Effect of EPA supplementation on body mass index in overweight hypertriglyceridemic subjects with different FABP2 and PPARα genotypes

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ABSTRACT

Background: Obesity is a consequence of the excessive accumulation of fat in adipose tissue which can result in significant morbidity and mortality. Obesity is a major health problem in Iran. Objective: The aim of this study was to investigate the effects of EPA consumption on BMI and fasting blood sugar by FABP2 genotypes and PPARα (Leu162Val, and G/C intron polymorphism) genotypes.

Methods: A total of 170 hypertriglyceridemic subjects were selected and genotyped for Ala54Thr, using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. After determination of their FABP2 genotypes, the first 23 eligible subjects who were determined as Ala54 carriers and the first 23 eligible Thr54 carriers were enrolled in the study. Participants took 2 g/d of pure EPA (four gel caps, each containing 500 mg of ethylester EPA 90%). Height and weight were measured by a Seca scale with light clothing and no shoes on. BMI was then calculated. Waist and hip circumferences were measured with a flexible tape.

Results: EPA supplementation decreased fasting blood sugar in Ala54 and Thr54 (p<0.001). No significant association was observed between BMI or fasting blood sugar and different FABP2 genotypes after EPA consumption. EPA supplementation increased BMI and decreased fasting blood sugar in Lue162 and Val162 (p<0.01), and interon 7 polymorphism (p<0.01). No interaction was observed between PPARα genotypes and degree of changes in BMI or fasting blood sugar after EPA supplementation.

Conclusion: Although EPA consumption showed the effect of EPA response on FBS in Ala54 or Thr54 and Leu162 or Val162 in FABP2 and PPARα genotypes but no interaction was observed between these genotypes and EPA supplementation.

Introduction

Obesity and insulin resistance (IR) are major health problems worldwide. The important variability in the prevalence of type II diabetes and obesity among different populations and ethnic groups has been the focus of many epidemiologists. The impact of both diseases on personal and public health is considerable and increasing in several areas of the world [2]. Both complex conditions are influenced by multiple genetic and environmental factors, and
both are major risk factors for chronic degenerative diseases such as type 2 diabetes, non-alcoholic fatty liver disease, arterial hypertension, dyslipidaemia and cardiovascular disease [3,4]. In obese subjects, the most frequently abnormalities are IR and hypertriglyceridemia [5]. Obesity and type II diabetes have been shown to cluster within families, suggesting a genetic component for their etiology [6].

Obesity is a major health problem in Iran [7]. In the South of Iran (Shiraz city); the prevalence of obesity (BMI ≥30) is 10.5% and 22.5% in men and women, respectively, which shows an increased secular change of 5.8% in men and 17.4% in women during a 14-year period [8]. The prevalence of obesity in Tehranian adults aged 20 years and over were measured in 1999-2001 (phase I), again in 2002-2005 (phase II) and 2006-2008 (phase III). The results showed 15.8%, 18.6% and 21% in men and 31.5%, 37.7% and 38.6% in women in phases I, II and III respectively (p< 0.001). This study demonstrates alarming rise in the prevalence of both obesity and abdominal obesity in both sexes especially in young men [9].

The consumption of n-3 PUFA, namely EPA and DHA, have been linked to reduced CVD risk [10,11], and to reduced fasting glucose levels, providing a protective effect against the development of type 2 diabetes [12]. A higher uptake of dietary fatty acids could contribute to both pathological conditions. The T54 allele of the FABP2 gene has been shown to have a greater affinity for long-chain fatty acids, increasing absorption and processing of fatty acids [13,14]. Carriers of the Thr54 allele in FABP2 have a twofold greater affinity to the absorption for the long-chain fatty acids than those with the Ala54-containing FABP2 [15], which supports the role of the FABP2 Ala54Thr polymorphism in the etiology of obesity and metabolic disorders. In vitro experiments have shown that this substitution increases the affinity of FABP2 for long chain fatty acids and is associated with increased triglyceride transport in human intestinal cells [14,16].

The hypolipidemic effect of EPA has been suggested to be a consequence of a decrease in the very low density lipoprotein (VLDL) fraction. Consistent with this hypothesis, studies have shown that EPA and DHA decrease VLDL production and secretion in vitro and in vivo [17]. There have been limited studies addressing the effect of EPA supplementation directly on obesity considering genotypes. The current study investigated the effects of EPA consumption on BMI and fasting blood sugar by FABP2 genotypes and PPARα (Leu162Val, and G/C intron polymorphism) genotypes followed by a controlled EPA intervention trail in hypertriglyceremic subjects.

