

Habitual Dietary Intakes of Fat were Associated with Apelin Gene Expression in Visceral and Subcutaneous Adipose Tissues

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Abstract

Background: The aim of the present study was to investigate the association of habitual intake of total fatty acids, saturated-, monounsaturated-, polyunsaturated fatty acids, n-3, n-6, and n-9 fatty acids with apelin gene expression in visceral and subcutaneous adipose tissue.

Methods: We obtained visceral and subcutaneous adipose tissues from 179 participants (71 non-obese and 105 obese), who had undergone open abdominal surgery. Dietary intake information was gathered with a valid and reliable food frequency questionnaire. The mRNA expression of apelin gene was analyzed by Real-Time PCR.

Results: Apelin gene expression was found to be more increased in subcutaneous and visceral adipose tissues in obese than in non-obese participants. Dietary intake of n-3 and polyunsaturated fatty acids was associated with apelin gene expression in subcutaneous and visceral adipose tissues among all categories of weight status after adjusting for total energy intake. Among obese individuals, visceral adipose tissue apelin mRNA levels were associated with total fat intake.

Conclusion: Higher apelin gene expression in adipocytes had an association with habitual intake of total fat and n-3 fatty acids in obese and non-obese individuals, indicating a determinative role of quality and quantity of fatty acid intake in a regular diet in adipose tissue adipokine.

Background

Obesity, a major public health problem, is considered one of the important risk factors for the incidence of insulin resistance (1). Accumulation of excess fat induced by obesity may affect the metabolism of glucose and lipids. It is well known that adipose tissue is not only the largest site reservoir for fats but also acts as an endocrine organ producing and secreting a variety of adipokines that induce insulin sensitivity both locally and in other organs (2).

Apelin, an endogenous ligand of APJ, which is a G-protein-coupled receptor, in humans, is transcribed by a gene on chromosome Xq25–26.1 (3). The apelin/APJ pathway has several physiological roles, including glucose and lipid metabolism, cardiovascular activity, cell growth, apoptosis, fluid homeostasis, and immune response⁽⁴⁾. After injecting apelin, glucose metabolism improves due to increased glucose utilization in adipose tissue^(4, 5). Consequently, it has been suggested that higher expression of the apelin gene and over-production of apelin could be one of the last protective defenses before the development of obesity-related disorders, including insulin resistance, type 2 diabetes, and hypertension (6–8).

Among energy-supplying nutrients, fatty acid intakes can modify metabolic responses in adipocytes (9–11). Notably in this context, in a recent systematic review, we described that despite the controversy existing in the findings of related studies, apelin concentrations might be decreased by hypocaloric diets in humans and apelin gene expression and concentrations may be increased by total fatty acids and

eicosapentaenoic acid (EPA)(12) in animals. Furthermore, apelin gene expression in adipose tissues of maybe increased by high-fat diets (13–15).

In the present study, we aimed to investigate the association of dietary intake of fatty acids (total fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 fatty acids, n-6 fatty acids, and n-9 fatty acids) with apelin gene expression in visceral and subcutaneous adipose tissues among non-obese and obese, adults without diabetes.

Methods

Subjects

For the current cross-sectional study, we selected 179 participants (71 non-obese ($18.5 < \text{BMI} \leq 30 \text{ kg/m}^2$) and 105 obese ($\text{BMI} > 30 \text{ kg/m}^2$) participants), who were admitted for elective abdominal surgery at the hospitals in Tehran, Iran. We selected individuals who were hospitalized less than 3 days, free of diabetes mellitus or cancer, not taking lipid-lowering drugs, not pregnant or lactating women, and not on special diets. Subcutaneous and visceral adipose tissues were collected during the surgery (approximately 100 mg). All questionnaires and other measures were completed before surgery.

Dietary Measurements

A valid and reliable semi-quantitative food frequency questionnaire (FFQ) was used, and an expert during face-to-face interviews assessed habitual dietary intake. A trained dietitian asked participants to determine the frequency of each food item that was consumed during the previous year on a daily, weekly, and monthly basis. Overall, the FFQ provides reasonably valid measures of the average long-term dietary intake (16). Fatty acids and other micro and micronutrients were calculated from the United States Department of Agriculture (USDA) FCT, and for traditional foods, Iranian FCT was used (17, 18). In the current 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, n-9 fatty acids, and cholesterol.

