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Inverse Associations of Overall and Abdominal Obesity with Plasma N-3 Polyunsaturated Fatty Acids in Iranian Adults

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ABSTRACT

Introduction: Obesity is a common health problem, which leads to diseases such as hypertension, cardiovascular diseases, hyperlipidemia, diabetes, gout, gall bladder and liver diseases, cancer, and depression. The prevalence of obesity has risen significantly across the world, especially in the Asia-Pacific region.Omega-3 is a polyunsaturated fatty acids with several beneficial effects on diseases such as diabetes, hypertension, rheumatoid arthritis, bipolar disorder, and asthma. The present study aimed to assess the levels of omega-3 fatty acids in subjects with various body mass indices (normal weight, overweight, and obese) and waist circumference (no risk, high-risk, and extremely high-risk).

Methods: This cross-sectional study was conducted on 151 subjects, including 58 men and 93 women (age range: 18-65 years), who were randomly selected from the patients attending the nutrition clinics in Mashhad, Iran at 2014. The participants were divided into three groups based on the body mass index (BMI; normal weight, overweight, and obese) and waist circumference (WC; no risk, high-risk, and extremely high-risk). Plasma levels of omega-3 fatty acids were determined in the blood sample collected from the participants using the gas chromatography apparatus equipped with a flame ionization detector (GC-FID). The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS), and written informed consent was obtained from all the participants.

Results: Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) had inverse associations with overall obesity (P<0.001, P<0.001, and P<0.001, respectively) and abdominal obesity (P=0.02, P=0.005, and P<0.001, respectively). Moreover, ALA, EPA, and DHA were inversely correlated with BMI (P<0.001, P<0.001, P<0.001, respectively) and WC (P=0.001, P<0.001, P<0.001, respectively). The differences in the levels of ALA and DHA were not statistically significant between men and women, while women had significantly higher levels of EPA compared to men (P=0.02).

Conclusion: According to the results, plasma omega-3 polyunsaturated free fatty acids were inversely associated with the anthropometric indices of obesity, including BMI and WC. It is recommended that further investigations be conducted to identify the possible mechanisms linking the levels of plasma omega-3 fatty acids with overall and abdominal obesity.

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Introduction

Obesity is a common health problem, which may lead to hypertension, cardiovascular diseases, hyperlipidemia, diabetes, gout, gall bladder and liver disease, cancer, depression,

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and other health complications (1-6). According to statistics, the presence of the comorbidities associated with obesity results in the reduction of life expectancy by 5-20 years (7).

The prevalence of obesity has been on the rise in various regions in the world, especially in the Asia-Pacific region (8). For instance, the prevalence of obesity in the Iranian population increased from 16.5% in 1998 to 20.8% in 2001 (9), and the combined prevalence of overweight and obesity in Japan increased from 16.7% during 1976-1980 to 24.0% in 2000 (8). It is estimated that over one billion individuals will be affected by obesity by 2030 worldwide with the continuation of the current trend of obesity (10).

Omega-3 fatty acid is a polyunsaturated fatty acid (PUFA) (11). The 18-carbon alpha-linolenic acid (ALA) and linolenic acid (LA) are known as essential fatty acids since mammalian cells cannot synthesize these components de-novo (12). ALA is the parent n-3 fatty acid, which could be metabolized into longer n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (13). Since the conversion of ALA into EPA and DHA is extremely inefficient (14, 15), these fatty acids cannot be synthesized adequately in the human body and must be obtained from dietary sources. Seafood sources (e.g., fish and fish oil supplements) have been reported to provide variable amounts of n-3 fatty acids in the form of EPA and DHA (16, 17).

In humans, n-3 fatty acids are primarily concentrated in the central nervous system, retina, and testicles (18). According to the literature, n-3 PUFAs have numerous health benefits (19). For instance, n-3 fatty acids have been shown to positively influence diabetes (20), hypertension (21), rheumatoid arthritis (22), bipolar disorder (23), and asthma (24). Furthermore, there are reports regarding the prevention of cardiovascular diseases and cancer by n-3 fatty acids (25-27).

Several studies have indicated that obese individuals have lower levels of plasma n-3 fatty acids compared to those with normal weight (28-30). In a study conducted on 124 adults, an inverse correlation was observed between the plasma concentrations of n-3 PUFAs and anthropometric indices of obesity, including body mass index (BMI), waist circumference (WC), and hip circumference (28). In another study performed on 100 obese and 100 non-obese individuals, significantly higher levels of ALA and docosapentaenoic acid (DPA) were reported in the obese patients, while the DHA levels were negatively correlated with BMI, WC, and plasma triglyceride levels. However, no significant difference was observed in the plasma levels of EPA and total n-3 fatty acids between the obese and non-obese subjects (29). Despite the studies conducted in this regard, limited research has been focused on investigating the plasma levels of n-3 PUFAs in the individuals with various BMI and WC.