Methods

Subjects

Participants were selected from hypertriglyceridemic subjects referred from Tehran Central Laboratories to the Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences (TUMS), Tehran, Iran. The inclusion criteria were a serum TG level higher than 200 mg/dL (>2.3 mmol/L) and a fasting blood glucose level lower than 110 mg/dL (<6.2 mmol/L). Those who received lipid-lowering agents, oral contraceptive pills, diuretics, sex hormones, thyroid medications, n-3 supplements, or had a history of gastrointestinal diseases, and smokers were excluded from the study. In total 170 hypertriglyceridemic subjects were selected and genotyped for Ala54Thr, using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. After determination of their FABP2 genotypes, the first 23 eligible subjects who were determined as Ala54 carriers and the first 23 eligible Thr54 carriers were enrolled in the study. Participants were given 2 g/d of pure EPA (four gel caps, each containing 500 mg of ethyl ester EPA 90%, a gift from Minami Nutrition, Edegem, Belgium). Two capsules were taken in the morning and two in the evening. The participants were followed weekly at the Emergency Medicine Research Center (EMRC). A checklist for weekly consumption of capsules was filled out. From each participant, a blood sample after 14 h of overnight fasting was taken at baseline and another sample was taken after 8 weeks of EPA supplementation. The study was approved by Ethic Committee of EMRC, Tehran University of Medical Sciences (TUMS). All participants were informed of the aims of the study and a written consent was obtained from them. The biochemical analyses were carried out at the EMRC laboratory, TUMS. Genetic studies were conducted at the Department of Medical Genetics, TUMS.

Anthropometric measurements

Height and weight were measured by a Seca scale (Germany) with light clothing and no shoes.
on. BMI was then calculated, and waist and hip circumferences was measured with a flexible tape.

Laboratory Analyses
Sera was separated from blood samples by centrifuging at 4 °C and 1,800×g for 15 min and stored in 1-ml aliquots in sterile tubes at -80 °C until used. Sera glucose level was measured by use of the colorimetric method with commercial kits (Pars-Azmoon, Tehran, Iran).

Genotyping
Ala 54 Thr (Gene ID = 2169): Genomic DNA was extracted as described previously [17]. A 180-bp DNA fragment containing the G to A nucleotide substitution in exon 2 (codon 54) of the FABP2 gene (Ala54Thr) was genotyped by PCR-RFLP as described previously [17].

Leu162Val (gene ID55465): The Leu162 Val mutation of the PPARα gene is caused by a C to G transversion at nucleotide 484 in exon 5. The PCR-RFLP method was performed as described previously [18].

Intron 7: The PCR-RFLP method was performed to determine intron7 mutation as described previously [18].

Sample Size:
\[ d = \frac{\overline{D}_1 - \overline{D}_2}{\sqrt{2}\sigma_d} \quad \text{where} \]
\[ n = \left( \frac{[Z_{1-\alpha/2}] + [Z_{1-\beta}]}{d} \right)^2 \]
\[ \overline{D}_1 - \overline{D}_2 = 2mm/l \quad \text{if} \]

Statistical analyses
The normality of distribution of continuous variables was tested by one sample Kolmogorov-Smirnov test. To normalize the continuous variables which were not normally distributed, a log transformation was applied. Differences between fasting blood sugar levels concentration and BMI between the two study groups with different FABP2 genotypes were tested separately by analysis of covariance, and baseline levels, gender, and age were considered as covariates.

Because only a few subjects with Thr54/Thr were found among the participants, they were pooled with Ala54/Thr subjects and analyses were carried out on the pooled data. Results are presented as mean ± standard deviation unless otherwise noted. Analyses were performed by SPSS for Windows (SPSS Inc., Chicago, IL, USA). P<0.05 was considered statistically significant.

