Obesity and smoking increases the risk of coronary heart diseases by lowering the omega-3 index: a cross-sectional study

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Background: Omega-3 index is considered as a potential risk factor for coronary heart disease (CHD) morbidity and mortality, especially sudden cardiac death.
Methods: A cross sectional study was conducted on randomly selected groups of overweight or obese Jordanian adult smokers and normal-weight, non-smoker controls in order to evaluate the omega-3 index among the selected groups. A total of one hundred and fifty Jordanian adults aged between 19 to 45 years from both genders was recruited from a private internal clinic and divided into four groups, where the ratio of healthy control group to the other selected groups was 2:1. Total fatty acids (FAs) in red blood cells (RBC) was determined using gas chromatography (GC), and omega-3 index was determined by adding the concentration percentages of Eicosapentaenoic (EPA) and Docosahexaenoic acid (DHA) in the membranes of RBCs expressed as the percentage of total FAs.
Results: Normal-weight participants had significantly ($p < 0.05$) the highest omega-3 index (4.27%±0.21), while the lowest index was among smokers' participants (1.82%±0.25).
Conclusion: Overweight and/or obesity, as well as smoking, decreases omega-3 index that will increase the risk of CHD. Therefore, attention should be given to normalise body weight and to avoid smoking to improve omega 3 index and decrease the risk of CHD.

Keywords: Coronary heart disease, Omega-3 index, obesity, smoking.

1. INTRODUCTION

Omega-3 index is a concept defined as the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the red blood cell (RBC) membranes expressed as the percentage of total RBCs fatty acids (FAs) [1].
The FA composition in RBC membrane represents an indicator for the long-term consumption (3-4 months) of dietary FAs as well as the nutritional and health status in adults [2].
Omega-3 index has been suggested as a risk factor for cardiovascular diseases. It is inversely associated with the risk of cardiovascular diseases and sudden cardiac death. In addition, Omega-3 index represents the individual status DHA and EPA levels which is reflected by the long-term RBCs membranes concentrations of EPA and DHA [3]. In 2009, William Harris defined the Low, intermediate and high-risk levels of omega-3 index, to be > 8%, 4% – 8%, and < 4%, respectively [4].
The omega 3 index levels were varied among different populations. It was found to be low to moderate between 3-6% in USA and other western countries [5]. In Canada, Germany and Spain it was reported to be 4.5%, 7.15% and 7.1% respectively [6]. The highest Omega-3 index was reported in Japan (9.58%) and in Korea (11.81%) that could be explained by the high consumption of fatty fish

that is the richest source of omega 3 fatty acids [7].
Omega-3 index is generally not affected by the fasting or feeding state since the half-life of RBCs is 4 to 6 times longer than plasma. This low biological variability of omega-3 index makes it useful to be used in epidemiological studies [4]. Although, it has been found that omega 3 index is affected by age, gender, race, smoking, obesity, alcohol consumption and the intake of omega-3 supplements [6].
Alpha-linolenic acid (ALA), EPA and DHA are examples of Omega-3 polyunsaturated fatty acids (PUFAs). The ALA is an essential dietary FA mainly found in flaxseed, walnuts and canola oil (plant-derived Omega-3 FAs). Also, it is the precursor of other long-chain omega-3 FAs in vivo such as EPA and DHA [8].
EPA and DHA are considered essential FAs. The in vivo conversion of ALA to EPA and DHA is inefficient, and therefore, the consumption of these two FAs is a must from their food sources to satisfy health requirements. Omega 3 found in eggs and meat but Fatty fish such as salmon, albacore tuna, sardines, herring and mackerel, are considered the most and the richest food sources of omega 3 [9].
Omega-3 FAs (and its related metabolic changes) play a major role in improving body composition and counteracting obesity by the regulation of adipokines (such as leptin and adiponectin), modulation of lipid metabolism, mitigating the inflammation of adipose tissue, alteration of epigenetic mechanisms and by promoting adipogenesis [10].
To the best of our knowledge, no previous studies were conducted to evaluate omega 3 index among Jordanians Therefore, it is critically important to evaluate the level of omega-3 index in Jordanian blood and to examine the effect of body weight and smoking on omega 3 index levels. Thus, we can develop effective strategies and nutrition awareness campaigns particularly about the benefits of omega-3 daily intake. The current study aims to examine if there is a difference between the omega-3 index levels among overweight and /or obese adult smokers and the normal-weight non-smokers controls.