The present study aimed to determine the levels of n-3 fatty acids in the individuals with various BMI (normal weight, overweight, and obese) and WC (no risk, high-risk, and extremely high-risk).

Material and methods

Study Population

This cross-sectional study was conducted on 151 individuals (mean age: 35.6±9.7 years), including 58 men (38.4%) and 93 women (61.6%), who were randomly selected from the patients attending the nutrition clinics in Mashhad, Iran. The selected participants had no history of diabetes, hypertension, and systemic diseases (e.g., lupus erythematosus). In addition, pregnant and lactating women and those consuming nutritional supplements and medications were excluded from the study.

The participants were divided into three groups based on the BMI, including normal weight (BMI≥18.5 kg/m2and <25.0 kg/m2; n=52), overweight (BMI≥25.0 kg/m2 and <30 kg/m2; n=52), and obese (BMI≥30.0 kg/m2; n=47), as well as WC, including no risk (WC<94 cm [M]; <80 cm [W]; n=82), high-risk (WC>94 and <102 cm [M]; >80 and <88 cm [W]; n=22), and extremely high-risk (WC ≥102 cm [M]; ≥88 cm [W]; n=47).

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS), and written informed consent was obtained from all the subjects prior to participation.

Anthropometric Assessment

Anthropometric measurements were

performed while the participants were in light clothing without shoes. BMI was calculated as weight in kilograms (kg) divided by height in meters squared (m2). WC was measured horizontally at the midpoint between the lowest rib and top of the hip bone. The classification of BMI and WC was based on previously defined cutoff points (31). Moreover, bioelectrical impedance analysis (model: BC-418 MA, Japan) was used to measure the body composition of the subjects, including the fat mass (FM), fat-free mass (FFM), and total body water (TBW). The measurements were performed after overnight fasting with no alcohol consumption or physical activity within 24 hours before the tests.

Plasma Fatty Acid Analysis

Plasma fatty acid was assessed using gas chromatography (CG) (Varian 450-GC, USA) as previously described (32). Briefly, plasma lipids were extracted using a mixture of chloroform/methanol (MeOH) (33). For KOH derivatization, the extracted lipids of the samples were dissolved in 200 milliliters of hexane and 100 milliliters of methanolic KOH (2mol/l). After five minutes of incubation at room temperature, 40 milligrams of sodium bisulfate was added, and the supernatant was collected (34). A gas chromatography device equipped with a flame ionization detector (GC-FID) was applied for the final analysis of the supernatant and to determine the plasma levels of n-3 fatty acids. The results of the measured level of fatty acids were expressed as microgram per milliliter (µg/ml).

Statistical Analysis

All the experiments were performed in triplicate. Data analysis was performed in SPSS version 18 (SPSS Inc., IL, USA), and the normality of the data was evaluated using the Kolmogorov-Smirnov test. In addition, descriptive statistics (mean, frequency, and standard deviation [SD]) were used for all the variables and expressed as mean±SD for the normally distributed variables and as median and interquartile range (IQR) for the variables with non-normal distribution. For the normally distributed variables, the analysis of variance (ANOVA) was applied, and Mann-Whitney U test was employed for the variables with non-normal distribution. All the statistical analyses were two-sided, and the P-value of less than 0.05 was considered significant.

Results

General Characteristics of the Subjects

Demographic and anthropometric characteristics of the participants are presented in Table 1. In total, 52 subjects with normal weight, 52 overweight subjects, and 47obese subjects were enrolled in the study. Among 151 adults, the mean age was 35.6±9.7 years, 38.4% of who were male. In addition, the number of overweight men was higher than the number of the women with normal weight and obese women (Table 1). No significant difference was observed in the mean age of the participants between the study groups (P=0.06).