Results
The baseline characteristics of participants stratified by gender are described previously [17-18]. For comparison of groups with different FABP2 genotypes, the data obtained from Thr54/Thr and Ala54/Thr subjects were combined. EPA supplementation decreased fasting blood sugar in Ala54 and Thr54 (p<0.001). No significant association was

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ala/Ala (n=23)</th>
<th>Ala/Thr (n=19)</th>
<th>Ala/Ala (n=23)</th>
<th>Ala/Thr (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>intervention</td>
<td>intervention</td>
<td>intervention</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>81±15.9</td>
<td>81±13.2</td>
<td>NS</td>
<td>79.4±12.7</td>
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<td>Height (cm)</td>
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<td>169.4±8.3</td>
<td>NS</td>
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<td>96.5±9.1</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>102.2±7.9</td>
<td>101.8±6.2</td>
<td>NS</td>
<td>101.6±6.1</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.2±4</td>
<td>28.1±3.2</td>
<td>NS</td>
<td>29.1±5.7</td>
</tr>
<tr>
<td>FBS(mg/dL)</td>
<td>102.9±6.9</td>
<td>93.2±7.7</td>
<td>0.001</td>
<td>101.6±6.7</td>
</tr>
</tbody>
</table>

* Fasting blood sugar; † Significant differences before and after intervention; NS- no significant differences
observed between BMI or fasting blood sugar and different FABP2 genotypes after EPA consumption (Table 1). EPA supplementation increased BMI and decreased fasting blood sugar in Lue162 (p< 0.01, p< 0.001 respectively); and Val162 (p< 0.03, p< 0.05 respectively) (Table 2), and interon 7 polymorphism (p< 0.01, 0.05 respectively) (Table 3). No interaction was observed between PPARα genotypes and degree of changes in BMI or fasting blood sugar after EPA supplementation (Tables 2-3).

Discussion

Fasting blood sugar has been measured in this study, we observed EPA consumption reduced the level of fasting blood sugar in Ala54 or Thr54 FABP2 genotype and Leu162 or Val162 PPARα genotype, and no interaction was observed between Ala54 and Thr54, Lue162 and Val162 groups. These result showed EPA consumption had effect on blood sugar without considering of genotypes. Although, Mori showed that EPA consumption increased fasting glucose insignificantly. The mechanisms responsible for the increase in fasting glucose after EPA supplementation are not known, but it may be because EPA increases hepatic glucose production or decreases hepatic insulin secretion [19]. Morcillo, et al showed that the effect of dietary fatty acids on the population pattern of insulin resistance is not independent of Ala54Thr polymorphism of FABP2. An interaction excited between this polymorphism and the intake of dietary fats in a population with a high intake of monounsaturated fatty acids [20]. Fasching et al showed that basal hepatic glucose output remained unaffected by fish oil (fish oil containing 3.8 g EPA and 2.5 g DHA ) to obese subject with impaired glucose tolerance (IGT) for 2 weeks [21]. Wanjihia et al. measured IGT according to World Health Organization diagnostic criteria. The intake of n-3 FA showed

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**Table 2. Changes in anthropometric measurements after EPA supplementation in hypertriglyceridemic subjects by PPARα genotypes (Lue162/Val)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lue162 (n=38)</th>
<th>Val162 (n=8)</th>
<th>P value</th>
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<tr>
<td></td>
<td>Before intervention</td>
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<td>Before intervention</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>78.5±12.2</td>
<td>79.1±12.1</td>
<td>0.014†</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.8±10.6</td>
<td>166.8±10.6</td>
<td>-</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>95.7±8.4</td>
<td>95.9±8.4</td>
<td>NS</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>101.6±6.3</td>
<td>101.5±6.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.3±4.8</td>
<td>28.6±4.8</td>
<td>0.012†</td>
</tr>
<tr>
<td>FBS (mg/dL)*</td>
<td>11.8±6.6</td>
<td>9.15±7.5</td>
<td>0.001†</td>
</tr>
</tbody>
</table>

*Fasting blood sugar; † significant differences between before and after intervention; NS- no significant differences

**Table 3. Changes anthropometric measurements after EPA supplementation in hypertriglyceridemic subjects by PPARα genotypes (G/C)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GG (n=24)</th>
<th>GC (n=22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Before intervention</td>
<td>After intervention</td>
<td>Before intervention</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>76.05±12.1</td>
<td>76.6±12</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.2±8.4</td>
<td>166.2±8.4</td>
<td>-</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96±9</td>
<td>95.9±8.9</td>
<td>NS</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>101.4±6.9</td>
<td>101±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.6±2.9</td>
<td>27.8±2.9</td>
<td>0.018†</td>
</tr>
<tr>
<td>FBS (mg/dL)*</td>
<td>103.5±6.45</td>
<td>92.89±7.2</td>
<td>0.001†</td>
</tr>
</tbody>
</table>

