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# Omega-3 index in the Czech Republic: No difference between urban and rural populations

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ABSTRACT

Naturally occurring long-chain omega-3 PUFA such as eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3) exert multiple effects on health, which are related to the intake of these lipids in the diet and correlate with the levels of omega-3 PUFA in the body. These levels are reflected by the omega-3 PUFA index, i.e. the EPA and DHA content as % of all fatty acids in red blood cells. The aim of this study was to evaluate omega-3 index in the Czech Republic, using blood samples collected from the capital city (n = 476) and the rural region (n = 388). The mean omega-3 index was 3.56 mol % with a maximal value of 8.10% and a minimal value of 1.12%. There was no difference in the index value between rural and urban / industrial regions, but this value was higher in subjects who reported eating fish or omega-3 PUFA supplements. In conclusion, the results indicated suboptimal values of the omega-3 index in the Czech population independent of the sampling region.

## 1. Introduction

Naturally occurring long-chain omega-3 PUFA, namely eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3), exert multiple biological effects that are mediated either by these PUFA themselves or their bioactive metabolites including specialized pro-resolving mediators, oxidized derivatives, ethanolamines, acylglycerols, and branched-esters of fatty acids, exerting mostly anti-inflammatory effects (reviewed in (Calder, 2013; Kuda, 2017; Kuda et al., 2018)). In humans, omega-3 PUFA attenuate systemic inflammation (Calder, 2013; Hung et al., 2015), ameliorate non-alcoholic fatty liver disease (Scorletti et al., 2014), and protect against cardiovascular disease (reviewed in (Mozaffarian et al., 2013; Tribulova et al., 2017; von Schacky, 2014)). The role of omega-3 PUFA in the primary prevention of cardiovascular diseases was supported by the recent randomized, placebo-controlled VITAL trial (see Discussion and refs. (Bassuk et al., 2016; Manson et al., 2018)). Therefore, several (inter)national health authorities have recommended dietary omega-3 PUFA intake to be between 0.2 g and 2.0 g per day (Mozaffarian et al., 2012; Perk et al., 2012; Smith et al., 2011) and omega-3 PUFA supplementation is advised as part of the secondary prevention of coronary heart disease (Siscovick et al., 2017).

Omega-3 PUFA index, i.e. the EPA and DHA content adjusted to the total content of other fatty acids in red blood cells (RBC), was defined as a risk factor for death from coronary heart disease (Harris and Von Schacky, 2004; von Schacky, 2014). This value serves also as a biomarker of omega-3 PUFA intake within the timeframe of erythrocyte half-life (˜60 days) and can be used by clinicians to trace dietary habits of their patients. Recently, a systematic world-wide analysis of omega-3 fatty acids in the blood stream of healthy adults revealed differences between populations adapted to Westernized eating habits and indigenous coastal diet (Stark et al., 2016). Very low blood levels of EPA + DHA (< 4%) were observed in Europe, including the Czech Republic (Stark et al., 2016).

Although there were published several papers describing omega-3 index in small clinical trials in the Czech Republic (Crispim et al., 2011; Hlavaty et al., 2008; Rossmeisl et al., 2018; Stankova et al., 2018; Veleba et al., 2015), a population-based survey was missing. We were

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able to collect and analyze RBC samples from a large socio-physiological survey QUALITAS focused on wellbeing, health and disease, and therefore to explore levels of omega-3 index in the Czech population. The general aim of the survey was to compare two distinct populations – people living in the capital city and people living in rural areas and small villages. Our hypothesis was that differences in living style, socio-economic factors and nutritional habits (e.g. availability of omega-3 PUFA-rich marine products in central European villages) might result in a difference in omega-3 PUFA availability, and thus in a difference in omega-3 index.

## 2. Material and methods

### 2.1. Study population

The study population was defined within a combined socio-physiological survey project "QUALITAS - Wellbeing in health and disease" using quota sampling (sex, age, education, place of residence size). Detailed analysis of the survey is out of scope of this study and will be published separately. Briefly, sex, age, and education quota followed data from the Czech population and housing census 2011 and the place of residence was defined either as highly urbanized area (Prague, ˜1.4 million inhabitants) or rural region (Ceske Budejovice and neighboring villages, up to 100,000 inhabitants). Participants (aged 18 years and older) were asked to fill a questionnaire related to their health and socio-economic status and to provide blood samples. In total, 864 RBC samples were collected and included in the study.