Table 1. Demographic and Baseline Characteristics of Study Population

		N(M:F)	Age (year)	Height (cm)	BMI (kg/m^2)	WC (cm)	FM (%)	FFM (kg)	TBW (kg)
Total		151(58:93)	35.6±9.7	163.8±8.9	27.2±5.1	88.3±17.1	28.8±8.9	51.4±10.3	37.7±7.5
BMI	Normal Weight	52 (13:39)	33.0±10.4	162.4±8.6	22.2±2.1	72.6±4.0	23.3±7.1	45.0±7.8	33.0±5.7
	Overweight	52 (31:21)	37.0±8.8	166.6±8.1	26.7±1.2	84.6±8.4	27.4±6.3	54.3±9.0	39.8±6.6
	Obese	47 (14:33)	36.9±9.4	162.3±9.6	33.2±3.8	109.7±17.1	36.4±7.7	55.5±10.8	40.7±7.9
P-value			0.06	0.01	<0.001	<0.001	<0.001	<0.001	<0.001

Legend: Values expressed as mean ±SD or N; WC: waist circumference; FM: fat mass; FFM: fat-free mass; TBW: total body water. Results with P<0.05 were considered statistically significant.

As expected, WC and FM were significantly higher in the obese subjects compared to the overweight subjects and those with normal weight. Furthermore, the overweight group had a significantly higher WC and FM compared to the group with normal weight. On the other hand, FFM and TBW were significantly lower in the subjects with normal weight (Table 1). According to the information in Table 2, there were significant inverse correlations between ALA, EPA, and DHA and higher overall obesity (P<0.001, P<0.001, and P<0.001, respectively)

and abdominal obesity (P=0.02, P=0.005, and P<0.001, respectively).

Table 2. Comparison of N-3 Fatty Acid Levels between Various Categories of Body Mass Index (BMI) and Waist Circumference (WC)

	Overall Obesity			Abdominal Obesity		
	Normal Weight (18.5≤BMI<25)	Overweight (25≤BMI<30)	Obese (BMI≥30)	No Risk (Men<94; Women<80)	High-risk (Men: 94-102; Women: 80-88)	Extremely High-risk (Men≥102; Women≥88)
	N=52	N=52	N=47	N=82	N=22	N=47
ALA (µg/ml)	32.1 (15.8-48.6)	15.4 (10.7-24.5)	14.2 (9.7-19.9)***	22.6 (13.5-40.8)	17.7 (10.9-29.4)	14.2 (9.7-18.7)*
EPA (µg/ml)	59.1 (30.5-80.5)	35.0 (14.7-59.3)	22.3 (8.5-59.4)***	51.4 (25.6-68.3)	39.5 (12.4-66.3)	24.8 (7.7-52.2)**
DHA (µg/ml)	138.8 (86.7-214.8)	88.8 (45.9-141.8)	61.0 (33.0-98.4)***	122.7 (65.5-178.3)	74.5 (43.7-138.2)	61.0 (37.6-104.2)***

Legend: Values expressed as median and interquartile range; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. Results with P<0.05 were considered statistically significant.*P<0.05, **P<0.01, ***P<0.001

The associations between plasma n-3 polyunsaturated fatty acids and anthropometric indices (BMI, WC, and FM) are presented in Table 3. Accordingly, there were inverse correlations between ALA, EPA, and DHA and BMI (P<0.001, P<0.001, P<0.001, respectively) and WC (P=0.001, P=0.001, P<0.001, respectively). Moreover, there were significant, inverse correlations between ALA and DHA and FM (P=0.03 and P<0.001, respectively), while the inverse correlation between EPA and FM was not considered statistically significant (P=0.3) (Table 3).

Table 3. Associations between Plasma N-3 Fatty Acid Levels and Anthropometric Indices (BMI, WC, and FM)

	ALA	EPA	DHA
BMI (kg/m²)	-0.33*	-0.34*	-0.42*
WC (cm)	-0.31*	-0.38*	-0.43*
FM (%)	-0.20*	-0.1	-0.32*

Legend: Spearman's correlation-coefficient was used to explore the correlations between omega-3 fatty acids and anthropometric measurements. BMI: body mass index; WC: waist circumference; FM: fat mass; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; *P<0.05.

According to the information in Table 4, there were no significant differences in the age, BMI, and WC between men and women. Although the differences in ALA and DHA were not considered statistically significant between the study groups, women were observed to have significantly higher EPA levels compared to men (P=0.02).

