±5.71	0.91	**0.01**	40.31±0.40	40.79±0.46	0.10	0.71
FFM (kg)	47.87±0.85	45.46±0.90	**0.02**	**0.05**	46.32±0.56	45.34±0.65	0.68	0.52
SMM (kg)	26.30±0.50	24.91±0.53	**0.02**	**0.06**	25.41 ±0.33	24.78±0.38	0.64	0.46
SLM (kg)	45.10±0.79	42.85±0.84	**0.02**	**0.06**	43.64±0.52	42.76±0.61	0.73	0.55
BMC (kg)	2.68±0.36	2.61±0.32	0.09	**0.04**	2.67±0.03	2.59±0.04	0.33	0.35
Trunk fat(kg)	15.77±0.18	17.29±0.19	0.26	**0.01**	15.16±0.14	15.62±0.16	0.08	0.47
Visceral fat (kg)	14.62±0.29	15.54±0.31	0.69	**0.03**	14.58±0.20	14.91±0.23	**0.05**	0.25
FFMI	18.21±0.16	17.58±0.17	**0.004**	**0.01**	17.76±0.13	17.60±0.11	0.78	0.52
FMI	12.21±0.16	12.85±0.17	0.35	**0.01**	12.23±0.11	12.38±0.13	**0.07**	0.55
Biochemical variables								
FBS (mg/dL)	84.88±1.55	86.85±1.64	0.33	0.40	86.94±1.01	86.85±1.17	0.97	0.86
TC (mg/dL)	179.83±5.49	174.49±5.83	0.75	0.51	180.44±3.35	172.32±3.88	0.18	0.17
TG (mg/dL)	123.11±12.10	132.78±12.85	0.65	**0.06**	110.96±6.7	130.53±7.85	0.08	**0.04**
HDL (mg/dL)	47.66±1.58	43.47±1.67	**0.04**	**0.07**	48.08±1.04	45.20±1.20	**0.02**	**0.04**
LDL (mg/dL)	99.60±3.92	92.87±4.16	0.44	0.25	95.83±2.37	93.55±2.75	0.23	0.20
Categorical variables								
Economic status								
Low level	51(62.2)	31(37.8)	**0.01**	**0.01**	28(32.6)	58(67.4)	0.31	0.20

Moderate level		72(43.4)	94(56.6)			46(26.3)	129(73.7)			
High level		44(43.6)	57(56.4)			24(22.9)	81(77.1)			
Education level										
Illiterate		3(75)	1(25)	**0.004**	**0.001**	1(25)	3(75)	0.11		0.20
Under diploma		26(55.3)	21(44.7)			16(33.3)	32(66.7)			
Diploma		78(56.1)	61(43.9)			45(30.8)	101(69.2)			
Master and higher		65(38)	106(62)			37(20.6)	143(79.4)			
Marital status										
Single		124(48.1)	134(51.9)	0.80	0.91	28(26.2)	79(73.8)		0.99	0.86
Married		48(46.6)	55(53.4)			71(26.2)	200(73.8)			
Losing weight history	yes	66(44)	84(56)	**0.01**	**0.01**	97(51.9)	90(48.1)		0.64	0.34
	no	76(57.1)	57(42.9)			79(49.4)	81(50.6)			
rs 3807992 genotype										
AA+AG		89(44.9)	109(55.1)	0.10	0.15					
GG		42(56.0)	33(44.0)							

EDII: energy-adjusted dietary inflammatory index, BMI: body mass index; WC: waist circumference, HC: hip circumference, NC: Neck circumference, WHR: waist-hip ratio, WHtR: waist-hight ratio, BFM: body fat mass, FFM: fat-free mass, SMM: skeletal muscle mass, SLM: soft lean mass, BMC: Bone mineral content, ECW: Extracellular water, ICW: intracellular water, FFMI: fat-free mass index, FMI: fat mass index, FBS: fasting blood glucose, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

P-value: obtain from ANOVA.

P-value *: obtain from ANCOVA; adjusted for age, physical activity, total energy intake, and BMI.

Continuous variables showed a MEAN± standard error (SE), categorical variables showed as the number and (%).

P<0.05 consider as significant, P=0.06, and 0.07 consider as marginally significant.