### 2.2. Study design

The period of data and sample collection was from September 2016 to June 2017. Fasted blood samples were routinely processed at participating medical facilities. One ml of RBC was frozen at −80 °C at the central laboratory in Prague or frozen at -20 °C upon collection at remote facilities and in 1–2 days delivered on dry ice to the central laboratory to be stored at −80 °C to prevent PUFA degradation (Pottala et al., 2012). This was the optimal logistics of sample collection for this large study as small medical facilities in rural areas do not have −80 °C freezers. Among other data, participants responded to a question "How often do you eat fish?" allowing 6 possible answers: more than twice a week / once a week / few per month / once a month / less often / never; and "Do you take any omega-3 PUFA supplements?" Y/N, optionally followed by detailed description of the form and quantity.

### 2.3. RBC fatty acid composition

An aliquot of 100 mg of RBC was extracted according to Matyash (Matyash et al., 2008). The amount was a frozen sample equivalent of 80–250 μl of fresh RBC (Pottala et al., 2012). The sample was milled with 250 μl of water, 100 μl of methanol with 0.001% butylated hydroxytoluene and 200 μl of 0.1 mg/ml 19:0 phosphatidyl choline internal standard (MM400, Retsch, Germany) for 1 min at 30 Hz. One ml of cold methyl-tert-butyl ether was added and samples extracted for 15 min using an orbital shaker at 1 Hz. Tubes were centrifuged at 2500 g for 10 min at 4 °C, the organic phase collected and dried in SpeedVac (refrigerated CentriVap, Labconco). Extracted lipids were trans-esterified with 0.5 N sodium methoxide in methanol (Sigma-Aldrich) for 10 min at room temperature. Reaction was quenched with 3 M HCl and fatty acid methyl esters (FAME) extracted to 1 ml of hexane after 10 min in the orbital shaker.

FAME were analyzed using comprehensive two-dimensional gas chromatograph with mass detection (Pegasus 4D, LECO, USA) (Rossmeisl et al., 2018). FAME sample was injected onto Tr-FAME column, 60 m, 250 μm ID, 0.25 μm PT (Thermo, USA) coupled to Rxi-5MS column, 1.7 m, ID- 250 μm ID, 0.25 μm PT (Restek, USA). Temperature program was as follows: 50 °C (1 min) – 20 °C/min – 180 °C (0 min) – 10 °C/min – 250 °C (6 min), He flow 1 ml/min. Injection temperature was 250 °C, transfer line temperature was 280 °C, modulation period 4 s, offset between primary and secondary column 15 °C, hot pulse time 1.5 s. Data files were automatically processed in ChromaTOF software using S/N ratio 10 and omega-3 index was calculated as a sum of EPA and DHA levels divided by total levels of all fatty acids (see Table 1) according to a methodology used in a recent review to present directly comparable values (Stark et al., 2016). FAME standard mixture GLC 744 (Nu Check Prep, USA) was used to optimize the assay (Rossmeisl et al., 2018). Figure S1 shows representative 2D chromatograms and mass spectra of EPA and DHA.

**Table 1**
Fatty acid composition of RBC.

| Fatty acid | Mol % |
|---|---|
| 14:00 | 0.47 ± 0.23 |
| 16:00 | 24.38 ± 1.30 |
| 18:00 | 17.35 ± 1.30 |
| 20:00 | 0.08 ± 0.03 |
| 22:00 | 0.01 ± 0.01 |
| 16:1 n-7 | 0.81 ± 0.41 |
| 18:1 n-9 | 18.25 ± 1.60 |
| 20:1 n-9 | 0.24 ± 0.06 |
| 18:2 n-6 | 13.56 ± 1.84 |
| 18:3 n-6 | 0.20 ± 0.10 |
| 20:2 n-6 | 0.21 ± 0.06 |
| 20:3 n-6 | 1.60 ± 0.34 |
| 20:4 n-6 | 15.02 ± 1.58 |
| 22:4 n-6 | 2.22 ± 0.49 |
| 18:3 n-3 | 0.08 ± 0.06 |
| 20:5 n-3 | 0.56 ± 0.27 |
| 22:5 n-3 | 1.62 ± 0.33 |
| 22:6 n-3 | 3.00 ± 0.77 |

