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Eicosapentaenoic acid improves insulin sensitivity and blood sugar in overweight type 2 diabetic mellitus patients: a double-blind randomised clinical trial

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INTRODUCTION Diabetes mellitus is the most common metabolic disorder in humans, and its incidence is increasing rapidly worldwide. Although polyunsaturated fatty acids have beneficial effects on diabetes mellitus, previous data regarding the possible positive effects of n-3 fatty acids on glycaemic indices were inconclusive. We conducted a double-blind randomised clinical trial to determine the effects of eicosapentaenoic acid (EPA), an n-3 polyunsaturated fatty acid, on overweight patients with type 2 diabetes mellitus (T2DM).

METHODS This double-blind, placebo-controlled randomised clinical trial was conducted on a total of 67 overweight patients with T2DM for a duration of three months. Of these 67 patients, 32 received 2 g purified EPA daily, while 35 received a placebo of 2 g corn oil daily. The patients' fasting plasma glucose (FPG), serum insulin, glycated haemoglobin (HbA1c) and insulin sensitivity indices were assessed.

RESULTS After three months of EPA supplementation, the group that received EPA showed significant decreases in FPG ($p < 0.001$), HbA1c ($p = 0.01$) and homeostasis model assessment of insulin resistance (HOMA-IR) ($p = 0.032$), when compared to the placebo group. EPA supplementation resulted in decreased serum insulin levels, with the levels between the EPA and placebo groups showing a significant difference ($p = 0.004$).

CONCLUSION The results of our study indicate that EPA supplementation could improve insulin sensitivity. It was able to decrease serum insulin, FPG, HbA1c and HOMA-IR. EPA could have beneficial effects on glycaemic indices in patients with T2DM.

Keywords: diabetes mellitus, eicosapentaenoic acid, insulin sensitivity

INTRODUCTION

Most countries all over the world are experiencing a rapid increase in the number of patients suffering from diabetes mellitus (DM).[1] According to the statistics of the World Health Organization (WHO), more than 180 million people worldwide have DM and this number would double by 2030 if no urgent action is taken. Type 2 DM (T2DM) is the most frequent form of the disease, accounting for 85% of all DM cases, while type 1 DM and specific/gestational DM, account for 10% and 5%, respectively.[2] It has been shown that DM-related morbidity and clinical complications can be reduced substantially by tight glycaemic control, which is typically defined as a glycated haemoglobin (HbA1c) level of less than 7%.[3,4]

Nutrition is likely to affect glycaemic control. Epidemiological studies have reported a lower prevalence of T2DM and other glucose metabolism disorders in populations that consume larger amounts of n-3 polyunsaturated fatty acids (PUFAs), found mainly in fish.[5] However, the findings of clinical studies are controversial. Early studies reported that fish consumption had adverse effects on blood glucose control and insulin activity in patients with T2DM.[6-8] It is now believed that the undesirable effects observed in those studies were due to the use of high doses of fish oil (> 10 g/day) and small sample size. Studies using lower doses of n-3 PUFAs (1–2 g/day) reported no deterioration of glucose control in their subjects.[9] Additionally, the beneficial effects of n-3 PUFAs on glucose metabolism have been shown in some clinical trials. One study reported a significant improvement in insulin sensitivity after 12 weeks of n-3 PUFA supplementation with 1.3 g eicosapentaenoic acid (EPA) and 2.9 g docosahexaenoic acid (DHA) in obese, premenopausal, nondiabetic women.[10] Another study compared the effects of consuming five portions of either oily fish (containing n-3 PUFA) or red meat daily, for a duration of two months on insulin resistance in young women.[11] The data obtained from that study suggested that an oily fish diet induced a significant decrease in both serum insulin levels and insulin resistance, as determined by homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI). The results of these studies,[10,11] which investigated the effects of fish oil, EPA and DHA

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intake on the development of T2DM, were ambiguous and inconclusive. Since the role of n-3 PUFAs in T2DM remains unresolved and few studies examining the impacts of EPA and DHA have been conducted, we designed a double-blind, randomised clinical trial to determine the effects of EPA on the glycaemic indices of overweight patients with T2DM.

METHODS

A total of 67 patients (26 men, 41 women) with T2DM, defined as individuals taking oral hypoglycaemic agents or with a fasting plasma glucose (FPG) level > 7.0 mmol/L,[12] participated in this study. The participants were aged 35–55 years, had a body mass index (BMI) of 25–30 kg/m^2, had been diagnosed with T2DM for 2–15 years, were nonalcoholics and nonsmokers, had no history of drug abuse, and were not pregnant or breastfeeding. Patients had no recent digestive, hepatic, renal, cardiovascular, thyroid or respiratory diseases, and no diagnosis of cancer in the previous year. Informed consent was obtained from all the patients.