Table 2. Galectin-3, leptin, and leptin resistance across rs 3807992 genotypes and median EDII score in obese and overweight women (n=363)

Variables	EDII median		P-value	p-value*	rs 3807992 genotypes		p-value	p-value*
	<0.07	>=0.07			GG	AA+AG		
	N=172	N=191			N=75	N=198		
	Mean±SE				Mean±SE			
Leptin (ng/ml)	27.13±3.45	29.87±3.01	0.64	**0.03**	30.38±2.82	26.69±3.69	0.52	0.40
Leptin resistance	0.81±0.05	0.91±0.05	0.17	0.21	0.81±0.06	0.90±0.05	0.72	0.31
Galectin-3 (ng/ml)	4.01±1.88	4.99±2.05	0.67	**0.06**	4.86±1.43	2.73±1.34	0.12	0.29

EDII: energy-adjusted dietary inflammatory index, SE: standard error

P-value: obtain from ANOVA.

P-value *: obtain from ANCOVA; adjusted for age, physical activity, total energy intake, BMI, economic status and education, age of starting obesity.

P<0.05 consider as significant, P=0.06, and 0.07 consider as marginally significant.

Table 3. The association between EDII across and rs 3807992 genotypes on the leptin, and Galectin3 in obese and overweight (n=363)

Variables	Models	EDII score		P-value^*	rs 3807992 genotypes		P-value^*
		β	95% CI		β	95% CI	
Leptin (ng/ml)	**Crude**	2.34	-22.52,27.21	0.85^	1.32	-3.73,6.38	0.60
	Model1	14.03	-8.57,36.65	0.22*	-0.71	-5.4,3.91	0.76
	Model2	16.73	1.56,39.32	**0.04***	2.53	-3.42,8.48	0.39
Leptin resistance	**Crude**	0.57	-0.36,1.51	0.22^	0.02	-1.24,0.17	0.72
	Model1	0.49	-0.24,1.23	0.18*	-0.01	-0.16,0.13	0.85
	Model2	0.55	0.00,1.30	**0.06***	0.08	0.00,0.28	0.08
Galectin3 (ng/ml)	**Crude**	21.45	-19.47,62.37	0.30^	-0.80	-3.93,2.31	0.60
	Model1	0.82	-0.59,2.25	0.25*	0.06	-3.84,3.97	0.97
	Model2	0.91	-0.64,2.48	0.24*	1.08	-3.40,5.58	0.62

EDII: energy-adjusted dietary inflammatory index

Model 1: additionally adjusted for age, BMI, total energy intake, and physical activity.

Model 2: additionally adjusted for, economic status, education level, age of starting obesity, losing weight history

^ Significant level in the crude model

*Significant level after adjustment by Model1, 2

P<0.05 consider as significant, P=0.06, and 0.07 consider as marginally significant.

Table 4. the interactions between adherence of EDII across rs 3807992 genotypes on the leptin, leptin resistance, and Galectin3 score in obese and overweight women (n=363)

Variables	Models	EDII adherence * AA+AG		
		B	95% CI	P-value
Leptin (ng/ml)	**Crude**	14.73	-41.06,70.54	0.60
	Model1	30.64	15.03,75.13	0.05
	Model2	18.84	3.25,65.33	**0.06**
	Model3	79.18	1.23,163.94	**0.04**
Leptin resistance	**Crude**	0.57	-0.36,1.51	**0.22**
	Model1	0.03	-1.94,2.01	**0.97**
	Model2	0.92	2.40,1.51	**0.45**
	Model3	0.55	-0.99,2.09	**0.48**
Galectin3 (ng/ml)	**Crude**	21.45	-19.47,62.37	0.30
	Model1	24.16	-25.04,73.38	0.33
	Model2	0.15	0.01,1.03	0.07
	Model3	31.35	.013,77.13	**0.05**

EDII: energy-adjusted dietary inflammatory index

Model 1: additionally adjusted for age, BMI, total energy intake, and physical activity.

Model 2: additionally adjusted for, economic status, education level

Model3: further adjustment with the age of starting obesity, history of losing weight

GG genotype has 0 risk allele, AG genotype has one and AA genotype have two risk allele.

GG genotype is considered as a reference group

*Significant level in the crude model and after adjustment by Model1, 2, and 3.

P<0.05 consider as significant, P=0.06, and 0.07 consider as marginally significant.