### 2.4. Statistical analyses

The data are expressed as boxplots (median, 25th and 75th percentile) with the whisker range defining the outer-most data point that falls within 1.5-times the inter-quartile range or as mean ± standard deviation for tabular data. Statistical analysis was performed with OriginPro 2018. The fish meal consumption data were evaluated using ANOVA on ranks with Dunn's all-pairwise test. Omega-3 PUFA supplements data were evaluated using unpaired two-tailed Student's *t*-test and $p < 0.05$ was considered significant. Data on fish production and consumption were extracted from FishStatJ, a tool for fishery statistics analysis, Release: 3.04.7 (FAO UN, 2018).

## 3. Results

The characteristics of our study population providing blood samples showed that the age was equally distributed (Fig. 1A) and that according to body mass index (BMI), 1.4% of the studied subjects were underweight (BMI ≤ 18.5), 47.1% normal weight (BMI between 18.5–25), 34.5% overweight (BMI between 25–30) and 17.0% obese (BMI ≥ 30; Fig. 1B), corresponding to the results of European health interview survey 2014 for the Czech Republic. Participants (366 males and 498 females) lived either in mostly rural South Bohemian region with administrative center Ceske Budejovice (˜94,000 inhabitants) or in the capital city of Prague (˜1,4 million inhabitants) and the selection followed the quota (Fig. 1C).

Analysis of RBC fatty acid composition revealed that the mean omega-3 index of the study population was 3.56% with maximal value 8.10% and minimal value 1.12% (Fig. 1D and Table 1). There was no difference detected in omega-3 index between the rural (n = 388) and the urban / industrial (n = 476) region (Fig. 1E) and no difference between males and females (males 3.50 ± 0.98%; females 3.60 ± 0.89%).

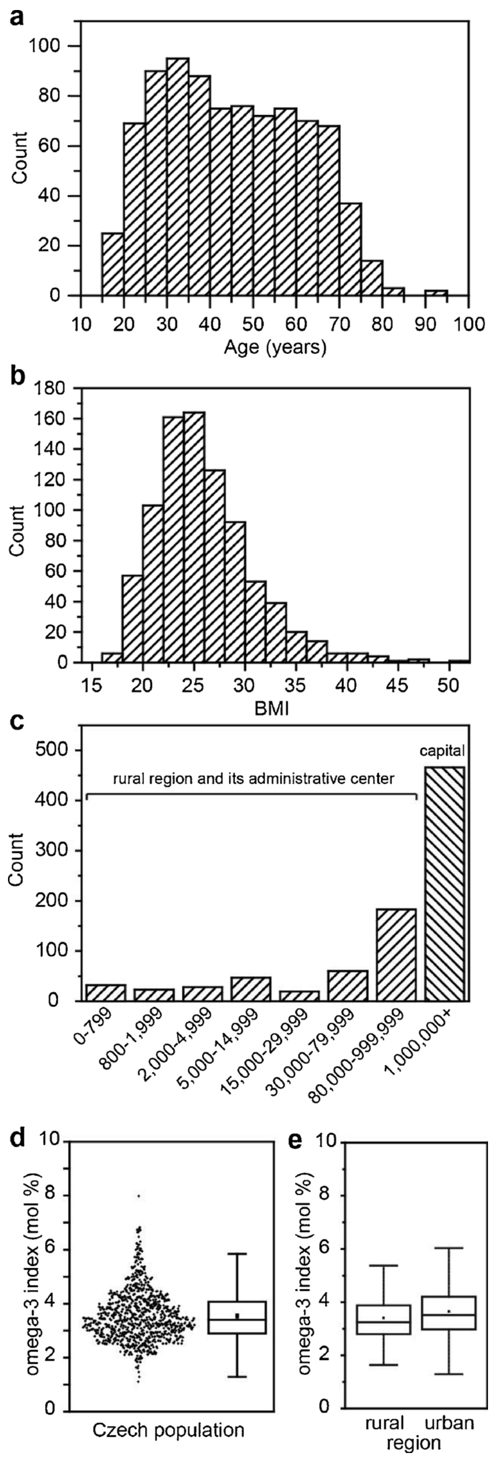


**Fig. 1.** Characteristics of the study population. (a) Distribution of participants according to age; (b) self-reported body mass index expressed as kg/m$^2$; (c) place of residence size / number of inhabitants (i.e. small villages up to capital city). Rural region was defined as a sum of all residences smaller than 1 million inhabitants. (d) Omega-3 index in RBC expressed as mol %. (e) Omega-3 index in rural and urban regions of the Czech Republic.

