The omega-3 index: a study on the Portuguese population

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ABSTRACT

The omega-3 index (O3I) is used as a biomarker for cardiovascular disease risk. The factors affecting O3I are not fully understood. A study was conducted in a representative sample of the Portuguese population (1,126 individuals) involving blood sampling for the determination of O3I and answering a questionnaire. Participants were asked to indicate their consumption frequencies and other relevant data. The average O3I of the population was 4.82±2.30%. There was a clear increasing trend of the O3I with higher amounts of consumed seafood, achieving an O3I of ~6% with three or more weekly meals. Age was a major determinant, presenting 50-79 year old males higher O3I values than 18-49. Physical activity led to higher O3I, 5.05±2.39% vs 4.64±2.21%. Smoking caused a lower O3I, 4.38±1.97% vs 4.89±2.34%. Physical activity had a larger effect upon O3I in consumers with high seafood consumption. In elderly (>70 year old), there was an inverse relation between O3I levels and high blood pressure. This study's findings point to the importance of changing dietary habits in the direction of increasing seafood consumption and combining these nutritional changes with a healthier lifestyle (with more physical activity and no smoking) for a higher O3I and a lower cardiovascular disease incidence.

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1. INTRODUCTION

The increased life expectancy in the more developed countries has led to a progressive ageing of societies. This evolution poses serious challenges, since it is associated to higher levels of morbidity and concomitant health costs. On the other hand, there are still many men and women that die well before their respective life expectancy. In particular, cardiovascular disease is a leading cause of morbidity and mortality worldwide [1]. In developing countries, both age-adjusted cardiovascular mortality rates and ageing of these populations are leading to a rapid rise in cardiovascular mortality. A large study reported that among all modifiable risk factors of cardiovascular disease, abnormal lipid levels were particularly important for the occurrence of myocardial infarction [2]. Such results seem to reinforce the connection between diet (and dietary lipids) and cardiovascular disease.

Seafood is a key component of the human diet and a nutrient-rich food source that is widely available [3], [4], being their consumption advised due to multiple nutritional benefits [5], [6]. Dietary recommendations advocate a weekly consumption of one to two meals of fatty fish [5], [6]. Fish and other seafood products, besides being an excellent source of high quality protein, low in saturated fat, and rich in micronutrients (such as selenium and vitamins E or B12), are also a key source of long chain polyunsaturated omega-3, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (omega-3 PUFA) [7]. Over the last decades, investigation has associated seafood, namely its contribution in EPA and DHA to the diet, to diverse health benefits for developing foetus, infants, and adults, including those at risk of cardiovascular disease [4], [8], [9].

The long chain omega-3 PUFAs EPA and DHA exert a modulating effect upon the immune and cardiometabolic pathways, having been comprehensively studied over the past decades for their influence on cardiovascular disease risk [10]. Recent meta-analyses show a significant protective action of EPA and DHA supplementation on cardiovascular disease [11] and coronary artery disease risk [12], morbidity, and mortality. Besides genetic factors, dietary intake of long chain omega-3 PUFAs and fish oil supplementation are critical determinants of the concentrations of EPA and DHA in blood [13]. The measurement of such omega-3 PUFAs in blood has been considered fundamental in research [14]. The omega-3 index (O3I), initially developed by Harris and von Schacky [15], the sum of EPA and DHA expressed as a percentage of total FAs in red blood cell membranes, has been shown to be inversely associated to cardiovascular disease mortality [16], [17]. Moreover, the O3I may predict certain coronary artery disease outcomes, such as fatal ischemic heart disease, acute coronary syndrome or sudden cardiac death [10], [18]–[20]. The OI3 may also convey relevant information regarding other diseases [4], [14]. Accordingly, EPA and DHA relative importance in red blood cells may be considered as an adequate marker for public health surveillance.

Given the importance of seafood consumption frequency for the O3I, Portugal, the European Union country with the highest average annual *per capita* seafood consumption, more than 50 kg, 53.8 kg in 2013 [21], corresponding to approximately 150 g meal of seafood per day, is a potentially significant case-study. There is also a knowledge gap regarding the influence of the interaction between lifestyle aspects (such as smoking and physical activity) and specific seafood consumption patterns upon the O3I. Detailed consumer surveys are of paramount importance. All these are major reasons for a public health assessment of the O3I status in the Portuguese population and the underlying causes. This problem can only be solved by a new comprehensive study that brings together a thorough survey of the population and an analysis of the O3I in the same sample of individuals. Therefore, the aim of this paper is twofold: to present and analyse the results of the performed study on O3I and seafood consumption in the Portuguese population and to identify which factors affect this critical cardiovascular health parameter.

2. METHOD

2.1. Human participants and ethical issues

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and since procedures involving human subjects/patients were confined to using part of the blood collected within a routine procedure of a medically-prescribed analysis for an additional fatty acid profile analysis, it was decided to just ensure that all participants were aware of these objectives and fully accepted these conditions and no approval by an ethics committee was sought after. Written informed consent was obtained from all subjects/patients. Given these circumstances, in accordance to the Portuguese and European regulatory framework, no special request was necessary to be submitted to the competent national research ethics service. In this case, the National Ethics Committee for Clinical Research ('Comissão Ética para a Investigação Clínica', CEIC) was not required to intervene and there is no ethical clearance number for the performed study. CEIC is required by law to intervene and issue the single ethical opinion for clinical trials and studies, if they involve medical devices, which was considered not to be the case in the current study.

2.2. Study design

The whole experimental work was conceived as two main interconnected components: i) collection of blood samples from a representative cross-section of the Portuguese population to determine the O3I and ii) survey of the seafood consumption frequency and other information (including socio-demographic and health-related data) from the individuals having their blood collected. Only the blood samples of individuals that accepted to participate in this public health study and duly filled out the printed survey were analyzed for the determination of the O3I. In compliance with the general data protection regulation [22], participants in the study were requested to give their written permission for the utilization of their responses and O3I values for scientific purposes. Random codes were issued to samples and surveys, but retaining a correspondence between these codes in order to relate O3I results to survey responses. Samples and surveys followed different paths so that blood analysts and survey readers had no access to the other codes and related information. The study supervisor held both sets of codes and made the correspondence only after completion of the survey database (with all valid participants' responses) and O3I data file. The anonymized results were then statistically treated and subjected to multiple checks by a different group of team members.