Quantitative real-time polymerase chain reaction analysis of gene expression

Total RNA was extracted via the RNX-plus solution kit (Cinnagen, Iran) according to the recommended protocol. The quality of the extracted RNA was evaluated by the NanoDrop spectrophotometer (Thermo Fisher Scientific, America), and the ratios of absorption (260/280 nm) of all samples were collected. We eliminated residual genomic DNA with the treatment of mixture by DNase. Fermentas kits (Thermo Scientific, America) were applied to synthesize complementary DNA (cDNA).

In the current study, the GAPDH gene expression was considered as a reference gene. The following primer sequences were used: Apelin Forward: 5'-GCCCATCACCAGCCATTCTTG-3';

Apelin Reverse: 5'-GGGCATCAGGCTCTTGTCTTCTCT-3';

GAPDH Forward: 5'-CTGCTCCTCCTGTTTCGACAGT-3';

GAPDH Reverse: 5'-CCGTTGACTCCGACCTTCAC-3'

To evaluate the efficiency of primers, five-fold serial dilutions of a cDNA, which contained transcripts from both apelin and GAPDH (20, 40, 80, and 100% of the main sample) were prepared, and the efficiency was 0.9.

In the current study, mRNA levels of considered genes were measured by Rotor-Gene 6000 (Sydney, Australia). The quantitative PCR (qPCR) was performed in a microtube containing 12.5µL 2X SYBR Green (Thermo Scientific, USA) along with 0.3µL forward and 0.3µL reverse primers, 8.9 µL RNase-free water, and 3µL of the cDNA. Thermal cycling was performed for qPCR amplification followed by 40 cycles for annealing, amplification, and quantification. All samples were run in duplicate, and the threshold cycle (Ct) were normalized to mRNA levels of the reference gene (GAPDH)

Anthropometric and laboratory measurements

In the present study, we measured weight and height in the precision of 0.1 kg and 0.1 cm, respectively. Moreover, by dividing the weight (kg) by the height (m) squared, the body mass index (BMI) was calculated. Waist circumference (WC) was determined and reported to the nearest 0.5 cm.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed twice, and the average was considered. Before the surgery, 5 mL of blood (after 10–12 h fasting) was obtained from all participants. In order to measure fasting plasma glucose (FPG), the glucose oxidase method was applied. Triglyceride and total cholesterol (TC) were measured by enzymatic methods. Intra- and inter assays coefficients of variation being < 1% and < 2.1%, respectively. Commercial kits, Pars Azmoon Co. (Tehran, Iran), were used to measure FPG, TG, and TC. Furthermore, the insulin level was determined by the ELISA method using Mercodia kits (Uppsala, Sweden). The apelin plasma levels were determined by the ELISA assay kit (ZellBio, Ulm, Germany). Inter- and intra-assays CVs were 1.7 to 2.3 and 1.9% for both, respectively.

Statistical analysis

The normality distributions of variables were checked by Kolmogorov–Smirnov tests and histogram with the normal curve. Continuous variables are described as mean ± standard deviation (SD) or median (interquartile 25–75) based on the distributions of variables. Because plasma TGs, insulin, and apelin were skewed, log transformation was used. Total dietary fat intake and its subtypes were energy-adjusted by the residual method (19). To determine the association of energy-adjusted TFA and its subtypes with apelin gene expression in subcutaneous and visceral adipose tissue, linear regression was performed, and age and BMI were adjusted in the model. Analyses were performed using Statistical Package for the Social Sciences program (SPSS) (version 15.0; SPSS Inc, Chicago IL), and the null hypothesis was rejected in each statistical test when *P*-values < 0.05.

Results

The mean age of the total sample studied was 41.3 years, being 47.1 and 37.1 years for non-obese and obese, respectively; their mean body mass index (BMI) was 36.13 kg/m² (24.6 and 43.0 kg/m² for non-obese and obese, respectively). Of the total sample (n = 179), 75% of participants were women. Mean TFA intake was 31.6% of total energy intake (107 g/d) in the total population. There was no significant difference between non-obese and obese participants regarding gene expression of apelin in visceral and subcutaneous adipose tissues (Fig. 1).

The characteristics of subjects were shown in Table 1; no significant difference was seen between groups in plasma levels of triglycerides and HDL-C. Non-obese participants had significantly lower mean FBS and insulin concentration than obese ones.