In this placebo-controlled, double-blind clinical trial, the patients were randomly assigned to either the control group, which received four 500-mg capsules of corn oil (Daana Pharmaceutical Company, Tabriz, Iran) daily, or the EPA group, which received four 500-mg capsules of EPA supplement (PlusEPA®; Minami Nutrition company, Edegem, Belgium) daily. Each EPA 500 mg capsule contained 95% pure EPA. Corn oil was used as placebo because it is the most popular dietary oil. EPA and corn oil were administered in the form of soft gels, which were identical in size, colour and taste. Patients were told to take the supplements postprandially for a duration of 12 weeks. They were also advised to maintain their diet, level of physical activity and antidiabetic agents throughout the duration of the study.

At the beginning of the study, the weight and height of all patients were taken using a measuring scale and BMI was calculated using the formula: weight/(height × height). Follow-up sessions were conducted every two weeks, during which the patients' weight, diet and compliance with the rules of the study were checked. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, Iran.

Blood samples were collected in the morning, after an overnight fast, and centrifuged at 3,000 rpm for 10 mins at room temperature for serum separation. The fasting serum samples were stored at –70°C until they were analysed for glucose (Glucose kit; Pars Azmoon Co, Tehran, Iran) and insulin (Immunotech Insulin IRMA IM3210; Beckman Coulter Inc, France). 1 mL of whole blood was preserved in tubes containing ethylenediaminetetraacetic acid as an anticoagulant agent for the determination of HbA1c percentage (NycoCard® kit 1042184; Axis-Shield PoC, Oslo, Norway). HOMA-IR was computed as FPG (mmol/L) × fasting serum insulin (mU/L)/22.5,[13] and QUICKI was calculated as 1/[log insulin (μIU/mL) + log glucose (mg/dL)].[14]

Table I. Baseline characteristics of patients (n = 67).

Characteristic	Mean ± SD		p-value*
	Control (n = 35)	EPA (n = 32)	
Age (yrs)	45.3 ± 3.93	45.03 ± 4.88	0.79
Male/female (no.)	13/22	13/19	0.77†
Weight (kg)	70.77 ± 8.43	74.44 ± 9.40	0.09
BMI (kg/m^2)	27.80 ± 1.65	27.9 ± 1.73	0.74
Duration of DM (yrs)	8.57 ± 3.86	7.43 ± 3.84	0.23

*Independent *t*-test showed no significant differences between two groups at baseline. †Chi-square test was used to detect differences in gender profile between the groups.
BMI: body mass index; DM: diabetes mellitus; EPA: eicosapentaenoic acid; SD:standard deviation

The Statistical Package for the Social Sciences version 16.0 (SPSS Inc, Chicago, IL, USA) was used in all analyses. The normality of the distribution of all variables was checked using Kolmogorov-Smirnov test. All values were presented as mean ± standard deviation. Differences between groups were assessed using independent *t*-test, and considered significant at $p < 0.05$. To remove the effects of baseline values, analysis of covariance (ANCOVA) was used.

RESULTS

A total of 70 patients were recruited initially, but only 67 patients completed the study successfully (i.e. no changes were made to their diet, level of physical activity and antidiabetic agents throughout the duration of the study). Three patients withdrew from the study – two due to digestive intolerance to the supplement and one due to unplanned travel. Analysis of the baseline characteristics of the control and EPA groups confirmed that the groups were well matched for the entry criteria (Table I). There were no significant differences between the groups in both the type and amount of oral hypoglycaemic agents taken. The patients took oral hypoglycaemic medications in the form of sulfonylureas (16%), biguanides agents (8%) or biguanides along with sulfonylureas (76%).

The baseline values of serum insulin, HbA1c and HOMA-IR were not significantly different between the two groups. However, FPG and QUICKI were higher in the control group (Table II). Correlations were observed between the duration of diagnosed DM and FPG ($p = 0.006$, $r = 0.33$), and between HbA1c and FPG ($p < 0.001$, $r = 0.62$). Serum insulin also correlated with weight ($p = 0.027$, $r = 0.27$) and BMI ($p = 0.02$, $r = 0.28$). At the end of the study, there was a decrease in the FPG, HbA1c and HOMA-IR of

Table II. Baseline and final values of the FPG, serum insulin, HbA1c, HOMA-IR and QUICKI of patients in the control and EPA groups.

