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Impact of Polyunsaturated Fatty Acids on Body Mass Index-A Correlation Study in Type 2 Diabetic Postmenopausal Women

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Keywords

Omega- 6 fatty acid, Omega -3 fatty acid, PUFA, Postmenopausal women, Obesity, Diabetes.

Abstract

Background: The relationship of n-3 and n-6 fatty acid in diabetic postmenopausal women remains unclear and the association of theses fatty acids with obesity is debated. The pattern of PUFA among diabetic and non-diabetic women helped to clarify the correlation. The study aimed to determine the pattern of Poly unsaturated fatty acids among the diabetic case and control women and to correlate the PUFA with body mass index (BMI).

Materials and Method

Plasma PUFA was measured by gas chromatography among 120 type 2 diabetes mellitus post-menopausal women and 120 non diabetic postmenopausal women. Fasting blood samples were collected among the postmenopausal women.

Results and Discussion

Noticeable differences between the plasma phospholipid compositions among the groups were observed. Lower percentage of n-3 fatty acid was observed in diabetic postmenopausal women when compared with controls. Spearman's correlation showed an inverse relationship of EPA and DPA showed with BMI among the case group which was statistically significant ($p<0.012$; $p<0.025$).

Interpretation and conclusion

Lower EPA and DHA levels promotes obesity and metabolic abnormalities related to obesity.

1. Introduction

Altered glucose metabolism is correlated with menopause.The sudden increase in glucose level after menopause may be attributed to hyperandrogenicity which is related with hyperinsulinemia, abdominal obesity when compared with pre-menopausal women [1-2]. Though estrogen therapy after menopause aid in reducing plasma glucose level[3], its side effects have been reported. A healthy lifestyle with proper diet and physical activity will play a challenging role in disease prevention after menopause. Experimental studies reportedLinoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are omega-3 fatty acids that have anti-inflammatory [4-5], and anti- obesity [6-7]properties which boost insulin sensitivity[8-9]. Over some time, these unsaturated fatty acids have become a molecule of public interest to maintain individual's personnel health.

As structural phospholipids, polyunsaturated fatty acids (PUFA) are present in membranes and are essential for maintaining membrane fluidity. These fatty acids are involved in a wide variety of physiological processes, such as cell signalling, blood coagulation, inflammation, and blood pressure control. According to how the double bond is positioned in the carbon chain PUFA is classified as omega-3 and omega -6 fatty acids, with ALA and LA as a parent carbon respectively

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[10]. EPA and DHA are synthesized from ALA. LA is transformed to arachidonic acid.During, pre-menopause, menopause, and pregnancy the fatty acid pattern changes accordingly[11].The strong connection between obesity, inflammation, and diabetes mellitus prompted us to propose that the PUFA pattern may represent a potential risk factor for T2DM in postmenopausal women.The present study aimed to investigate the PUFA pattern in post-menopausal women with and without diabetes and to correlate the fatty acid levels with the body mass index (BMI).

2. Materials and Method

The present study included 240 postmenopausal women with the age group of 45-65 years of which 120 postmenopausal women were diabetic and 120 were non diabetic females[13]. Each participant was recruited from the outpatient department of a tertiary care hospital from Mangalore. Blood samples were collected from the recruited subjects after obtaining their consent. Women who did not menstruate for about one year and subjects with a history of diabetes, subjects with a intake of glucose and lipid modification agent were included in the study. Postmenopausal women with hormone replacement therapy, serious disorders and who had undergone hysterectomy were excluded from the study. Control group included post-menopausal women without the history of diabetes and other serious disorders. The study was approved by the ethics committee of K.S Hegde medical academy, Nitte (Deemed to be University). Women with natural menopause and who had a history of diabetes were included in the investigation. Participants with estrogen hormone therapy,supplementation with omega-3 fatty acids or any other chronic disease were excluded.