Table 1
General characteristics of study participants according to presence of non-obese and obesity

	Non-obese (n = 32)	Obese (n = 15)	P value
Age ^b (years)	47.1 ± 14.7	37.1 ± 10.10.6	< 0.001
Body mass index ^{a,b,c} (kg/m ²)	24.6 ± 2.8	43.0 ± 6.1	< 0.001
HDL-Cholesterol ^{a,c} (mg/dl)	55.8 ± 19.5	51.1 ± 16.0	0.080
Triglycerides (mg/dl)	79.0 (65.0-136.0)	109.0(71.5–153.0)	0.106
Fasting plasma glucose (mg/dl)	85.1 ± 19.4	91.3 ± 17.9	0.032
Insulin (µU/mL)	8.2 ± 10.9	15.1 ± 9.8	< 0.001
Data are represented as mean ± SD or median (IQ 25–75)			

Dietary intakes of subjects were presented in Table 2. The mean total energy intake in non-obese individuals was lower than obese ones. Non-obese subjects also had significantly lower TFA, SFA, n-3 fatty acids, and n-6 fatty acids intakes and higher consumption of carbohydrate than these obese counterparts.

Table 2

Dietary intakes of study subjects according to the non-obese, obese, and morbidly obese participants

	Non-obese (n = 32)	Obese (n = 15)	P value
Total energy intake ^{a,b} (kcal)	2424 ± 747	3190 ± 1034	< 0.001
Carbohydrate ^{b,c} (% energy)	58.9 ± 6.6	55.9 ± 6.9	0.005
Protein (% energy)	14.2 ± 1.8	14.2 ± 2.5	0.995
TFA ^b (% energy)	29.4 ± 5.9	32.3 ± 5.9	0.002
SFA (% energy)	9.2 ± 2.6	9.9 ± 2.6	0.059
MUFA (% energy)	9.9 ± 2.0	10.5 ± 2.4	0.054
PUFA ^{b,c} (% energy)	6.0 ± 1.6	6.4 ± 1.7	0.131
n-3 fatty acids ^b (g/d)	1.8 ± 0.6	2.7 ± 0.9	< 0.001
n-6 fatty acids ^b (g/d)	12.7 ± 5.7	19.5 ± 9.2	< 0.001
TFA, total fatty acids; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.			
Data are represented as mean ± standard deviation.			

Associations of apelin gene expression in visceral and subcutaneous adipose tissue with energy-adjusted TFA and its subtypes (residual method) after controlling for age and BMI are presented in Tables 3. Based on weight status, non-obese participants showed that high subcutaneous and visceral adipose tissue apelin gene expression was significantly associated with high intakes of PUFA and n-3 fatty acids. Moreover, in obese subjects, it was observed that high apelin mRNA in both visceral and subcutaneous fat depots was associated with high intakes of TFA, PUFA, and n-3 fatty acids.

Table 3
Regression coefficients of total fatty acids and its subtype intakes with apelin gene expression in non-obese and obese participants after adjustment for age and BMI

	Non-obese (n = 32)		Obese (n = 32)	
	Standardized β	P value	Standardized β	P value
Total fatty acids				
Visceral	0.071	0.740	0.579	< 0.001
Subcutaneous	0.174	0.354	0.658	< 0.001
Saturated fatty acids				
Visceral	0.098	0.610	0.105	0.561
Subcutaneous	0.255	0.121	0.116	0.554
Monounsaturated fatty acids				
Visceral	0.131	0.495	0.256	0.134
Subcutaneous	0.271	0.149	0.265	0.142
Polyunsaturated fatty acids				
Visceral	0.424	0.021	0.394	0.024
Subcutaneous	0.512	0.004	0.407	0.024
n-6 fatty acids				
Visceral	0.140	0.462	0.232	0.188
Subcutaneous	0.179	0.343	0.302	0.144
n-3 fatty acids				
Visceral	0.523	0.005	0.558	< 0.001
Subcutaneous	0.500	0.004	0.607	< 0.001
β coefficients adjusted for age and BMI.				
Energy-adjusted were calculated using the residual model for all exposure.				

Discussion

This study showed that habitual dietary intakes of TFA and PUFA were positively associated with apelin gene expression in visceral and subcutaneous adipose tissues among obese participants. Findings revealed that dietary intake of n-3 fatty acids was positively associated with apelin mRNA expression in visceral and subcutaneous among non-obese and obese participants.