Table 4. Comparison of N-3 Fatty Acid Levels between Men and Women

	N (%)	Age (year)	BMI (kg/m²)	WC (cm)	ALA (µg/ml)	EPA (µg/ml)	DHA (µg/ml)
Men	58 (38.4)	36.91±3.39	27.75±5.00	87.68±14.50	15.5 (10.1-30.0)	28.4 (14.0-61.6)	90.6 (46.2-156.4)
Women	93 (61.6)	34.86±9.58	26.90±5.22	88.71±18.72	17.7 (12.7-40.6)	47.7 (24.9-74.3)	87.0 (50.8-146.8)
P-value		0.2	0.3	0.7	0.09	0.02	0.7

Legend: Values expressed as mean±SD or median and IQR; Results with P<0.05 were considered statistically significant; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Discussion

According to the results of the present study, obese and overweight subjects had significantly lower plasma concentrations of n-3 PUFA compared to the individuals with normal weight. Moreover, plasma n-3 PUFA was negatively correlated with overall obesity (reflected by BMI) and abdominal obesity (reflected by WC).These findings could be due to the differences in the dietary patterns and lifestyle. The consumption of fast food, meat, and saturated fats are often higher in obese individuals compared to the general population, and the presence of subcutaneous fat tissues further increases the level of palmitic acid in the bloodstream.

Similar findings have been reported by Micallef et al. (2009), who observed an inverse correlation between the plasma concentrations of n-3 PUFAs and anthropometric indices associated with obesity (BMI, WC, and hip circumference) in 124 adults (28). However, the inverse correlation observed between ALA and anthropometric indices of obesity was not considered statistically significant, which is inconsistent with our findings. In the mentioned study, total n-3 PUFA was reported to be significantly lower in obese patients compared

to the subjects with normal weight (28).

In a study conducted on100 obese and 100 non-obese individuals, significantly higher levels of ALA and docosapentaenoic acid (DPA) were observed in obese patients, whereas DHA levels were negatively correlated with BMI, WC, and plasma triglyceride levels. However, no significant difference was observed in EPA and total n-3 FA between the obese and non-obese subjects (29). Another study in this regard was performed on 10 obese adolescents and 15 lean control subjects, and the findings indicated the lower serum phospholipid (PL) concentration of n-3 PUFA in the obese individuals compared to the lean females, especially in terms of the level of DHA (30). In addition, n-3 PUFAs were observed to be inversely correlated with all subcutaneous adipose tissue (AT) compartments in the mentioned research, with the exception of visceral AT. However, interpreting the results must be with caution due to the small sample size of the present study (30). Similarly, Klein-Platat et al. reported significantly higher DHA and total n-3 PUFA levels in plasma PLs and cholesterol esters (CEs) of adolescents with normal weight compared to overweight adolescents (35).

Scaglioni et al. (2006) conducted a study on 67 normolipidemic obese children and 67 age- and gender-matched children with normal weight, and negative correlations were observed between the Z-score of BMI and plasma PUFA, n-3 PUFAs, and DHA with C22:6 n-3-to-C20: 6 n-6 and C22:6 n-3-to-C18: 3 n-3 ratios in obese children (36). However, Gil-Campos et al. denoted significantly higher concentrations of 16:1n-7, 16:1n-9, 18:3n-3, 22:6n-3, and n-3 PUFA in the total plasma lipids of the obese subjects with metabolic syndrome and children with normal weight (37). The generalization of the mentioned findings should be done cautiously due to the small sample size and characteristics of the subjects (obese prepubertal children).

In another research, Howe et al. denoted a gender-dependent correlation between erythrocyte n-3 content and obesity. Moreover, similar erythrocyte levels of EPA and DHA were observed in men and women; however, men tended to have slightly lower EPA and DHA (omega-3 index) compared to women (38). On the other hand, inverse correlations were observed between DHA and EPA+DHA with BMI, WC, and body fat (BF) in women, while DPA was inversely correlated with BF in men (38). In contrast, Ogura et al. observed no significant difference in the PUFA values in plasma and erythrocyte PLs between men and women, which is consistent with the results of the present study, with the exception of EPA, which was significantly higher in women in our research (39).

The main strengths of our study were the population-based method and considering both overall obesity (reflected by BMI) and abdominal obesity (reflected by WC) in the analyses. The main limitations of the research were the larger number of female subjects (61.6%) and not explaining the possible mechanisms through which obese patients have lower plasma n-3 PUFA levels. Other limitations of the study were the lack of food frequency questionnaire and not determining its association with serum FAs, which may affect the interpretation of the final results.

Conclusion

To the best of our knowledge, this was the first study to analyze the correlation between plasma n-3 PUFAs in healthy participants. According to the results, plasma n-3 PUFAs and anthropometric indices were correlated. However, due to the observational design of the study, we could not clarify the FAs with stronger effects. Therefore, it is recommended that further investigation be conducted to determine the possible mechanisms linking plasma n-3 FAs with overall and abdominal obesity.

Conflict of interest

None declared.

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