2. MATERIALS AND METHODS

2.1 STUDY DESIGN AND PARTICIPANTS

A cross sectional study was conducted on 150 participants, to evaluate the omega-3 index in separate groups of normal-weight, overweight, obese or smokers' adult subjects in Amman, Jordan. The ratio of normal-weight control group to the other selected groups was 2:1.

2.2 HUMAN PARTICIPANTS AND THEIR INCLUSION AND EXCLUSION CRITERIA

Subjects were recruited from a private clinic under the supervision of a physician and were divided into 4 age and gender matched groups. Medical reports for each participant were obtained from the same clinic, while personal, demographic, anthropometric and medical history were obtained through a personal interview. Those who agreed to participate were asked to sign a witnessed written consent form.

2.3 INCLUSION AND EXCLUSION CRITERIA

Participants aged between 19 to 45 years old, free from chronic diseases, non-alcoholics, and not taking omega 3 or fish oil supplements 4 months prior to the study time were included in the study. Meanwhile, those aged less than 19 and older than 45, suffer from chronic diseases, alcoholics, smokers (except for the smokers' group) and participants on medications and /or omega 3 or fish oil supplements were excluded from the study.

2.4 STUDY GROUPS

The study participants were divided into four groups as the following:
Group 1 (controls): 60 normal-weight (BMI 18.5-24.99 kg/m^2; WHO, 2004) adults, aged between 19-45 years old from both genders.
Group 2 (obese): 30 obese (BMI > 30 kg/m^2; WHO, 2004) participants, aged between 19-45 years old from both genders.
Group 3 (overweight): 30 overweight (BMI 25.00 – 29.99 kg\m^2; WHO, 2004) participants, aged between 19-45 years old from both genders.
Group 4 (smokers): 30 normal weight (BMI 18.5-24.99 kg/m^2; WHO, 2004) smokers, aged between 19-45 years old from both genders.

2.5 SAMPLE SIZE DETERMINATION

The required sample size for male and female groups used in this study was determined using the following equation [11]:

$$n = 2 \times (Z_\alpha + Z_\beta)^2 \times SD^2 \div \delta^2$$

Where n is the required number of subjects of male and female group of the study, Z_α, Z_β are the values of the standard normal distribution at specific levels of confidence 95% given a two-sided α of 0.05=1.96 and a power of 80%, $Z_{0.20}$ = 0.84. SD is the standard deviation (Pooled) and δ is the estimation of the difference between both groups (males and females). According to the above equation, the sample size calculation showed that approximately 55 subjects will be required for each arm of the trial to detect a change of 0.8 in the omega 3 index with 80% power and 5% significance. The standard deviation (SD) was assumed to be 1.5 [12].
$n = 2 \times (1.96 + 0.84)^2 \times (1.5)^2 \div (0.8)^2 \approx 55$ subject per group.
To increase the power of the analysis the number of

subjects was increased to 70 subjects at least per group

2.6 BLOOD SAMPLE COLLECTION

Blood samples were collected at laboratory centre in Amman by the responsible physician after ensuring that participants were free from diseases (through the revision of at least 3 months laboratory medical reports). Ghazzawi, 2011 methodology were followed for blood sample collection and fatty acids profile assessments [4].

2.7 DETERMINATION OF OMEGA-3 INDEX

The fatty acids methyl esters FAMEs were determined by comparison of retention time with a standard mixture of known concentrations of cis-4,7,10, 13,16,19-DHA and EPA Method (Al-Ismail et al., 2011). The Omega-3 index was calculated by taking the summation of DHA and EPA RBCs membranes concentration percentages as the following:
Omega-3 index = % DHA + % EPA [4, 5].

