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Original Article

Effect of Omega-3 Supplementation on Lipocalin 2 and Retinol-Binding Protein 4 in Type 2 Diabetic Patients

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Abstract

Background: Serum levels of lipocalin 2 (LCN 2) and retinol-binding protein-4 (RBP 4), increase in type 2 diabetes mellitus (T2DM). We sought to determine whether serum LCN 2 and RBP 4 change after an intervention with omega-3 fatty acids supplementation in diabetic patients.

Methods: Forty-five type 2 diabetic patients from Iranian Diabetic Association in Tehran, Iran in 2013 were randomly recruited into two groups: one group received 4 g/d omega-3 for 10 wk; and the control group received placebo. Blood samples, food intake records, anthropometric measurements were obtained from all participants at the beginning and end of the study.

Results: Fasting RBP 4 plasma levels significantly changed after 10 wk supplementation ($P = 0.01$). The LCN 2 concentrations decreased in omega-3 group, but the changes were not statistically significant. Omega-3 supplementation had no noticeable effect on anthropometric factors.

Conclusions: These findings provide a rationale for omega-3 supplements aimed at lowering serum RBP 4 levels in T2DM.

Keywords: Omega-3 fatty acid, Lipocalin, Retinol-binding protein, Diabetes mellitus

Introduction

Lipocalins family encompasses more than twenty different proteins. Theses mall, soluble protein shave different sequences with only 20% similarity to each other. However, all of them composed of8 antiparallel beta-barrels. Lipophilic substances such as free fatty acids, retinoids, arachidonic acid and steroids pass through a hole inside the barrel (1, 2). Lipocalin 2 is a 25kDa glycoprotein, identified as neutrophil gelatinase associated lipocalin (NGAL), neutrophil lipocalin (NL) and 24P3 oncogene. This protein secreted mainly from the liver and adipose tissue (3). LCN 2 expression is increased by substances that improve insulin resistance and is decreased by thiazolidinediones (TZD) (4). Lipocalin 2 up-regulates peroxisome proliferator-activated receptor gamma (PPARγ) and its target genes including leptin, adiponectin, fatty acid synthase (FAS) and lipoprotein lipase in adipose tissue (5).

Retinol-binding protein 4 (RBP 4) belongs to lipocalins family and transfers small hydrophobic molecules form membranes (6). Its gene locates

on the long arm of chromosome 10, near glucose homeostasis area in European-Caucasian ethnicity and near region of type 2 diabetes in Mexicans-Africans (7, 8). The protein consists of 201 amino acids and its molecular weight is 21kDa (9, 10). The highest expression is seen in the liver and adipose tissue. RBP 4 increased in the early stages of stroke and cardiovascular disease (11-13). Serum RBP 4 might have a negative correlation with insulin resistance and development of T2DM (14, 15). RBP 4 is connected to diabetes through insulin secretion, and pancreas beta cells play a key role in this relationship (15). Significant weight loss by diet, lifestyle modifications and bariatric surgery may reduce RBP 4 levels (16). Improvement in insulin resistance by exercising also reduces RBP 4 concentration (17). RBP4 concentrations increase in humans with obesity and type 2 diabetes. Rosiglitazone, an insulin-sensitizing drug that activates PPARγ, decreases RBP4 concentrations. Lowering RBP 4 might be a novel strategy that helps type 2 diabetic patients (18).

Ecological studies have shown that marine foods may negatively associate with insulin resistance in obese subjects. Meta-analysis reports omega-3 fatty acids in the Asian population, unlike the western population, protect against T2DM. However, Asian patients have lower levels of omega-3 than western patients in their plasma and cell membranes (19). EPA correlates with up-regulation of PPARγ and down-regulation of interleukin-6 and tumor necrosis factor-alpha (TNFα). Results suggest that EPA probably change PPARγ activation and expression (20).

We carried out this study to compare the effect of omega-3 supplementation versus placebo on LCN 2 and RBP 4 levels in type 2 diabetic patients. We hypothesized that the omega-3 supplementation would reduce these adipokines after 10 weeks as well as TZD.

Materials and Methods

In the present double blind randomized controlled trial in 2013, 45 subjects of both sexes, 40-65 yr old, tested and diagnosed with diabetes mellitus by physician, were enrolled during appointments at the Iranian Diabetic Association Center in Tehran, Iran. Fort four participants successfully completed the study. The inclusion and exclusion criteria are listed in Table 1.