3. Clinical measurement

Information on participants' age, menopausal status, diabetic history, medication, and supplements were collected via a self-administered questionnaire. A stadiometer and a calibrated digital scale were used to measure the subject's weight (kg) and height (cm), respectively. Waist circumference and hip circumference were measured using an elastic tape. Weight in kilogrammes divided by height in metres squared was used to determine body mass index (BMI).

4. Biochemical Measurements:

Following an overnight fast, blood samples were taken from the recruited patients.Fasting blood glucose was analyzed using semi-automated biochemistry enzymatic analyser (STAR 21 PLUS,Bio chrome, Hyderbad, Telangana, India). Serum insulin was measured by ELISA kits (Biotechnology company, DiametraS.r.l, ,ViaPozzuolo, Italy). To estimate insulin resistance homeostasis was applied as HOMA = [FPG (nmol/l) * insulin (IU/ml)/405].

5. Plasma fatty acid estimation and analysis:

Fatty acids were estimated from the plasma of the recruited study subjects by the method established by Metcalfe.et.al[12]. Under alkaline condition fatty acid were extracted from the plasma (100µl) in the presence of an internal standard (C17:0, triheptadecanoin, NuChek Prep, Elysian, MN, USA) as fatty acid methyl esters (FAMEs). It includes saponification followed by the extraction of neutral lipids and fatty acid with the derivation of methyl esters. This was then analyzed on a 7820A Agilent gas chromatography- flame ionization detector, California, USA. Based on the retention duration, individual fatty acids were calculated and expressed as a percentage of the total amount of fatty acids present.

6. Statistical Analysis.

Analysis of the data wascarried out using SPSS version 16. Mean $\pm$ standard deviation were used to express parametric tests. Student t test were used to compare the data.Non parametric test were expressed as median and interquartile range. Mann-Whitney U test is employed to compare case and control. Spearman's correlation coefficient was calculated to correlate body mass index with PUFA. A p value of 0.05 or less was regarded as statistically significant.

7. Results

Menopausal women with diabetes were significantly older 57.61$\pm$ 5.9 with higher BMI 26.1 $\pm$ 4.4. Also, they had a higher level of insulin and insulin resistance than women without diabetes (Table1).

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Table 1. Baseline data of case and control women			
	Case (n=120)	Control(n=120)	*P* value
Age	57.61± 5.9	54.21 ± 4.9	0.000***
BMI	26.1 ± 4.4	24.2 ± 3.8	0.000***
FBS(mg/dL)	152 ±62	99 ±15	0.000***
Insulin (IU/mL)	18.4(13.2-22.4)	1.1 (0.42-2.28)	0.000***
HOMA-IR	6.3 (1.85 -11.7)	1.1 (0.42-2.28)	0.000***
Data that is normally distributed is shown as mean + SD. Difference between the groups is assessed by student t test The median(interquartile range) is used to express non-parametric data.The groups are compared using the Mann Whitney— U test. .*** *p* value <0.001 considered significant.			

8. Plasma fatty acid composition

There were observable distinctions between the plasma phospholipid compositions among the groups. Women with diabetes had higher levels of linoleic acid (c18:2n-6). The percentage of DGLA, and AA was also raised in the case group. When compared to non-diabetic postmenopausal women, women with diabetes exhibited considerably reduced levels of EPA (c20:5n-3) and DHA (c22:6n-3).

Table 2: Plasma fatty acid composition (%) according to diabetic status inpostmenopausalWomen

n-6PUFA	Case	Control	P-Value
c18:2n-6 (LA)	16.6(12.0-21.8)	15.1 (12.1-24.9)	0.05
c18:3n-6 (GLA)	0.6 (0.46-0.73)	0.57 (0.5- 0.9)	0.21
c20:3n-6(DGLA)	4.24 (2.9-5.7)	3.7(2.4-6.9)	0.51
c20:4n-6(AA)	4.09 (2.95-4.5)	3.6 (1.56-5.06)	0.28
n-3PUFA			
c18:3n-3(ALA)	0.32 (0.45-1.02)	0.39 (0.2-0.69)	---
c20:5n-3(EPA)	1.74 (1.11-2.7)	1.8 (1.03-3.6)	0.041*
c22:6n-3(DHA)	1.9 (1.5-2.44)	2.1 (1.32-3.1)	0.016*