2.3. Elaboration of the questionnaire and conduction of the survey

The questionnaire was prepared as a thorough assessment of the seafood consumption frequency and patterns among the Portuguese population. Moreover, it was intended to collect information relevant to cardiovascular disease risk assessment and, for this reason, questions addressing physical activity and smoking were also considered. Accordingly, two different parts were considered important: seafood consumption frequencies and estimated amounts and socio-demographic data of the respondents including lifestyle habits and specific health conditions, as presented in Table 1. The questionnaire was conceived as a relatively short query. Thus, the number and complexity of the questions was restrained, thereby leading to a calibrated choice of the questions and of the wording. Accordingly, each question was previously discussed by a multidisciplinary team composed of nutritionists, survey specialists, representatives from commercial stakeholders in the seafood sector, statisticians, and other people with previous experience in the seafood sector. In addition, the questionnaire draft was presented to a group of twelve individuals outside the expert group with the purpose of assessing clarity, simplicity, and adequacy of the questions. Proposed alteration suggestions were followed in order to improve the questionnaire. Estimates of seafood apparent consumption were used in the selection of the consumption frequency options (ranging from never to two or more daily meals). A final group of questions was elaborated to comprise a multiplicity of aspects: gender, age, education level, professional activity, geographical location, lifestyle habits (smoking & physical activity), and health condition (high blood pressure, cardiovascular & inflammatory diseases).

Table 1. Elaborated questionnaire on the general seafood preferences and consumption patterns of the Portuguese consumers

Consumption questionnaire	
1)	When you took your last meal?
2)	How many times did you eat seafood (including sushi) in the last week?
	□ None
	□ 1-3 Times
	□ >3 Times
3)	Have you consumed seafood (including sushi) in the last 24 hours?
	□ Yes
	□ No
4)	In the last six months, in average, how often you have been eating seafood?
	□ Never
	□ Less than a monthly meal
	□ A monthly meal
	□ Two to three monthly meals
	□ A weekly meal
	□ Two weekly meals
	□ Three to four weekly meals
	□ Five to six weekly meals
	□ A daily meal
	□ Two or more daily meals
5)	Each time you eat seafood, which is the average meal size?
	□ Less than 100 g or less than a loin or less than three sardines
	□ 100 to 200 g or two loins or three to six sardines
	□ More than 200 g or more than two loins or more than six sardines
Socio-demographic query	
6)	Gender:
	□ Masculine
	□ Feminine
7)	Birth date:………/…../……….
8)	Schooling:
	□ Up to nine years
	□ Between nine and eleven years
	□ Twelve years
	□ College degree
9)	Professional activity:
10)	Postal code:
11)	Do you smoke?
	□ No
	□ Yes
12)	Do you perform physical activity regularly?
	□ No
	□ Yes
13)	Do you have high blood pressure?
	□ No
	□ Yes
14)	Do you have any cardiovascular disease?
	□ No
	□ Yes
15)	Do you have any inflammatory disease?
	□ No
	□ Yes

For the collection of blood samples, a nationwide network of sample collection points and respective personnel belonging to a clinical analysis private company was used. Questionnaires were printed and sent to specific clinical sample collection centres, which were chosen in order to ensure appropriate representativeness. The questionnaire was handed to individuals visiting their local analysis centre for doing routine clinical analyses requested by their physician and had to be filled out after blood collection. The filling of the whole questionnaire took only 5 minutes. In this way, it was possible to successfully conduct a nationally representative seafood consumption survey that started on the May 29th, 2019 and lasted thirty one months (lengthier than expected due to COVID 19 restrictions) until December 27th, 2021.

2.4. Blood collection and preparation

In clinical analysis centres, blood from the study participants was taken in the morning after an overnight fast as is usual in routine clinical analyses. Heparinized blood (5 mL) was left to rest for about 30 min. Afterwards, red blood cells were separated by a procedure involving sequentially a first centrifugation (10 min at 700×g), removal of supernatant, washing of the pellet twice with a 0.9% NaCl solution, and a second centrifugation (5 min at 700×g). Approximately 1 mL of the obtained pellet was freeze-dried and stored at -80 °C until analysis of the FA profile.

2.5. Fatty acid profile and calculation of O3I

The analysis of the fatty acid methyl esters (FAME) in the phospholipids in the red blood cell membranes was done by transesterification using a methodology adapted from Bandarra *et al*. [23] and Bandarra *et al*. [24]. Briefly, the amount of freeze-dried red blood cells corresponding to 1 mL of pellet (see 2.4.) was put into a test tube and 5 mL of a 5% acetyl chloride-methanolic solution (freshly prepared before use) was added. Tubes were left to react for 1 h in a water bath adjusted to 80 °C. Once sample extracts were cooled, 1 mL of Milli-Q water and 2 mL n-heptane were added. Then, tubes were agitated and centrifuged for 3 min at 3,000×g. The organic phase was then collected and filtered through anhydrous sodium sulphate (used to remove any residue from the aqueous phase). The final extract was then analyzed by gas chromatography, in a Scion 456-GC gas chromatograph (West Lothian, UK), equipped with a capillary column DB-WAX (Agilent Technologies, Santa Clara, CA, USA) (film thickness, 0.25 µm), 30 m × 0.25 mm i.d., an auto-sampler, and a flame ionization detector (GC-FID system) The separation of the FAME was carried out with helium as the carrier gas, using a temperature program for the column starting at 180 °C and increasing to 200 °C at 4 °C/min, holding for 10 min at 200 °C, heating to 210 °C at the same rate, and holding at this temperature for 14.5 min. FAME identification was based in their retention time, using a standard mix (PUFA-3, Menhaden oil, Sigma-Aldrich) containing all main fatty acids as comparative reference. FAME results were expressed as percentage (relative content) of the total FAME in mass terms (proportional to chromatographic peak areas in a GC-FID system). The O3I was calculated according to the (1) established by Harris and Schacky [15]:

$$\text{Omega} - 3 \text{ index } (\%) = [\text{EPA}] + [\text{DHA}] \quad (1)$$

where, [EPA] is percentage of EPA in the red blood cells' membrane and [DHA] is percentage content of DHA in the red blood cells' membrane.

2.6. Data analysis

The data collected from the survey and O3I analyses were brought together in an Excel© spreadsheet, which presented a line with several fields for each study participant. This operation was carefully performed in order to guarantee correct matches between survey responses and O3I analysis result. The first field of the spreadsheet informed the date when the respective questionnaire was filled out (no identification about the respondent was available, thereby ensuring anonymity) and the remaining fields contained the various answers and the O3I result. Overall descriptive statistics were calculated, for instance, for the gender distribution in the total universe of the respondents. Furthermore, a system of dynamic Excel© tables was used for a more rapid and flexible treatment of the data. Any participant that did not answer a specific question was not considered for that aspect (for instance, age), but had his/her responses used for other aspects (seafood consumption frequency). Specific filters per spreadsheet column were turned on and graphics were built in order to enable a better evaluation of survey and O3I data. This also enabled to select specific participant groups and study them as isolated sub-groups, for instance, older consumers (>70 year old) and study the relationship between particular survey responses and O3I average, since specific correlations may be obfuscated or confounded by other variables, such as age.