2.8 DIETARY ASSESSMENT

A three-day food record (2 working days and 1 weekend) was obtained from each participant for a dietary assessment in order to evaluate the total consumption of dietary n-3 FAs. Participants were provided with verbal and written instructions on how to record their food intake and describe, (accurately) in details, food portion size, food quality and preparation methods during the initial interview with each participant.
After analysing the data using ESHA- food processor software, results were correlated with blood n-3 index.

2.9 STATISTICAL ANALYSIS

Statistical analysis was conducted using Statistical Package for Social Science version 22 (SPSS for Windows, Rel. 22.0, 2013, Chicago: SPSS Inc.). Descriptive statistics were performed to identify the means+standerd error of the means for all continuous variables and frequencies with percentages were used to describe the categorial variables. One-way analyses of variance (ANOVA) and student t-test was used to compare the difference between the normally distributed means of omega-3 index among the four study groups. All reported p-values were 2-tailed and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 SELECTED CHARACTERISTICS OF THE STUDY PARTICIPANTS

Table I shows that a statistically significant ($p < 0.05$) difference in mean body weight was observed between the different groups of participants where, the obese group had the highest (93.83±2.33) mean body weight compared to overweight (80.47±1.92), normal-weight and smokers' participants (63.83±1.25; 62.20±1.10) respectively. However; no significant ($p \geq 0.05$) difference was found in the mean weight between normal weight and smokers' participants (63.83±1.25; 62.20±1.10) respectively. Similar results were found between the study groups regarding their BMI (Table I).

3.2 EVALUATION OF OMEGA-3 INDEX

Table II: shows the blood (RBCs) mean levels of DHA and EPA as well as the omega-3 index (calculated as %EPA + %DHA) among different participants. Data from the table indicated that omega-3 index was significantly ($p < 0.05$) different between the four study groups. Normal-weight participants were having significantly ($p < 0.05$) the highest omega-3 index (4.27%±0.21) followed by overweight (2.92%±0.23) and obese (2.39%±0.29) while, the lowest omega-3 index was observed among smokers' participants (1.82%±0.25) (Table II).

3.3 DIETARY INTAKE OF n-3 INDEX FAS

Table III shows the dietary intake of n-3 FAs in g/day obtained through the 3-day food record for the different participants, data reveals that overweight and obese participants had significantly ($P < 0.05$) higher n-3 FAs intake (0.71±0.07 and 0.69±0.07, respectively) than healthy and healthy smokers (0.44±0.05 and

Table I - General demographic and anthropometric characteristics of the study groups

	Variable	Normal-weight (n = 60)	Smokers (n = 30)	Overweight (n = 30)	Obese (n = 30)	*p*-value
Anthropometric measurements	Height (cm)	167.31±1.17[a]	167.2±1.54[a]	170.93±1.82[a]	170.07±1.8[a]	0.216
	Weight (kg)	63.83±1.25[c]	62.20±1.10[c]	80.47±1.92[b]	93.83±2.33[a]	**0.001**
	BMI (kg/cm^2)	22.70±0.24[c]	22.26±0.28[c]	27.44±0.25[b]	32.40±0.58[a]	**0.001**
Demographic characteristics	Age (year)	30.79±1.06[b]	26.87±1.02[c]	32.23±1.27[ab]	34.8±1.49[a]	**0.001**
	Gender n (%)					
	Male	32 (53.3)	14 (46.7)	15 (50.0)	16 (53.3)	0.935
	Female	28 (46.7)	16 (53.3)	15 (50.0)	14 (46.7)	

A, b, c: Data are presented as mean ± SEM, and were considered statistically significant at $p < 0.05$