It should be noted that the present study was the first investigation that assessed the relationship between habitual intake of fatty acids and apelin mRNA expression. Previous data on the relationship between TFA and apelin gene expression in adipose tissue was limited to findings in rats(13, 20); generally, apelin expression in adipose tissue and apelin concentrations increased in the rodents on a high-fat diet, compared with those on a standard diet (20) (13). Although animal studies show that apelin mRNA levels in adipose tissue and serum concentrations after a high-fat diet increased simultaneously (14, 21); another study demonstrated that despite the increase in apelin gene expression in adipose tissue in response to the high-fat diet, apelin concentration did not change significantly (15). Moreover, despite human studies investigating the impact of a low-calorie diet on apelin concentration among obese patients, no information regarding dietary components was reported (22–24); hence, we were unable to determine the relation of dietary components, especially dietary intakes of fatty acids on apelin. In the current study, we observed that TFA was associated with apelin gene expression in the obese subjects after controlling for BMI, thus, suggesting that the amount of body fat was not a mediator for an increase in apelin gene expression.

Although we found no significant difference between apelin gene expression among the obese and non-obese individuals, a higher concentration of apelin in patients with obesity was previously reported (22, 23, 25, 26). Regarding apelin gene expression and secretion, no direct association between gene expression pattern and secretion of apelin was found (27). Moreover, Celik et al. reported that by a 1.12% reduction (28) and Heinonen et al. by a 14.28% reduction BMI (24), no significant change in apelin concentrations was observed. Therefore, in addition to weight status, some other related factors might also modify the concentration of apelin. Insulin level is a potential factor in apelin changes. Decreased insulin concentration has possibly more influence than merely weight loss. Therefore, insulin levels may have a mediated role in the existing direct relationship between apelin and excess weight (13, 14, 20, 27, 29). Participants who were free of diabetes and hypertension were recruited for the present study. Furthermore, the nutrient content of the habitual diet may be among other factors that influence apelin.

High intakes of PUFA and n-3 fatty acids predicted apelin gene expression in both subcutaneous and visceral adipose tissue in all the weight groups. Apelin expression in response to EPA intervention was investigated in adipose tissue (13, 14); EPA supplementation (1 g/kg) in rats with a high-fat diet increased apelin gene expression (14). Moreover, in mice that received a high-fat diet enriched with 36 g/kg EPA, adipose tissue apelin gene expression was increased (13). Furthermore, intervention by EPA in a high-fat diet among mice led to a reduced level of insulin and glucose as well as improved insulin sensitivity through the increment of β oxidation in insulin-dependent organs and enhanced the expression of apelin and apelin receptor (13, 30); similarly, apelin was also demonstrated to enhance glucose tolerance in mice with obesity and insulin resistance (31). In addition, the present study showed n-3 fatty acids predicted apelin gene expression of a similar amount in both adipose tissues in obese subjects and 1-SD increase in n-3 fatty acids had association with approximately 0.6 unit increases in both visceral and subcutaneous apelin mRNA levels. In addition, it seems that n-3 fatty acid intakes increased apelin gene expression more in subcutaneous adipose tissue among obese subjects than in non-obese ones (0.7 vs. 0.5). Perez-Echarri et al. showed that in rats, overfeeding with a diet rich in SFA increased apelin gene

expression in visceral fat (14). However, apelin gene expression in rats fed with a high-SFA diet along with EPA treatment was higher than that fed to rats i.e., high-fat diet (14).

The potential mechanism of the effect of dietary fatty acids intake on apelin is not well understood; however, some mediator pathways can be suggested. Insulin, leptin, and peroxisome proliferator-activated receptor- γ (PPAR γ) are potential candidates for the regulation of the diet-induced apelin levels. (8, 13, 32–35).

There were limitations that should be stated. Small sample sizes limited our power to detect statistical associations. Despite the relatively large magnitude of the β standard in the dietary exposure and apelin mRNA levels, the exploratory insight of the current study should be considered. Therefore, our results need to be confirmed in the cohort and trial studies. Because the design of the current study was a cross-sectional, causal relationship cannot be inferred. However, since there is less probable that apelin induce dietary fat quality, we assumed that our conclusion to be likely that dietary fat may have an impact on apelin gene expression. Moreover, the lack of measuring apelin protein concentration and APJ gene expression was another limitation.

Conclusions

In conclusion, our findings showed that dietary intakes of total fatty acids and n-3 fatty acids were positively associated with apelin gene expression in adipose tissue in both non-obese and obese participants. Results suggest that changes in quality and quantity of dietary fatty acids can affect apelin gene expression in adipose tissue.

List Of Abbreviations

eicosapentaenoic acid; EPA

total fatty acids; TFA

saturated fatty acids; SFA

monounsaturated fatty acids; MUFA

polyunsaturated fatty acids; PUFA

food frequency questionnaire; FFQ

Quantitative real-time polymerase chain reaction analysis; qPCR

body mass index; BMI

fasting plasma glucose; FPG

Declarations

Ethics approval and consent to participate

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.1395.169) and conducted in accordance with the declaration of Helsinki as well as our institutional guidelines. Written informed consent was obtained from all participants.