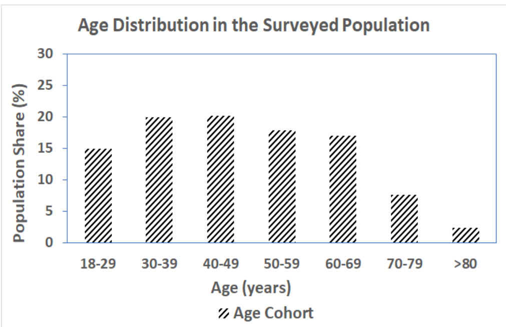
2.7. Statistical analysis

Sample representativeness was evaluated by standard statistical analysis [25]. On the other hand, one-way and factorial analysis of variance was performed using the STATISTICA© software (StatSoft, Inc., Tulsa, USA), version 6.1, 2003. This approach enabled identify which socio-demographic factors, health-connected lifestyle aspects, and seafood consumption patterns affected the O3I. Data normality and variance homogeneity were checked by the Kolmogorov-Smirnov test and F-test of Levene, respectively. The difference of means between pairs was resolved by using confidence intervals in a Tukey honestly significant difference (HSD) test. Level of significance was set for $p<0.05$ ($p<0.10$ in the case of high blood pressure and O3I). Results were presented as average ± standard deviation.

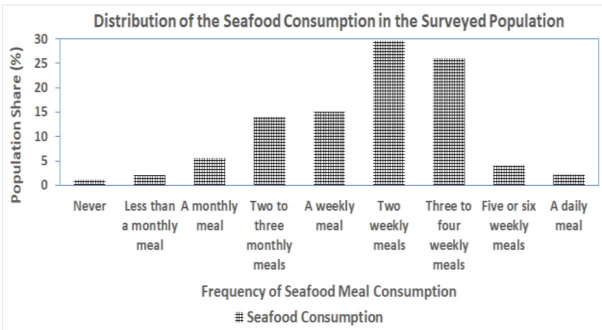
3. RESULTS AND DISCUSSION

3.1. Characterization of surveyed population

There was a total of 1,126 individuals whose blood was sampled and filled out the questionnaires. In general, the questions were fully answered with only some few exceptions (whose numerical expression was negligible). Regarding the representativeness of the study conducted in 2019-2021, some issues must be highlighted as shown in Figure 1. The total number of participants in the study deviated slightly (-8%) from the planned national scenario (1,228 participants) as a result of the effects and constraints imposed by COVID-19. The geographical distribution of the participants was also considered in order to replicate the Portuguese population, being a high representativeness level largely accomplished but with also some deviations. Concerning gender distribution, there was a major deviation, since men are 47.6% in the Portuguese population [26], but they did not surpass 27.1% in the surveyed population. This deviation is related to the context of the study, supported on clinical analysis customers (volunteering to participate in the study), which were mostly feminine. With respect to age, the young and middle-age cohorts were overrepresented and the elderly (>70 year old) were underrepresented, for instance, people aged 80 or older are more than 5% of the Portuguese population [26], but only 2.4% of the study participants, as illustrated in Figure 1(a). Such deviations derive from a lower availability to participate and answer the questionnaire in the case of the elderly. Nonetheless these deviations and slightly lower than planned number of participants do not diminish the whole robustness of the study as assessed statistically [25] and the relevance of its outcomes. A main key result of the study was that most of the Portuguese population (55.7%) consumes between two and four weekly meals of seafood as can be seen in Figure 1(b). Nevertheless, there is a large variability in the frequency of seafood consumption in the population. It can be observed that the statistical mode is attained with a frequency of two weekly meals of fish (about 30 % of respondents), followed closely by the category of three to four weekly meals. There are also two other relevant categories, with frequencies in the range of 10%-15%. These are the groups of individuals with frequencies of consumption of one meal per week and two to three meals per month. Symmetrically to this set of four categories, there is the category of a single monthly meal and the category of five to six meals a week (reaching approximately 5% of the whole population). There are approximately 2% to 3% of individuals who claimed to consume a fish meal every day, 1% to 2% who answered less than a monthly meal and just over 1% who do not consume fish. No survey respondents reported consuming two or more meals a day.



(a)



(b)

Figure 1. Characterization of the studied population: (a) regarding age distribution and (b) seafood consumption patterns

It should be remarked that the survey results regarding seafood consumption frequency seem to point to a broad decline of seafood consumption in Portugal over the first decades of the XXIst century or to an overestimation of consumption if landings, aquaculture production, exports, imports, and population statistics are used to calculate an apparent *per capita* consumption [26]. In fact, according to FAO (2022) [21], Portugal was the European Union country with the highest average annual *per capita* seafood consumption, about 54 kg in 2013, possibly corresponding to a 150 g daily meal of seafood, thus surpassing the average consumption frequency in the survey results. In any case, consumption reduction and adverse factors to seafood purchase may be a problem. One of such factors is price [27], which have been rising, in part due to seafood overexploitation.

Fish consumption frequencies can be analyzed with more detail regarding age cohorts. Given the possible difference in dietary patterns between age groups, the extreme cohorts that participated in the study (18-29 and > 80 year old individuals) can be assessed separately. Whereas the young cohorts were characterized by consuming between one monthly meal and two weekly meals, the elderly consumed three or more weekly meals of seafood. There was a broad trend to higher seafood consumption in older consumers. This is relatable to possible changes in seafood consumption preferences in the youth and may pose a future risk to cardiovascular disease incidence.