Characteristic	Control (n = 35)	EPA (n = 32)	p-value
FPG (mg/dL)			
Baseline	187.09 ± 56.28	146.56 ± 38.77	0.001
Final	191.77 ± 58.18	127.00 ± 31.91	< 0.001
p-value	0.51	0.001	
Serum insulin			
Baseline	5.36 ± 2.18	7.34 ± 3.06	0.1
Final	6.80 ± 2.67	6.76 ± 2.31	0.97
p-value	0.008	0.20	
HbA1c (%)			
Baseline	8.91 ± 1.81	8.92 ± 1.39	0.97
Final	9.11 ± 1.79	8.14 ± 1.22	0.01
p-value	0.27	< 0.001	
HOMA-IR			
Baseline	2.43 ± 1.76	2.73 ± 1.31	0.55
Final	3.10 ± 1.96	2.10 ± 1.73	0.03
p-value	0.010	0.004	
QUICKI			
Baseline	4.17 ± 1.79	2.48 ± 1.02	0.02
Final	3.85 ± 2.96	3.58 ± 2.17	0.73
p-value	0.66	0.93	

Note: Data is presented as mean ± standard deviation. Baseline and final values were compared using paired *t*-test, and the differences between the groups were assessed using independent *t*-test. A p-value < 0.05 was considered to be significant.
EPA: eicosapentaenoic acid; FPG: fasting plasma glucose; HOMA-IR: homeostatic model assessment for insulin resistance; QUICKI: quantitative insulin-sensitivity check index

patients in the EPA group when compared with the control group ($p < 0.001$, $p = 0.01$ and $p = 0.03$, respectively). Moreover, after the effects of baseline values were removed, changes in the FPG, HbA1c, serum insulin and HOMA-IR were noticeably greater in the EPA group than the control group ($p = 0.007$, $p < 0.001$, $p = 0.004$ and $p < 0.001$, respectively). In other words, conspicuous decreases were observed in the FPG (11%), HbA1c (8%) and serum insulin (3%) of patients in the EPA group, whereas these indices were observed to have increased slightly in the control group (4%, 3% and 4%, respectively).

DISCUSSION

In our study, we found that FPG and serum insulin levels were reduced in the EPA group when compared with the control group. This suggests that the consumption of EPA supplements could improve glycaemic control in patients with DM. The effect of baseline glycaemic control was removed in our study using ANCOVA.

The results of previous studies were ambiguous, with some studies showing that fish oil consumption could increase plasma glucose concentrations. One study reported that high intake of long-chain n-3 fatty acids may even modestly increase the incidence of T2DM.[15] A review article by Heine, which was based on a limited number of observations, concluded that the consumption of n-3 PUFAs had an adverse effect on glycaemic control in patients with T2DM.[16] However, it is now believed that the undesirable effects observed in those studies were due to the use of high doses of fish oil (> 10 g/day) and small sample sizes.

A randomised, double-blind, placebo-controlled, crossover study revealed that the consumption of 6 g of n-3 PUFA (EPA plus DHA) daily for a duration of six months, in addition to the usual oral therapy, caused a nonsignificant net glucose increase of 3% in patients with T2DM.[17] This suggests that n-3 PUFA intake, along with hypoglycaemic agents, had no adverse effects on glycaemic control. In a study by Sirtori et al,[6] 89 patients with T2DM received 2.6 g of EPA plus DHA daily for the first two months, followed by 1.7 g daily for the next four months. This treatment regimen was continued for up to one year. At the end of the study, no significant changes were observed in the FPG or serum insulin levels between the treatment and control groups, with the latter receiving olive oil as placebo. Some recent studies, however, have shown that n-3 PUFAs have beneficial effects on glucose metabolism. It was reported that consumption of n-3 fatty acids or higher doses of fish oil could improve glucose and insulin metabolism, as well as increase insulin sensitivity.[10,18] Epidemiological studies have also reported a lower prevalence of T2DM and other glucose metabolism disorders in populations that consume larger amounts of n-3 PUFAs, which are found mainly in fish.[5]

HbA1c, which represents the average plasma glucose concentration over a prolonged period of time, decreased significantly following EPA supplementation in our study. Since our study lasted for a duration of three months, there was sufficient time for a noticeable change in HbA1c to be observed. Egert et al reported no change in the HbA1c level of individuals who consumed 2.8 g of EPA a day for three weeks.[19] Another study, in which patients consumed 500 mg of EPA a day with or without vitamin C for eight weeks, showed that EPA consumption could reduce HbA1c conspicuously.[20] From these studies, it seems that the duration of intervention plays a key role in the changes in HbA1c levels, since the lifespan of red blood cells is almost 120 days.