Table II - RBC level of EPA, DHA and Omega-3 index among participants

Variable (%)	Normal-weight (n = 60)	Smokers (n = 30)	Overweight (n = 30)	Obese (n = 30)	*p*-value
EPA	1.17± 0.19[a]	0.49± 0.14[b]	0.81± 0.15[ab]	0.50± 0.14[b]	0.009
DHA	3.10± 0.15[a]	1.33± 0.24[c]	2.11± 0.26[b]	1.89± 0.30[bc]	0.001
Omega-3 index	4.27±0.21[a]	1.82±0.25[c]	2.92±0.23[b]	2.39±0.29[bc]	0.001

A, b, c: Data are presented as mean ± SEM, and were considered statistically significant at $p < 0.05$
EPA: Eicosapentaenoic acid, DHA: *Docosahexaenoic acid*

Table III - Dietary intake of n-3 FAs in g/day obtained through the 3-day food record for the different participants,

Variables	Healthy n=60	Healthy Smoker n=30	Overweight n=30	Obese n=30	*P*-value
Energy (kcal)	1268.20±53.81[c]	1173.99±39.15[c]	2169.76±57.08[b]	2426.30±98.20[a]	0.001
Calories from fat (kcal)	476.31±23.58[c]	434.65±21.60[c]	824.57±36.30[b]	976.48±49.41[a]	0.001
Calories from sat fat (kcal)	156.75±7.70[c]	141.97±9.08[c]	270.02±15.91[b]	315.63±19.13[a]	0.001
Protein (g)	55.50±2.05[b]	52.05±2.07[b]	87.78±6.10[a]	97.87±4.58[a]	0.001
Carbs (g)	145.07±6.49[b]	135.30±5.04[b]	259.07±8.02[a]	271.42±11.94[a]	0.001
Fat (g)	53.12±2.63[c]	48.50±2.41[c]	92.00±4.04[b]	108.91±5.50[a]	0.001
Saturated Fat (g)	17.42±0.86[c]	15.77±1.01[c]	30.00±1.77[b]	35.07±2.13[a]	0.001

A, b, c: Data are presented as mean ± SEM, and were considered statistically significant at $p < 0.05$
* Data are presented as mean ± SEM, and were considered statistically significant at $p < 0.05$

0.34±0.04, respectively). In addition, obese and overweight had a significantly (*P* <0.05) higher PUFA intake (14.20±0.99 and 14.14±0.95, respectively) than healthy and healthy smokers (7.80±0.69 and 7.31±0.53, respectively).

Moreover, overweight and obese participants had significantly (*P* <0.05) higher ALA intake (0.79±0.08 and 0.78±0.07, respectively) than healthy and healthy smokers (0.49±0.06 and 0.39±0.04, respectively). On the other hand, there was no significant (*P* ≥0.05) differences in the dietary intake of SDA, EPA, DPA and DHA between different participants.

With respect to the dietary intake of ETA, obese and overweight had a significantly (*P* <0.05) higher intake (0.12±0.02 and 0.11±0.02, respectively) as compared with healthy and healthy smokers (0.06±0.01 and 0.05±0.01, respectively).

4. DISCUSSION

The association between body weight and omega-3 index was examined. Results of the current study revealed that omega-3 index was higher in normal-weight participants as compared with other participants groups. The objective of the study was to evaluate the omega-3 index among normal-weight, overweight, obese or smokers' Jordanian adults.

William Harris in 2009; proposed high, intermediate and low risk levels of the omega-3 index to be < 4%, 4%–8%, > 8%, respectively. Data from Table II has revealed that omega-3 index among smokers, overweight and obese was between f 1.82% - 2.92% which indicated that these groups are at higher risk and are less protective. Whereas, normal-weight participants had a omega-3 index value of 4.27% that is slightly above that of the high-risk level (least protection) and falls within the intermediate risk level.

In general, omega-3 Index is higher in countries that consume more omega-3 FAs sources [13]. Furthermore, the richest dietary source of DHA and EPA is fish oil particularly from fatty fish [14]. This could be attributed to the fact that Jordan is a country with larger inland populations and comparatively small fish resources, which explains our results. In addition, the dietary intake of plant sources of omega-3 FAs such as flaxseeds and flaxseed oil, walnuts and walnut oil, soybeans and soybean oil, pumpkin seeds, rapeseed and (canola) oil are limited.