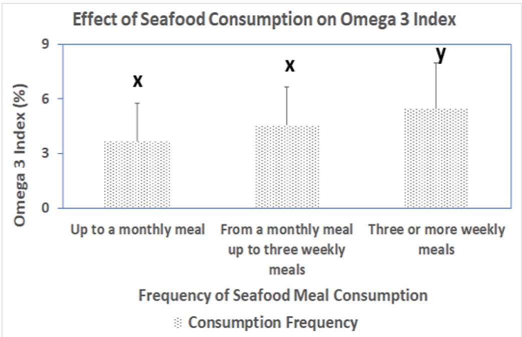
3.2. O3I and seafood consumption

The overall O3I of the population sampled by the survey was 4.82±2.30%. This is an intermediate value for this index, since it is in the 4%-8% range [15]. Such a population could even be recruited for improving its omega-3 levels [28]. From studies on the relationship between EPA, DHA, and the O3I and the risk of death from cardiovascular disease [17], [28]–[33], it was concluded that there is a strong reduction in risk with an increase in the O3I from 4% to values above 8%. Harris *et al*. [31] pooled data of over 27,000 participants from 10 studies and found that people with >8% O3I had a 35% lower risk of death from coronary heart disease than those with an O3I <4%. The O3I was clearly related to risk in a dose dependent manner with a risk reduction of 90% at the highest levels of the O3I [30], [32]. Additionally, according to Harris *et al*. [17], a comparison between the highest O3I quintile (>6.8%) and the lowest one (<4.2%) entailed a 39% lower risk for a cardiovascular disease incident. Thus, the O3I initially proposed by Harris and Schacky [15], a biomarker of the cardiac membrane content in omega-3 FAs, can be a risk factor for coronary heart disease. These authors defined a risk scale for death from coronary disease: 0%-4%, high risk; 4%-8%, intermediate risk; greater than 8%, low risk.

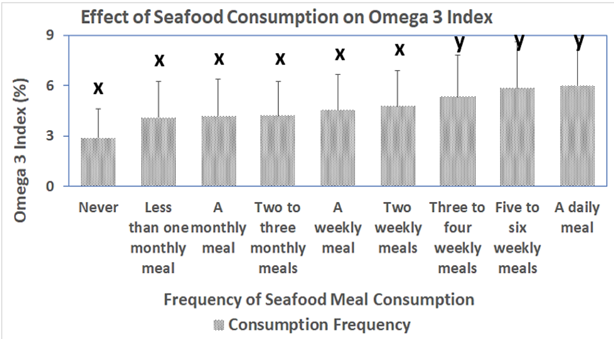
The high standard deviation in the O3I measured in the current study highlights the existence of a great variability of this parameter in the Portuguese population. Other studies covering diverse populations [10], [34], [35] have also found large variability of the O3I values. Furthermore, the mean value in the current study is similar to those reported in other papers [10], [36]. Namely, a mean O3I of 4.5% has been calculated for a population of Canadian adults between 20 and 79 years old and also for the USA population studied by the National Health and Nutrition Examination Survey (NHANES 2003-2004) [10], [36]. Considering the 95th percentile of O3I levels, it reached 8.5% in the Portuguese population. This compares to a 95th percentile of 7.3% in the Canadian population [10], [36]. Hence, it seems that the O3I situation in the Portuguese population is slightly better.

An explanation for this comparison favorable to the Portuguese population may lie in the high seafood consumption in Portugal [21]. Indeed, the average O3I may be a reflection of the mean seafood consumption of two weekly meals reported by the study participants. Moreover, the variation of the O3I in the surveyed population may be related to the consumption frequency of each participant. The combined analysis of these two datasets reveals clear and significant increasing trends of the O3I with higher amounts of consumed seafood as seen in Figure 2. Since consumption frequency was a discontinuous variable 10 categories as shown in Table 1, a more broad as in Figure 2(a) or detailed as in Figure 2(b) analysis can be considered. In the former case, three or more weekly meals enabled an O3I of almost 6%, which was statistically higher than the 3%-5% level for lower consumption frequencies. In the latter case, a similar statistically relevant consumption of three weekly meals is evident. Higher consumptions up to a daily meal did not lead to an improved O3I. Of course, a larger and statistically more robust study could enable to observe a general increase trend encompassing all 10 categories. It is also possible to evaluate the share of the surveyed population with a high O3I (>8%) as a function of seafood consumption as shown in Figure 3. This share augmented exponentially with increasing seafood consumption, namely augmenting from less than 5% for two weekly meals to more than 10% for three/four weekly meals and to more than 20% for five/six weekly meals.

It is acknowledged that a major determinant of the O3I is the EPA+DHA intake, being the impact of other dietary FAs worthy of investigation [34]. A nexus between seafood (especially fatty fish) consumption and O3I has been found by Demonty *et al*. [10]. These authors used as frequency cut-off a consumption of at least two weekly servings of fish (at least one was oily fish) and found that the study participants above this cut-off had an O3I of 5.6%, a value higher than that of those with a lower seafood consumption, 4.4%. These results in a Canadian population broadly agree with the Portuguese study. The relevance of pondering fatty fish may explain why ~80% of the participants in the Portuguese study eating five/six seafood meals/week had an O3I below 8%. Of course, populations with very high seafood consumption and large amounts of fatty fish, such as Japanese or Alaskan Eskimos, have very high average O3I values, reaching 8.5%-9.0% [37], [38].



(a)



(b)

Figure 2. Variation of the O3I with the seafood consumption frequency (n>50; different letters correspond to statistically significant differences, p<0.05): (a) considering broad and (b) detailed consumption categories

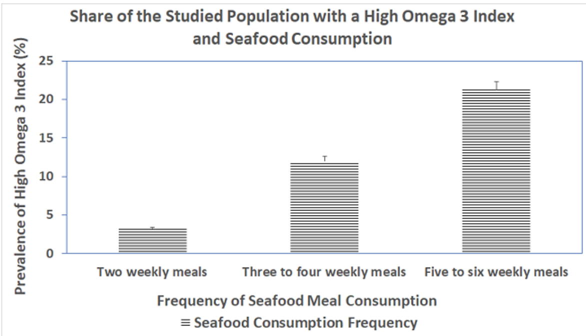


Figure 3. Share of the studied population with a high O3I (>8%) as a function of seafood consumption frequency

However, the results in the Portuguese population also deviate from other studies in Western nations. Wagner *et al*. [39] reported a mean O3I of 6.02±1.75% in a French population. These authors observed a progressive increase of the O3I with seafood consumption, ranging from 4.81±0.21% in participants reporting up to a monthly meal to 6.94±0.11% in those eating two or more weekly meals. This O3I value is higher than the equivalent result for the same seafood consumption frequency in Portugal (<6%). This may be due to the lipid content and composition of the consumed fish. Oseeva *et al*. [40] calculated a mean O3I of 3.56% (but calculated in molar base, which lowers up to 10% the O3I calculated in mass base) for a population in the Czech Republic. There was also a direct dependency of the index on seafood consumption frequency in this population, but varying from <3% for those consuming less than a monthly meal to just 4% for individuals eating two weekly meals [40] 1% less than in the equivalent Portuguese group. A study on the Basque population [35], a Southern European population, showed an O3I of 6.9%. This surpasses the Portuguese average O3I. There was also an increase of the O3I (ΔO3I of ~+1%) when relative fish consumption more than doubled in the Basque population [35]. The high intake of EPA and DHA could, in part, explain the low incidence of coronary heart disease and cardiac death overall in Southern Europe [34]. These comparisons highlight the need of further study detailing the type of fish (lean vs fatty) and specific FA composition.