*Fasting blood sugar; † significant differences between before and after intervention; NS- no significant differences
significant inverse relationship between IGT and consumption of n-3FA and polyunsaturated fatty acids (PUFA) but no association was found between saturated fats intake and IGT [22]. The results of Takeuchi’s study showed that the influence of the dietary n-6/n-3 ratio on the serum lipid and glucose levels varies, depending on the duration and life stage of feeding [23]. Ramel et al believed that the fish oil diet reduces fasting insulin and an extent similar to that observed with a weight loss of 4.7 kg [24].

The Ala54Thr variant was not associated with overweight or obesity in our population, although it is possible that Thr54 genotypes confers some degree of susceptibility to obesity associated with an influence of the genotype on parameters related to lipid metabolism [25-27]. These results are in agreement with Karani et al.

Our study showed that EPA supplementation did not effect on BMI in Ala54 or Thr54 groups, and FABP2 genotypes not conferred to EPA consumption response. The Ala54 polymorphism of FABP2 gene showed a sexual dimorphism regarding obesity in Fisher’ study population [28], in which, the prevalence of Thr54 homozygote was 2.4 times higher in obese women than in obese men. Moreover, the Thr54 allele was significantly associated with higher BMI and elevated leptin levels in women [28]. In fact, n-3 PUFA supplementation may play an important role in preventing weight gain and improving weight loss when n-3 PUFA are supplemented concomitantly with a structured weight-loss program. Furthermore, inclusion of n-3 PUFA in a weight loss program may provide additional health benefits [1,29].

Single-nucleotide polymorphisms (SNPs) have helped to explain differences in human individuals [30]. Case-control studies of obesity and type 2 diabetes have indicated that carriers of the Val-allele have a lower BMI and body fat percentage [31] and a higher mean age at diagnosis of type 2 diabetes [32]. Yet, other studies found no associations between the polymorphism and BMI [14,15,18,21] or type 2 diabetes [33-37].

In our study a significant positive association was found between the presence of the Leu162Val allele with lower BMI, in overweight and diabetic women [39]. Vohl et al found no significant association between Leu162Val polymorphism and greater BMI, either in patients with type 2 diabetic or in non-diabetic subjects. Discrepancies among these studies may originate from differences in studied population with most studies focusing on older or unhealthy populations, and also may be due to differences in methods of measuring both regional and total adiposity [18,38]. Evans et al. [37] observed similar allelic frequency of the Leu162Val polymorphism in subjects with hyperlipidaemia and type 2 diabetes, in morbidly obese patients as well as in healthy individuals. He therefore concluded that this polymorphism has no major role in the development of these conditions. Similar results were reported in another study [40]. A study performed on healthy adults has shown that the Val carriers had significantly lower BMI and percentage of body fat in comparison to wild type, but this association was completely abolished after adjustment for total body fat and gender [31]. This is in agreement with a study that showed no effect on the BMI level [36]. According to Aldhoon, et al study the Leu162 Val polymorphism of PPARα does not influence the level of weight reduction after 2.5 year follow-up, as no differences between the genotype groups were observed. The favorable changes in energy and nutrient intake were not due to the gene effect, as no differences between genotypes were detected. They assume that this improvement is entirely due to the intervention. Similar changes in body weight, BMI, waist circumference, lipid profile, restraint and hunger scores were found in both genotype groups [41].

We observed EPA consumption increased BMI in leu162 or Val162 PPARα genotypes but no interaction was observed between leu162 and Valin162 carriers. Arai, et al showed that EPA consumption decreased body weight and plasma insulin levels in KK mice but plasma glucose did not change after EPA consumption [42].

Conclusion
Although EPA consumption has been shown the effect of EPA response on FBS in Ala54 or Thr54 in FABP2 genotypes and Leu162 or Val162 in PPARα genotypes, no interaction observed between these genotypes and EPA supplementation.
Acknowledgments

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Conflict of interest: None of the authors had a personal interest or a potential personal conflict.

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