Data presented as median(inter quartile range).n-6 PUFA: omega 6 polyunsaturated fatty acids. c18:2n-6 :lionoleic acid, c18:3n-6:gamma-linolenic,c20:3n-6:dihomo-gamma-linolenic acid,c20:4n-6 arachidonic acid, n-3 PUFA: omega 3 polyunsaturated fatty acids: c18:3n-3:alpha linolenic acid, c20:5n-3: Eicosapentaenoic acid, c22:6n-3: Docosahexaenoic acid

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Higher concentration of Omega-6/omega-3 ratio was observed in diabetic women when compared with non-diabetic postmenopausal women (figure:2) .Statistically significant difference existed.

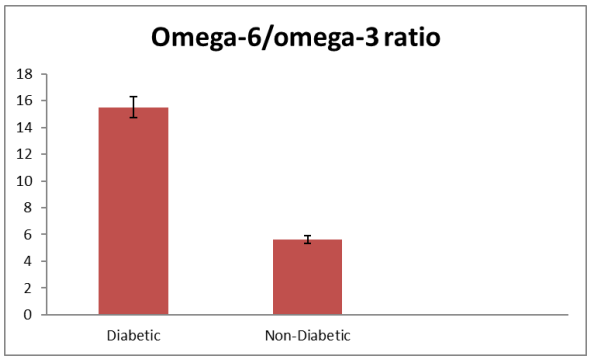

Figure II: omega-6/omega-3 ratio among diabetic and non-diabetic postmenopausal women. Data represented as mean ± std error with p <0.05 which was statistically significant.

9. Body mass index and specific fatty acid correlation

According to Spearman's correlation (r = 0.197, p0.05), there is a positive relationship between LA and BMI. In the case group, there was a statistically

significant inverse connection of EPA + DPA and BMI (r =-0.098; p0.05; r =-0.135; p0.05).

A statistically significant association was found between insulin and insulin resistance with BMI among the case group. A negative correlation was observed among healthy control women.

Table 3: Spearman's correlation of BMI with fatty acids

BMI	Case	*P* value	Control	*P* value
LA	0.197	0.05	0.16	0.28
GLA	0.50	0.71	-0.115	0.47
DGLA	0.023	0.03*	-0.003	0.98
AA	0.072	0.64	0.401	0.09
ALA	-0.011	0.92	0.17	0.39
EPA	-0.098	0.012*	0.015	0.09
DHA	-0.135	0.025 *	0.123	0.06

Spearmans correlation coefficient is calculated to correlate BMI with n-3 and n-6 fatty acids. **P Value*<0.05 and ****P*<0.001 is considered statistically significant. Abbrevations n-6 PUFA: omega 6 polyunsaturated fatty acids. c18:2n-6 :lionoleic acid, c18:3n-6:gamma-linolenic,c20:3n-6:dihomo-gamma-linolenic acid,c20:4n-6 arachidonic acid, n-3 PUFA: omega 3 polyunsaturated fatty acids: c18:3n-3:alpha linolenic acid, c20:5n-3: Eicosapentaenoic acid, c22:6n-3: Docosahexaenoic acid

10. Discussion

According to the study, in postmenopausal women with type 2 diabetes mellitus, having a higher BMI is linked to greater levels of LA, DGLA, and insulin resistance as well as lower levels of EPA and DHA. We observed that LA and DGLA had a significant positive correlation with BMI. So the present study proposes that obesity and metabolic abnormalities are related to an uncontrolled intake of omega 6 fatty acids. The parent n-6 fatty acids found mainly in plant oils are metabolized to GLA by delta-6-desaturase which is then elongated to DGLA. Studies support that DGLA is thought to have an anti-inflammatory effect [14-15]but its conversion to AA promotes obesity through the mechanism of adipogenesis [16-17]. Besides, prostaglandin E2 and leukotriene B4 is pro-inflammatory. The development of fat and chronic inflammation observed in a type 2 diabetic patient is thus explained by an excessive consumption of LA. However, changes in the hormonal milieu (estrogen) after menopause may also have a significant role in obesity-related disorders.