Consumption of EPA supplements could also reduce HOMA-IR, an index of insulin resistance. However, in our study, QUICKI did not change significantly. Previously, Griffin et al cited that a diet enriched in EPA, DHA, or both, caused no modification in HOMA-IR or QUICKI.[21] In contrast, another study, which examined the effects of oily fish consumption for two months on insulin resistance, showed that the consumption of oily fish induced significant improvement in HOMA-IR and QUICKI when compared with the consumption of red meat.[11] One of the major putative mechanisms that could account for the possible beneficial effects of n-3 PUFAs on insulin resistance

is the anti-inflammatory properties of n-3 PUFAs, which have been previously reported.[22,23]

In conclusion, our study showed that a diet supplemented with 2 g of EPA daily can improve the indices of glycaemic control, namely FPG, serum insulin, HbA1c and HOMA-IR, in T2DM patients. While the complete mechanism of action of EPA has yet to be elucidated, it is well known that an increase in n-3 PUFAs intake causes an increase in the fluidity of the skeletal muscle membrane.[24] Hence, the resulting improvement in glucose uptake might be related to an increase in the residency time of glucose transporters 1 and 4 in the plasma membrane.[25,26] Moreover, the genes of adiponectin, adiponectin receptors and peroxisome proliferator-activated receptors, which are likely associated with insulin resistance, might be influenced by n-3 PUFA intake.[27,28] Further clinical and basic studies are needed to elucidate the underlying mechanisms.

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REFERENCES

1. International Diabetes Federation. Diabetes Atlas. Belgium: IDF, 2000.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27:1047-53.
3. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998; 352:837-53.
4. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000; 321:405-12.
5. Nettleton JA, Katz R. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: a review. J Am Diet Assoc 2005; 105:428-40.
6. Sirtori CR, Crepaldi G, Manzato E, et al. One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycaemic alterations. Atherosclerosis 1998; 137:419-27.
7. Borkman M, Chisholm DJ, Furler SM, et al. Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. Diabetes 1989; 38:1314-9.
8. Vessby B. n-3 fatty acids and blood glucose control in diabetes mellitus. J Intern Med Suppl 1989; 731:207-10.
9. Axelrod L, Camuso J, Williams E, et al. Effects of a small quantity of omega-3 fatty acids on cardiovascular risk factors in NIDDM. A randomized, prospective, double33 blind, controlled study. Diabetes Care 1994; 17:37-44.
10. Browning LM, Krebs JD, Moore CS, et al. The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. Diabetes Obes Metab 2007; 9:70-80.
11. Navas-Carretero S, Pérez-Granados AM, Schoppen S, Vaquero MP. An oily fish diet increases insulin sensitivity compared to a red meat diet in young iron-deficient women. Br J Nutr 2009; 102:546-53.
12. Funnell MM, Brown TL, Childs BP, et al. National standards for diabetes self management education. Diabetes Care 2012; 35 suppl 1:S101-S8.
13. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-9.
14. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000; 85:2402-10.
15. Kaushik M, Mozaffarian D, Spiegelman D, et al. Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. Am J Clin Nutr 2009; 90:613-20.
16. Heine RJ. Dietary fish oil and insulin action in humans. Ann N Y Acad Sci 1993; 683:110-21.
17. Connor WE, Prince MJ, Ullmann D, et al. The hypotriglyceridemic effect of fish oil in adult-onset diabetes without adverse glucose control. Ann N Y Acad Sci 1993; 683:337-40.
18. Mori TA, Bao DQ, Burke V, et al. Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. Am J Clin Nutr 1999; 70:817-25.
19. Egert S, Fobker M, Andersen G, et al. Effects of dietary-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid on parameters of glucose metabolism in healthy volunteers. Ann Nutr Metab 2008; 53:182-7.
20. Mahmoudabadi MM, Djalali M, Djazayery SA, et al. Effects of eicosapentaenoic acid and vitamin C on glycaemic indices, blood pressure, and serum lipids in type 2 diabetic Iranian males. J Res Med Sci 2011; 16 suppl 1:S361-7.
21. Griffin MD, Sanders TA, Davies IG, et al. Effects of altering the 1 ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. Am J Clin Nutr 2006; 84:1290-8.
22. Rasic-Milutinovic Z, Perunicic G, Pljesa S, et al. Effects of N-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. Ren Fail 2007; 29:321-9.
23. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006; 83 (6 suppl):1505S-19S.
24. Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis 2000; 150:227-43.
25. Nugent C, Prins JB, Whitehead JP, et al. Arachidonic acid stimulates glucose uptake in 3T3-L1 adipocytes by increasing GLUT1 and GLUT4 levels at the plasma membrane. Evidence for involvement of lipoxygenase metabolites and peroxisome proliferator-activated receptor gamma. J Biol Chem 2001; 276:9149-57.
26. Figueras M, Olivan M, Busquets S, López-Soriano FJ, Argilés JM. Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status. Obesity (Silver Spring) 2011; 19:362-9.
27. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 2007; 56:1198-209.
28. Rosenson RS. Effects of peroxisome proliferator-activated receptors on lipoprotein metabolism and glucose control in type 2 diabetes mellitus. Am J Cardiol 2007; 99:96B-104B.