There are also methodological issues in these studies. The EPA and DHA intakes should be calculated or, at least, estimated. This would require not only knowing the amount of each seafood product consumed at each meal, but also to determine EPA and DHA levels in the products. The cross-sectional nature of such studies does not allow to set causality links between traits in the participants' diet (e.g. omega-3 supplements) and the O3I. Nonetheless, such causation is shown in intervention trials [41], [42]. Other O3I analysis difficulty is related to the higher sensitivity of EPA and DHA than other FAs to the time period between blood collection and red blood cell isolation and freezing, thus underestimating O3I [43]. Differences in the list of FAs included in the calculation of relative amounts is another difficulty. However, FAME analysis is currently very accurate and such differences would only affect trace FAs [10]. Comparison across studies is rendered more difficult by the different survey structures, consumption categories and cut-offs (which may also weigh seafood consumption in % of total calories instead of counting the number of meals), and representativeness in each study and by the lack of standardized and harmonized methodologies for the determination of the O3I [44].

3.3. O3I and socio-demographic factors

The variation of the O3I with age and gender is shown in Figures 4 and 5. With exception of the 18-29 year old cohort (in the >80 year old cohort, there were opposite findings according to cohort clustering), gender did not affect the O3I level. Younger cohorts had less 0.5% O3I, having males a lower index. Age showed itself to be a more influential factor in O3I. Either grouping individuals in large age cohorts as in Figure 5(a) or small cohorts as in Figure 5(b), significant differences were observed. Small cohorts revealed lower O3I levels in 18-29 and >80 year old men, not exceeding 4.5%, which contrasted with index levels above 5.5% in the 50-79 year old men. This was corroborated when larger cohorts were compared. Reinforcement of this observation is attainable through the increasing trend of the population proportion with high O3I (>8%) from less than 3% for the 18-29 year old cohort to more than 12% for the 60-69 year old cohort, as observable in Figure 4.

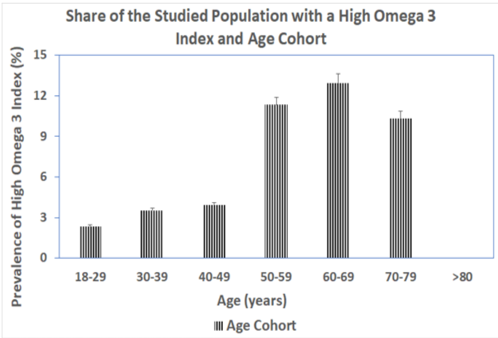
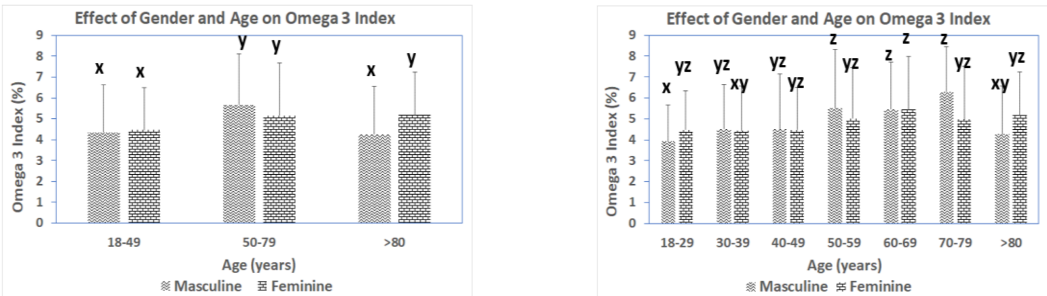


Figure 4. Share of the studied population with a high O3I (>8%) as a function of age cohort



(a) (b)

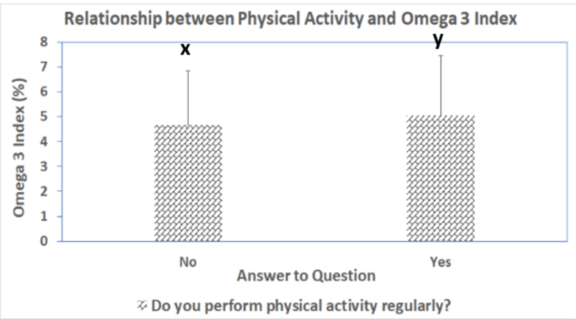
Figure 5. Variation of the O3I with gender and age (n>15; different letters correspond to statistically significant differences, p<0.05): (a) considering broad and (b) detailed cohorts

Other comparable studies have also reported narrow or no differences between the O3I values of men and women [10], [39], [40]. Whereas, in the Canadian population [10], 0.15% difference with advantage to the female group was measured and, in a French study [39], 0.15%-0.44% variation with gender was registered (also to advantage of the female group), in the Czech population [40], no difference was registered between men and women. In this regard, it may be claimed that higher seafood consumption by women may lead to small O3I differences. However, such higher consumption levels were not found by other researchers [33], [37] in their studied populations as well as in our Portuguese study. It should be remarked that there are suggestions that sex hormones affect the enzymatic synthesis of long chain PUFAs, causing gender-specific differences in EPA and DHA levels [45]. These differences are mainly related to the ability to synthesize EPA and DHA from α-linolenic acid, an omega-3 precursor [46].