Consent for publication

Not applicable

Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

E.Y. conceptualized and designed the study, gathered adipose tissue, RNA extraction, performed Real-time PCR, analyzed and interpreted the data, prepared the manuscript, and approved the final manuscript as submitted. M.Z. prepared the lab materials; cDNA synthesized, prepared the manuscript and approved the final manuscript as submitted. G.A. entered data, drafted the initial manuscript, and approved the final manuscript as submitted. M.H. supervised the project, consulted lab protocol, and approved the final manuscript as submitted. P.M. drafted the initial manuscript and approved the final manuscript as submitted. A.Kh. biopsied the patients during the abdominal surgery and approved the final manuscript as submitted. M.S. supervised the project and approved the final version of the manuscript as submitted.

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Figures

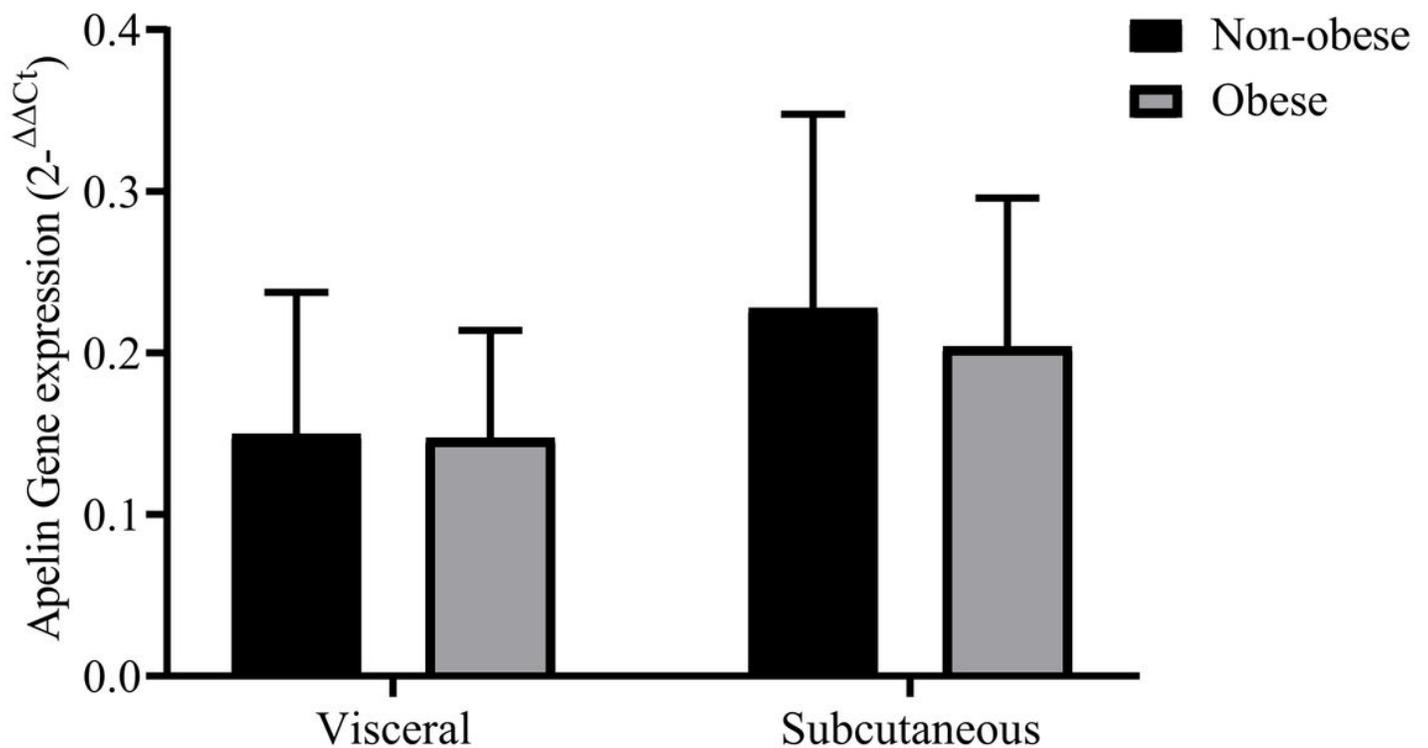


Figure 1

Student t-test was used to compare group means; Mean with standard deviation for apelin gene expression in adipose tissue