Results from the current study have revealed that normal-weight participants had significantly (*p* <0.05) higher omega-3 index than obese (2.39%±0.29) participants, this is in agreement with the findings of Burrows *et al.*, (2011) [15], who showed that omega-3 index in non-obese children (5.0%±1.4) was higher but not significant (*p* ≥0.05) than obese children (4.4%±1.4). In a cross-sectional sample of older Australian adults, non-obese female participants had significantly (*p* <0.05) higher omega-3 index than obese ones. In addition, and according to the same study, it was reported that DHA was inversely associated with BMI; therefore, suggesting an inverse association between anthropometric measurements and omega-3 index [12], this finding agrees with our

results. In addition, the inverse association between long-chain omega-3 PUFA levels in plasma or RBC and different anthropometric measurements was found in 3 small studies including 124 adults, 48 children and adolescents [15, 16, 17].

It has been demonstrated that low RBC levels of DHA and EPA are associated with high levels of oxidative stress among obese participants [18]. Nevertheless, data from animal studies has revealed that impaired hepatic metabolism of long-chain omega-3 PUFAs may also be a factor, because activity and expression are key enzymes that play a major role in EPA and DHA synthesis from ALA are altered by obesity [19]. It was reported that omega-3 FAs exhibit anti-inflammatory properties, which explains our results regarding normal-weight controls as having higher omega-3 index as compared with overweight and obese participants [20]. In the current study, smoking participants were recruited with a normal BMI, to study the effects of smoking on omega-3 index apart from overweight and obesity. The results of the current study showed that omega-3 index was significantly ($P < 0.05$) higher in normal-weight participants as compared with smokers, this finding is in agreement with the findings of Scaglia, [21], who showed that omega-3 index was significantly ($P < 0.05$) higher in non-smokers as compared with smokers. In addition, Scaglia have found that the level of DHA in RBC of non-smokers participants was significantly ($P < 0.05$) higher than in smokers, which is consistent with our results (7286.52%±933.55 vs 3711.47%±856.88) [21]. The low omega-3 FAs level among smokers could be explained by the metabolism of omega-3 FAs. It was reported that the absorption, metabolism and synthesis of FAs could interfere with tobacco smoking. Another proposed explanation is that PUFAs are more susceptible to oxidation, and smoking plays an important role in the oxidation process (has a potent oxidant role) [22, 23]. Some researchers have found that smokers have higher rates of reactive oxygen species as well as other free radicals [24]. Moreover, Simon, *et al*. (1996), found that the concentration of DHA and other PUFAs in the smokers' blood was significantly ($p < 0.05$) lower than in non-smokers, that is consistent with our results. He also found an inversely proportional relationship between the level of DHA in RBC and smoking; smokers are more susceptible to a decrease of 30% in DHA concentration as compared with non-smokers [25]. It has been reported, according to a cross-sectional MONA LISA-NUT survey, that omega-3 index was positively associated with a number of several factors, like: the level of education, age, DHA and EPA intake, and seafood consumption, while smoking was inversely associated with omega-3 index [26], this finding explains our results, as smokers had a lower omega-3 index than normal-weight participants. It is believed that the suppression of PUFAs in smokers is largely due to the increase of lipid peroxidation that results from the higher oxidative stress existing among smokers [26].

5. CONCLUSION

Among participants, normal-weight participants had the highest omega-3 index, while smokers had the lowest. And the omega-3 index values obtained for different participants indicated that most Jordanians lie within the high-risk level (<4%), making them highly susceptible to CHD and sudden cardiac death (as well as other health complications). Smoking and obesity have been found to affect the omega-3 index (inverse association) negatively. The promotion of healthy eating campaigns are needed at a population level to instruct the vast majority of the population on the health benefits of fish consumption and its relationship with omega-3 FAs and omega-3 index.

Ethical approval

This research study is approved by the ethical committee of the University of Jordan. Report number (19/2018/4102).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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