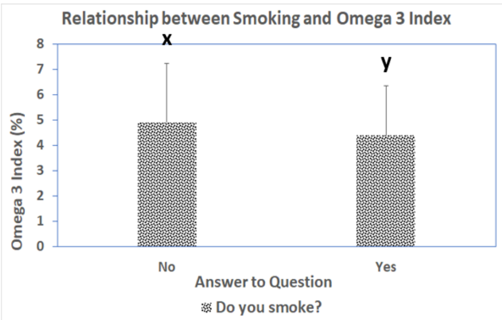
Concerning age, several studies [10], [38], [39] support an increasing trend of the O3I as men and women get older, at least up to a certain age. Demonty *et al*. [10] recorded an upward trend of the index in older cohorts: 4.29% in 20-39 year old; 4.45% in 40-59 year old; and 4.96% in 60-79 year old participants. Wagner *et al*. [39] observed an O3I increase from the 5.0%-5.5% interval for 35-44 year old men and women to 6.0%-6.5% for 55-64 year old individuals. Itomura *et al*. [38] were able to observe an even stronger effect in Japan, since the O3I was 7.0%-7.5% in the 18-29 year old cohort, but reached approximately 10% in older individuals (>60 year old). Though seafood consumption amounts are larger in older cohorts, a finding that was also clear in the Portuguese population while nearly 40% of the 50-79 year old individuals ate three or more weekly meals of seafood, less than 30% did the same in the 18-49 year old group, it seems to be insufficient to justify the observed trend in the O3I. Studies presented by Itomura *et al*. [38] and Salisbury *et al*. [47] showed that age was an independent predictor of the O3I even after offsetting the effect of seafood consumption on it. This phenomenon may be due to age-specific factors per se or to the interplay between them: i) a slower turnover of omega-3 PUFA in older people; ii) lower linoleic acid intake or higher α-linolenic acid intake in older cohorts (considering the competitive pathways for the synthesis of long chain omega-6 and omega-3 PUFAs); and iii) a possible survivor bias in that people with higher O3I live longer [48]. A recent study on the Basque population [35] also pointed out that many membrane lipids in red blood cells are remodeled with age and display a shift in the acyl chain composition towards a higher PUFA content. For the oldest cohort (>80 year old), poor absorption of omega-3 PUFA and other age-specific aspects may become more important and depress the O3I. In all this discussion, it should be noted that there is also a dynamic balance between EPA and DHA levels and the absorption of these FAs, which may be reduced for very high levels [24].

3.4. O3I and lifestyle aspects

The dependence of O3I on lifestyle aspects, such as physical activity and smoking, was also analyzed as shown in Figure 6. The participants declaring to be more physically active had a significantly higher O3I than the other participants, 5.05±2.39% vs 4.64±2.21%, as visible in Figure 6(a). On the other hand, smokers had a lower O3I than non-smokers, 4.38±1.97% vs 4.89±2.34%, depicted in Figure 6(b). As with age and gender, seafood consumption frequency differences may cause these O3I differences between groups with different lifestyle habits. In fact, seafood consumption is higher in individuals practicing physical activity.



(a)



(b)

Figure 6. Relationship between O3I and specific lifestyle aspects (n>100; different letters correspond to statistically significant differences, $p<0.05$): (a) considering physical activity and (b) smoking

In order to discount the influence of seafood consumption, the effects of smoking and physical activity were studied in sub-groups with specific seafood consumption frequencies, as shown in Figure 7, for instance, considering only those consuming up to a weekly meal of seafood, as depicted in Figure 7(a). Significant differences were detectable in the case of physical activity for the situations of two to four and more than five weekly meals of seafood. These are observable in Figures 7(b) and 7(c). This means that performing physical activity per se had a positive effect upon the O3I, reaching an improvement from 4.97±2.39% to 6.79±2.86% for those individuals eating seafood more than five times every week. While Figure 7(d) shows that smoking had a negative effect on O3I for those eating up to a weekly meal of seafood, no effect was observed for higher consumption frequencies, as shown in Figures 7(e) and 7(f). In accordance to the study's findings, the share of the population with a high O3I (>8%), depicted in Figure 8, was much reduced in the smoking sub-population, 3.1% vs 7.4%, as shown in Figure 8(a), and the equivalent share was higher among those participants reporting regular performance of physical activity, 9.3% vs 4.9%, clearly observable in Figure 8(b).

Smoking and/or physical activity are addressed in other studies [10], [38], [39], [49]–[51]. Smoking is known to negatively impact the O3I, being its depressing effect as large as 2.45% [49] and also smaller but still significant as 0.86% [51], 0.68% [39], and 0.45% [10]. In the case of Canadian adults, whose mean O3I index was reported to be 4.5%, a dependence on age, ethnicity, fish consumption, supplement use, and also smoking was found [52], [53]. However, Itomura *et al*. [38] did not find any significant correlation between the O3I and smoking. Schuchardt *et al*. [51] observed a depressing action of smoking even after mathematical adjustment. It is possible that smoking affects the metabolism of omega-3 PUFAs, being an enhanced susceptibility of these FAs to oxidation and the role of smoking in the promotion of oxidation processes [54] a possible reason. Pasupathi *et al*. [55] claimed that smokers have higher levels of reactive oxygen species. A high use of omega-3 PUFAs in the synthesis of anti-inflammatory metabolites in smokers may reduce O3I [56].