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
30-65 year old BMI 18.5- 40kg/m^2	Consuming dietary supplements at least 2 weeks before and throughout the intervention; Consuming omega-3 supplements in the last 3 months; Chronic renal, hepatic, gastrointestinal, hematological diseases, thyroid disorder; Using orlistat, sibutramine or any other drug for weight loss; Pregnancy and lactation; Thiazolidinediones insulin therapy

Randomization was carried out using a random permuted block. All participants (45 T2DM) were randomized to the following groups based on BMI: 1) 23 type 2 diabetic patients constituted the case group; and 2) 22 T2DM patients served as the control group. The two homogeneous groups were prescribed four capsules of omega-3 fatty acids or four capsules of placebo per day. To avoid possible adipokines and progesterone level changes in the luteal phase, premenopausal female patients were asked to begin treatment in the early follicular phase of their menstrual cycles. All participants were requested to maintain their usual exercise and dietary habits. The supplement formulation contained 310 mg eicosapentaenoic acid (EPA), 210 mg docosahexaenoic acid (DHA),

110mg other polyunsaturated fatty acids and 5mg vitamin E (Maxepa Forte Capsules, Seven Seas, UK). Placebo was composed of paraffin oil that absolutely resembled omega-3 (Zahravi, Iran). The compliance was approximately over 90% for those who remained in study. Researchers were instructed to pay special attention to measure levels of patients' compliance to ensure the validity of the final data of the investigation. Therefore, subjects were followed each 2 wk after enrolling to study during the 10 wk of follow-up. Blood sample was drawn at the beginning and the end of the study after at least 12 h fasting. All blood samples were centrifuged at 3000 g for 10 min and sera were separated into the clean tube aliquots and were stored at -80 ˚C.

Dietary intake was assessed using the 3-day food records (comprising two working days and a weekend) at the beginning and end of the intervention. The portion sizes of the consumed foods were explained to all participants by a trained nutritionist. All food items were converted to grams. Modified Nutritionist IV software was used to estimate the energy and nutrient intakes.

Weight was measured using a digital scale (803, Seca Clara, Germany) with an accuracy of 100 g, in light clothes and without shoes. Height was measured without shoes using a stadiometer (206, Seca, Germany) with an accuracy of 0.1 cm. Hip and waist circumferences were measured using a measuring tape (201, Seca, Germany) with an accuracy of 0.1 cm. Body mass index (BMI) was calculated using these recorded values [weight(kg)/hight^2(m)].

LCN-2 and RBP-4 were measured by research enzymatic calorimetric ELISA kits (Boster Biological Technology Ltd, China). The tests were conducted according to the company's instructions.

Data were analyzed using the SPSS 18.0 for Windows (Chicago, IL, USA). The results are explained as mean ± SEM. Student's *t*-tests were used to compare the two groups and paired *t*-tests were used for competing before and after data within each group. $P< 0.05$ and more than 80% power were considered statistically significant.

Results

A total of 45 type 2 diabetic patients were invited to participate in the study. One patient inomega-3 group did not complete the study. Participant characteristics are shown in Table 2.The sample (n=44) of diabetics who completed study had similar characteristics, as there were no significant differences between the group characteristics.

Table 2: Characteristics of the participants at baseline and after the intervention

		Omega-3 (n=22)	Placebo (n=22)	*P* value‡
Age (yr)	Before	54.23 ± 1.64	53.32 ± 1.45	0.68
Height (cm)	Before	162 ± 2.11	156 ± 1.37	0.02
	Before	69.21 ± 2.84	63.57 ± 2.65	0.15
Weight (kg)	After	68.96±2.91	63.60±2.78	0.19
	Difference	-0.25±0.29	0.40±0.36	0.53
	P value§	0.40	0.91	
BMI (kg/m^2)	Before	26.19 ± 0.78	25.93 ± 0.92	0.83
	After	26.11±0.84	25.95±0.98	0.91
	Difference	-0.08±0.1	0.02±0.14	0.55
	P value§	0.45	0.85	
WHR	Before	0.84±0.01	0.85±0.01	0.1
	After	0.84±0.16	0.85±0.01	0.09
	Difference	0.003±0.002	-0.005±0.003	0.08
	P value§	0.27	0.18	

WHR: waist to hip ratio, All values are expressed as means ±SEM, § paired t-test ‡ two independent sample tests

At the end of the study, retinol-binding protein-4 decreased significantly in omega-3 group ($P< 0.001$). But the changes of lipocalin 2 was not significant following omega-3 supplementation comparing the baseline amount of the same group ($P=0.14$). However, after 10 wk of intervention, there was significant difference in retinol-binding protein 4 and lipocalin 2 between the two groups ($P = 0.01$, and $P = 0.03$, respectively).

Comparing the amount of change between two groups showed a significant difference for RBP 4 but not LCN 2 ($P = 0.01$ and 0.08, respectively (Table 3).There were no significant changes in dietary intake of omega-3 and omega-6 during the study (Table 4).