Figures

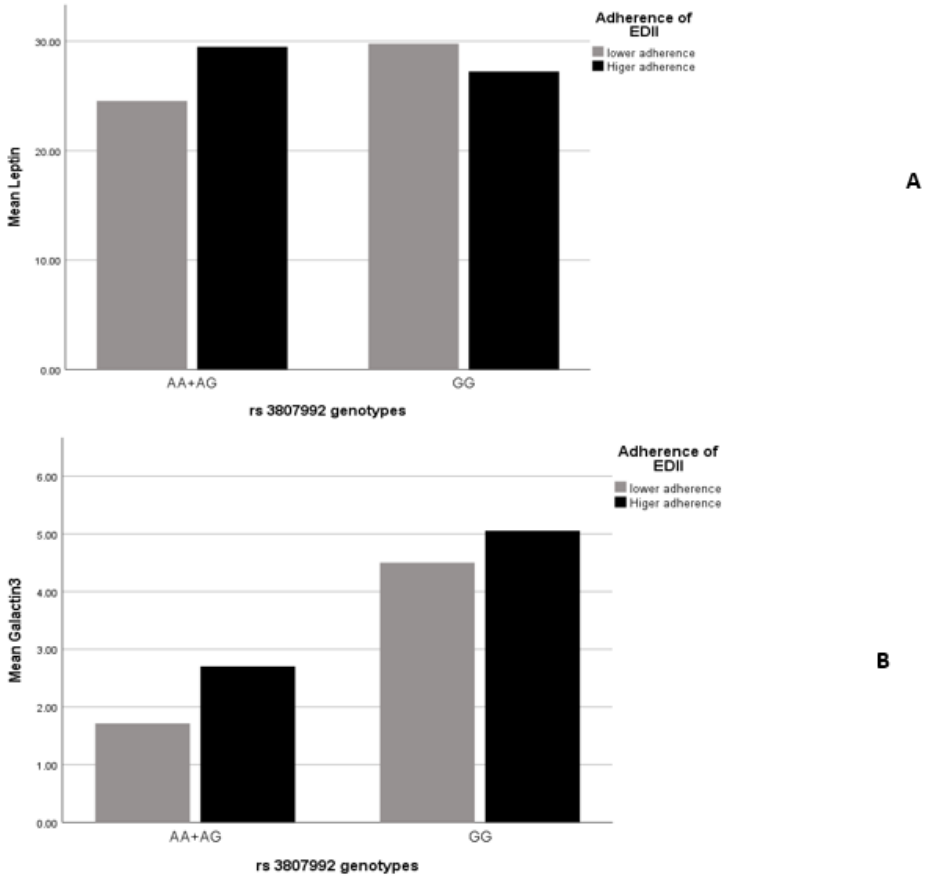

Figure 1

Interaction between rs 3807992 genotypes (GG consider as the reference group) with EDII on leptin and Galectin3 (A, B respectively).

(A) Leptin (The P-value for AG+AA genotype: 0.02; P-value for adherence of EDII: 0.05; P-value for interaction between AG+AA genotype and Leptin: 0.65).

(B) Galectin3 (The P-value for AG+AA genotype: 0.31; P-value for adherence of EDII: 0.02; P-value for interaction between AG+AA genotype and Galectin3:0.24)

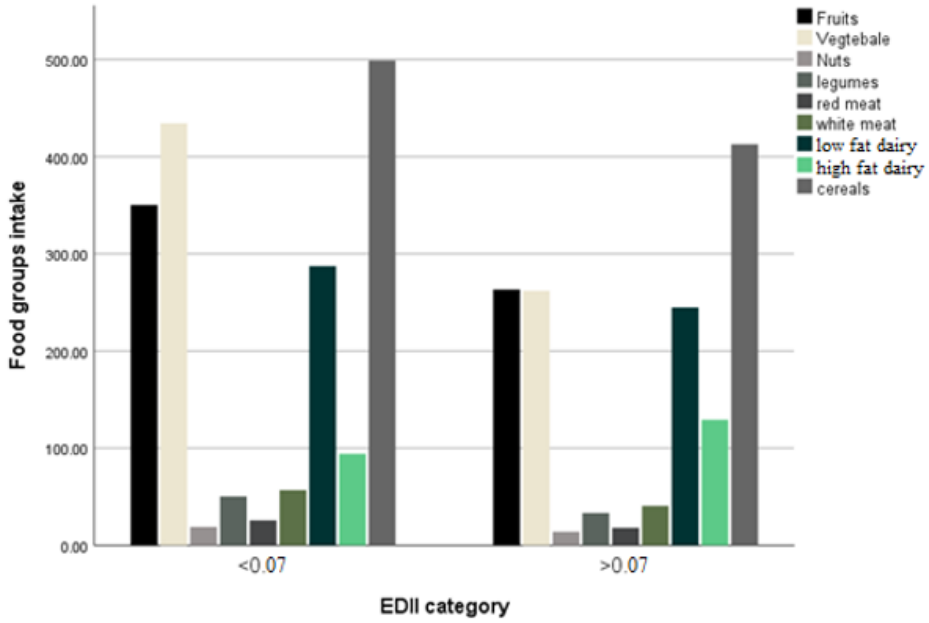

Figure 2

Food groups intakes among EDII categories in obese and overweight women.