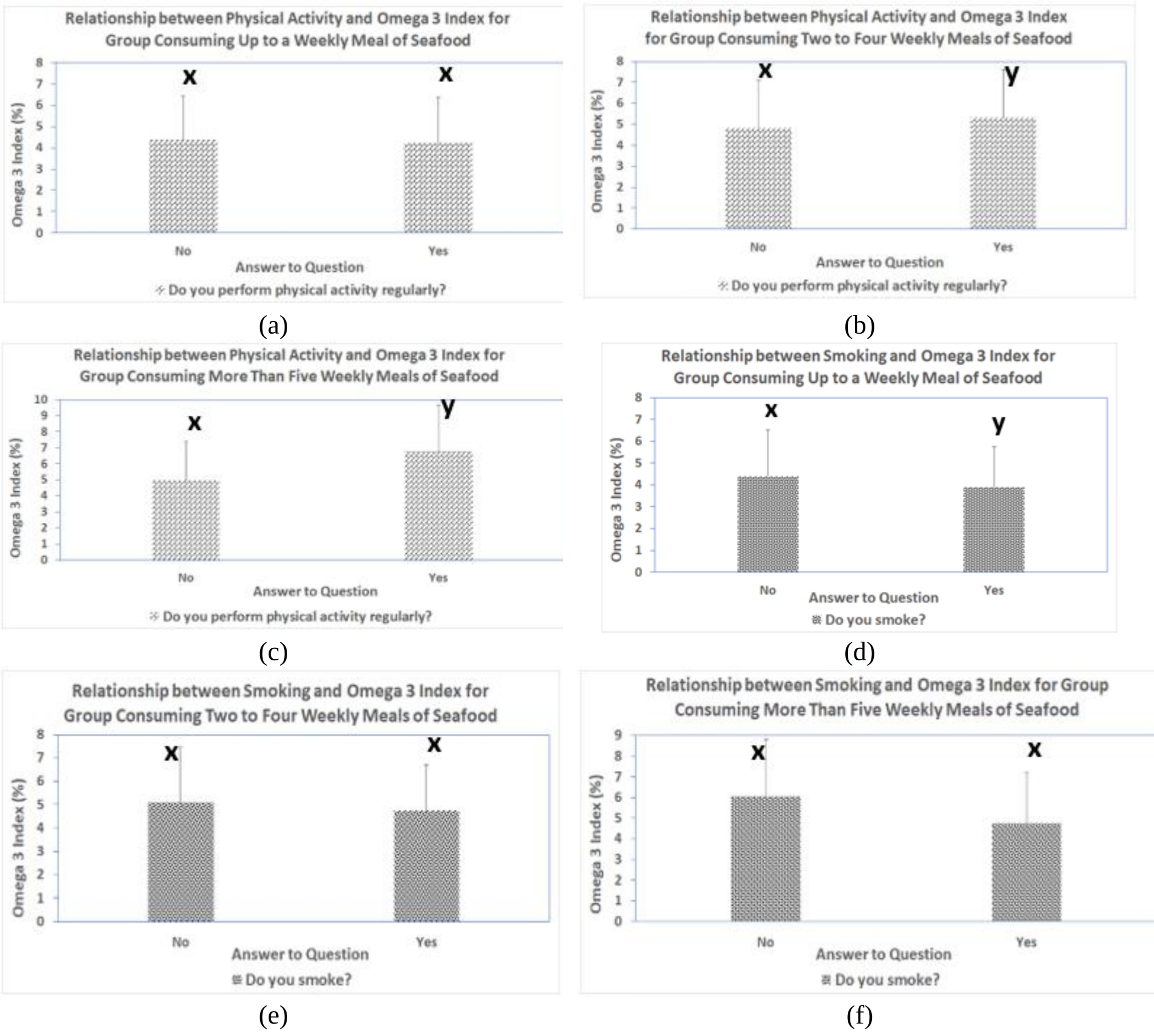
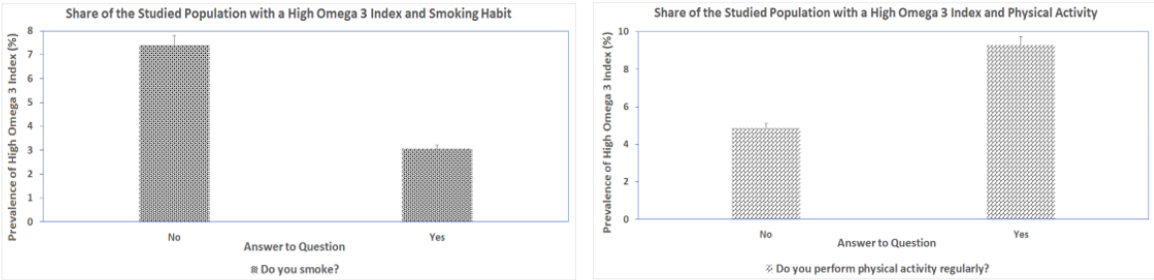


Figure 7. Relationship between O3I and specific lifestyle aspects (n>10; different letters correspond to statistically significant differences, $p<0.05$): (a), (b), and (c) after controlling seafood consumption frequency as a confounding factor: physical activity and (d), (e), and (f) smoking



(a) (b)

Figure 8. Share of the studied population with a high O3I (>8%): (a) share as a function of smoking and (b) physical activity habits

Physical activity has also been pointed out as a determining factor of the O3I [38], [39], [51]. However, determined intensity of the effect on the index was claimed to vary from weak but still significant [38], [51] to moderate [39]. In all instances there was an inverse relationship between performance of regular physical activity and the level of the O3I. After controlling for higher seafood consumption among individuals doing regular exercise, as done in the current study, there seems to be an independent action of physical activity upon the EPA and DHA levels and different from what was observed in other studies [38], [39], [51]. Such action may be ascribed to a lower utilization of EPA and DHA for energy production when physical activity is increased. In fact, due to their properties, EPA and DHA are less suitable for storage in the adipose tissue, being their percentages in the human adipose tissue below 1% even after more than one year of daily consumption of 10 g of fish oil [57]. It is possible that the relative enrichment in EPA and DHA in the red blood cells as a consequence of doing physical exercise is stronger when a high seafood consumption ensures a very clear excess of EPA and DHA intake versus consumption of other less unsaturated FAs for energy production.

3.5. Omega-3 index and key health parameters

In the conducted survey there were also three questions relating to the health condition of each participant, asking about the existence of: i) high blood pressure; ii) cardiovascular disease; and iii) inflammatory disease, as presented in Table 1. The answers were related to the O3I not only without controlling any confounding factor, but also taking into account age as displayed in Figure 9, given the connection between age and health. No significant difference between the groups answering negatively and positively to each health-related question was observed. However, in the case of blood pressure, shown in general in Figure 9(a), after taking into account age as a confounding factor (blood pressure increases with age), it was detected a reduction of O3I levels in the elderly (>70 year old) associated to high blood pressure, from 5.57±2.53% to 5.03±2.27% as seen in Figure 9(b). For cardiovascular disease, no difference was found in the general population, as highlighted in Figure 9(c), nor in the elderly, as displayed in Figure 9(d). Finally, regarding inflammatory disease, the same absence of effect was seen in the whole population, as visible in Figure 9(e), and in the elderly, observable in Figure 9(f).

The relationship between these health issues and the O3I levels in different populations was also previously studied [10], [58], [59]. Regarding either high blood pressure or heart disease, Demonty *et al*. [10] did not find any significant difference in the Canadian population between the reference group and the group presenting one of these two health conditions. The existence of a negative correlation between O3I and blood pressure was dubious in the case of the randomized controlled trial conducted by Stanton *et al*. [59]. These studies did not explicitly isolate and study the correlation between the index and high blood pressure in a specific age cohort as done in the current study of the Portuguese population. Though focusing on normotensive young and healthy individuals, a fairly recent study [60] found out that a higher O3I is associated with statistically significant, clinically relevant lower systolic and diastolic blood pressure levels. On the other hand, other researchers [58], which studied 30-35 year old and overweight populations in Spain, Iceland, and Ireland (32% of participants with high blood pressure), found no association O3I and systolic or diastolic blood pressure.

Nevertheless, considering cardiovascular health overall, there is stronger evidence, being a higher O3I associated with significantly lower risk for total cardiovascular disease events according to a robust multivariable-adjusted analysis [17]. Furthermore, a recent global study [61] concluded that omega-3 PUFAs may lower cardiovascular risk through a number of pleiotropic mechanisms, including by lowering blood pressure, but also by mediating antithrombotic effects or by providing precursors for the synthesis of pro-resolving mediators that inhibit inflammation. The absence of such association in the surveyed Portuguese population must be considered together with some inherent weaknesses of the study, such as: i) the low number of respondents with reported cardiovascular disease, 6% of a total of 1,126 participants, statistically less representative; ii) the absence of a clinically diagnosed independent confirmation of cardiovascular disease (the study depended on the participants' self-knowledge and availability to disclose their health condition); and iii) cardiovascular disease, differently from age or smoking, is not a determinant, but an outcome of low O3I levels, thereby being preferable other study designs for illuminating any association.