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Observational studies showed that higher levels of LA were associated with a decreased risk of diabetes[18-19]. Improvements in insulin sensitivity are linked to higher blood levels of LA [20-21], reduced visceral adipose tissue[22-23], and reduction in the inflammation markers[24-25]. Moreover, another study observed that when LA-oil was supplemented for women, lean mass increase, reduced trunk adipose tissue, as revealed by dual x-ray absorptiometry. However, in the present study, we did not observe any statistical differences in level of LA between the groups. Future research including a bigger sample size is required to determine the advantages of LA for postmenopausal women's glycemic management.

The present study observed a lower concentration of ω- 3 fatty acids (EPA, DHA) acids in post-menopausal women with diabetes. ALA the parent ω- 3 fatty was also seemed to be lower in the case group. ALA stimulates insulin secretion by the pancreatic β-cell-expressed G- protein receptors thereby enhancing insulin sensitivity through IGF-1 (insulin-like growth factor-1) pathways [26].

In the case group, the current investigation revealed an inverse relationship between BMI and EPA and DHA.. The effect of omega 3fatty acid on body composition has been widely studied in animal models but the report on humans is rare. The increased fatty acid oxidation in the liver and adipose tissue is what causes EPA and DHA to have anti-obesity benefits.[27]. Several studies report that the reduction of appetite by omega-3 fattyacids, increase the apoptosis of lipocyte, where some other research indicates that expression of several genes can be changed by omega -3 fatty acids that are involved in the fat mobilization of different tissues by increasing fatty acid oxidation[28]. The anti-inflammatory properties of EPA and DHA are well established.Evidences from earlier studies showed that the G- protein (GPR-120) acts as a receptor for n-3 PUFA expressed mainly in adipocytes, macrophage, and in hepatic stellate cells. EPA and DHA bind GPR-120 and inhibit TLR, TNF-α inflammatory signal pathway resulting in M1-M2 macrophage polarization in the reduction ofthe inflammatory gene expression(IL-6, TNF- α, MCP-1, IL-1b) and accerlatingthe anti-inflammatory gene expression in adipose tissue (1L-10, MGL-1)[29].

Modern western diet consist of high amount of omega -6 fatty acids and very low concentration of omega-3 fatty acids resulting in an unhealthy omega-6/omega -3 ratio. An unbalanced omega-6/omega-3ratios leads to the occurrence of atherosclerosis, diabetes and obesity [29-31]. The present study also observed a high level of omega 6/omega 3 fatty acid in the diabetic female. It has previously been reported that Omega -6 fatty acids play a vital role in the conversion of pre-adipocyte to mature adipocyte [32] and this differentiation can be removed by omega -3 fatty acids [33-36]. Omega -3 fatty acids supresses the lipogenic enzyme,increases beta-oxidation and thereby decrease the deposition of fats[37].

There were several limitations to our study. The total intake of food and fat was not estimated. So we were unable to correlate the individual fatty acid with dietary data. Secondly, Dietary fatty acid intake before the test may affect the pattern of serum fatty acid composition. Further studies with a larger sample are recommended in obese diabetic postmenopausal women that whether these fatty acids actually play a role in obesity that may help in preventing type 2 diabetes mellitus.

11. Conclusion

In postmenopausal women with diabetes, the findings of this investigation revealed that the serum level of the omega 3 fatty acid (EPA and DHA) is reduced.Thus, a different pattern of PUFA is observed among post-menopausal women with and without diabetes. These findings shows that it is necessaryto estimate the dietary habits of women after menopause which aids in preventing the obesity-related metabolic conditions.

12. Conflict of interest

We declare that we have no conflict of interest

13. Acknowledgement

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