Table 3: Plasma LCN 2 and RBP 4 at baseline and after the intervention

		Omega-3 (n=22)	Placebo (n=22)	*P* value‡
Lipocalin 2 (pg/ml)	Before	6161.77±207.07	6302.59±316.28	0.711
	After	5635.73±284.73	6537.73±298.74	0.034
	Difference	-616.95±456.14	728.31±595.48	0.08
	P value§	0.14	0.64	
Retinol-binding protein 4 (µg/ml)	Before	28.54±1.16	27.51±1.57	0.60
	After	17.69±0.98	24.21±2.27	0.012
	Difference	-10.85±1.62	-3.29±2.6	0.01
	P value§	0.000	0.21	

All values are expressed as means ±SEM § paired t-test ‡ two independent sample tests

Table 4: Omega-3 and omega-6 dietary intakes at baseline and 10thwk of study

		Omega-3 (n=22)	Placebo (n=22)	*P* value‡
Omega-3 (gr)	Before	0.96±0.12	1.19±0.11	0.18
	After	1.07±0.12	1.04±0.12	0.84
	Difference	0.11±0.15	-0.14±0.18	0.29
	P value§	0.47	0.44	
Omega-6 (gr)	Before	20.06±1.37	20.53±1.11	0.69
	After	20.09±1.06	22.90±2.39	0.33
	Difference	0.02±1.37	2.37±2.75	0.45
	P value§	0.86	0.46	

All values are expressed as means ±SEM § paired t-test ‡ two independent sample tests

Discussion

This study for the first time, in our knowledge, investigated the effect of omega-3 supplementation on LCN 2 and RBP 4 concentrations in type 2 diabetic patients. In previous studies changes in the plasma expression of other adipokines such as leptin, adiponectin, and resistin has been reported faced with n-3 fatty acids (21). The results of this double-blind randomized clinical trial demonstrate that intake of 4g omega-3 for 10 wk leads to significant decrease in RBP 4 in T2DM patients. Considering that RBP 4 and LCN 2 come from an analogous family, our hypothesis was their modifications are paralleled. LCN 2 and RBP 4 were decreased in omega-3 group versus placebo group, but mean differences between the two groups only for RBP 4 was statistically significant.

Study of the adypocyte cells showed that substances cause insulin resistances; elevate LCN 2 expression while TZD reduce its expression. In addition, insulin resistance might be induced by

exogenous LCN 2. Animal models demonstrate a sharp decline in the level of LCN 2 contribute to insulin action improvement (4). Because of n-3 PUFA is an anti-inflammatory agent and decrease insulin resistance in some studies (22, 23), it seems that omega-3 has a similar mechanism with TZD. Hyperinsulinemic state in human participants definitely increased circulating lipocalin 2 concentrations which indicate the up regulating effect of insulin on lipocalin 2 (5). In a cross sectional study, LCN 2 did not correlate with total body fat or body weight and it could not play a prominent role in prediction of metabolic risk factors (24). Our finding about LCN 2 dealing with body weight was consistent with it.

In subjects with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes mellitus, lipocalin 2 concentrations are higher than people with normal glucose metabolism. Glucose metabolism disturbance in T2DM, independently correlates with increased level of LCN 2 (25). In the present study, LCN 2 level was not measured in normal subjects.

Various agents with different mechanism can influence on RBP4 such as orlistat, an anti-obesity drug, that promote lipid profile, glycemic control and insulin resistance (26). Acarbose, an anti-diabetic drug, also improve lipid profile, glycemic control and insulin resistance. In addition, acarbose reduce RBP 4 and adiponectin levels (27). Both drugs have local effect, but omega-3 has systematic effects via different pathways.

Pan et al. investigated the effects of a flaxseed-derived lignan supplement on RBP 4 in type 2 diabetic patients, so after 12 wk flaxseed oil lignan did not change RBP 4 level (28), this issue could be related to different type of omega-3 applied in the study.

Agonists of PPARγ improve RBP 4. Administration of pioglitazone, an insulin sensitizer, for 12 wk in diabetic patients, showed that RBP 4 correlates with insulin resistance and complication of diabetes (29). One study compared the effect of pioglitazone versus gliclazide, another anti-diabetic drug. After approximately 3 months, pioglitazone significantly decreased serum RBP 4 and HOMA-IR values, but gliclazide did not change these variables in type 2 diabetic patients (30). Because omega-3 is also PPARγ agonist, our finding about significant decrease in serum RBP 4 in intervention group was consistent with previous study (30).

Conclusion

Fish oil has several benefits in metabolic syndrome and cardiovascular diseases, but its role in diabetes remains controversial. The present study introduced a new viewpoint about omega-3 supplementation effects on adipokins that relates to insulin resistance in diabetic patients. The results of our study showed beneficial effects ofomega-3 on LCN 2 and RBP 4. Further studies are needed to elucidate cellular-molecular pathways, particularly changes in PPARγ gene expression with different doses of omega-3 in diabetics.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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