For inflammatory disease and other relatable health issues, there is some evidence showing association between O3I and inflammation biomarkers, for instance, Olliver *et al*. [62] reported an inverse association between the O3I and monocyte cell counts in older Australian men and women aged ≥65 years. Moreover, in the case of asthma, a chronic inflammatory disease of the airways, a higher O3I was observed in subjects with controlled or partially controlled asthma compared to subjects with uncontrolled asthma [63]. Moreover, a cross-sectional study of subjects with peripheral artery disease (PAD) showed that O3I was inversely associated with biomarkers of inflammation, which increases the risk of progression to PAD and its seriousness [64]. However, other aspects of the FA profile in the blood and tissues may be relevant for inflammatory processes, such as the balance between omega-3 and omega-6 FAs. Indeed, besides the omega-3 FA suppression of transcription factors that control the production of circulating inflammatory cytokines [62], [65], omega-3 PUFA also compete with omega-6 PUFA, thereby regulating levels of arachidonic acid-derived immune and inflammatory mediators [66].

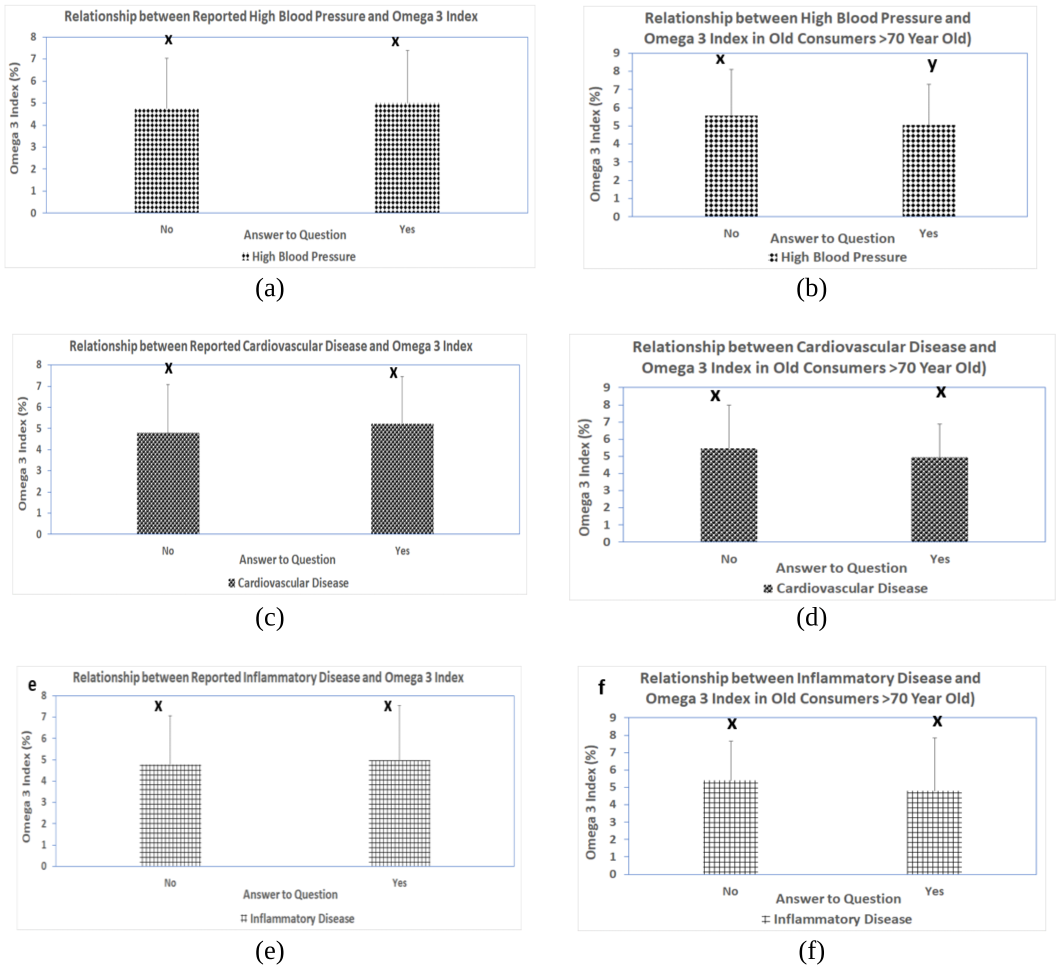


Figure 9. Relationship between O3I and reported health issues (n>10; different letters correspond to statistically significant differences, p<0.10) before and after controlling age as a confounding factor (by focussing on the >70 year old cohort): high blood pressure: (a) and (b), cardiovascular disease (c) and (d), and inflammatory disease (e) and (f)

4. CONCLUSION

It was possible to successfully undertake a broad survey study of the seafood consumption by the Portuguese population. Despite some deviations, such as a larger female participation, a representative sampling population was attained, yielding a total of 1,126 valid questionnaires with associated O3I analyses. The overall average O3I of the population was 4.82±2.30%, an intermediate value of the index. There was a clear and significant increasing trend of the O3I with higher amounts of consumed seafood, determining three or more weekly meals an O3I of almost 6%, statistically higher than the 3%-5% level for lower consumption frequencies. Regarding socio-demographic factors, while gender did not affect the level of the O3I, age was a major determinant, presenting 50-79 year old males significantly higher O3I values than 18-49 and >80 year old males. Concerning lifestyle issues, those study participants performing regular physical activity had a higher O3I than the other ones, 5.05±2.39% vs 4.64±2.21%, and smokers had a lower O3I than non-smokers, 4.38±1.97% vs 4.89±2.34%. After controlling seafood consumption levels, physical activity had a positive effect upon the O3I, rising it from 4.97±2.39% to 6.79±2.86% for those individuals eating more than five weekly meals of seafood. Results also suggest a strengthening of the physical activity effect upon the index with higher seafood consumption levels. With respect to specific health issues, it was possible to detect a reduction of O3I levels in elderly (>70 year old) with high blood pressure, from 5.57±2.53% to 5.03±2.27%. It should also be remarked that there are some limitations of the performed study in the Portuguese population: i) as the high standard deviations highlight, there must be other factors generating variability in the O3I, being genetic factors an important possibility and ii) the study's cross-sectional design precludes making any causal inferences. Finally, it should be stressed that, given the continuous association between the O3I and cardiovascular disease risk as well as between seafood consumption and the index, any meaningful increase of seafood consumption, even if up to 8%, could contribute to lower cardiovascular disease risk in the population.

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