The study population was divided into 6 groups based on the self-reported fish consumption frequency. Participants, who consumed fish meal at least two times per week, had mean omega-3 index 4.10% and the value linearly decreased to 2.58% in participants, who don't eat fish. This trend was progressively statistically significant (Fig. 2A).

Participants (10%), who reported consumption of omega-3 PUFA supplements, had significantly higher omega-3 index than those not taking any supplementation, 4.22% versus 3.46%, respectively (Fig. 2B). Brand, form and dose of omega-3 supplements were not statistically evaluable, but reflected the omega-3 PUFA dietary supplements available on the market in general.

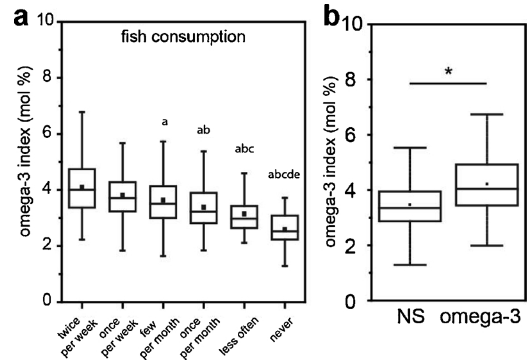


**Fig. 2.** Self-reported fish and omega-3 PUFA consumption. (a): Omega-3 index expressed based on the frequency of fish meal consumption. ANOVA on ranks: a, statistically significant from the 1 st group; b, statistically significant from the 2nd group, etc. up to e, statistically significant from the 5th group. (b): Omega-3 index of participants who reported omega-3 PUFA supplementation (omega-3) or no supplements (NS).

## 4. Discussion

We explored the omega-3 index in the Czech population and compared the urban and the rural regions. The median omega-3 index is lower than 4% and should be considered as very low when compared to world-wide statistics (Harris and Von Schacky, 2004; von Schacky, 2014). Previously, the omega-3 index within the Czech population was quantified in plasma phospholipids of healthy individuals (4.2%; n = 118) (Crispim et al., 2011) and also in clinical trials: plasma phospholipids of obese women (4.0%; n = 40) (Hlavaty et al., 2008), plasma phospholipids of diabetic patients (5.1%; n = 60) (Veleba et al., 2015), and RBC of typical urban population (5.1%; n = 95) (Stankova et al., 2018). Results of the present study using 864 respondents are in agreement with the previous data and highlight very low levels of omega-3 PUFA in the Czech population. However, the main objective of the socio-physiological survey was to compare rural and urban regions in the Czech Republic. Our data showed that there was no difference in omega-3 index between these two regions. Sociological evaluation of the data (the effect of sex, age, self-reported diseases and medications, etc.) was beyond the scope of this paper and will be published later.

The low levels of omega-3 PUFA are striking and could be partially explained by dietary habits of Czech citizens. According to Fishery and Aquaculture Statistics 2013 (FAO UN, 2018), food supply quantities of freshwater fish and marine fish in the Czech Republic were 2.96 kg/capita/year and 0.56 kg/capita/year, respectively. Majority of the Czech freshwater aquaculture production in ponds was focused on common carp (˜95%), followed by rainbow trout or pike in 2016 (FAO UN, 2018), while imported marine species were unknown. Carp and its farmed variants is a very poor source of omega-3 PUFA, especially of DHA, according to US Department of Agriculture (USDA, 2015) and the related characterization of fish products on the market (Strobel et al., 2012). Moreover, the South Bohemian region is famous for carp production in local ponds. Therefore, respondents who reported fish meal consumption are most probably eating farmed carps.

One of the limitations of our study is that the number of participants, who reported use of omega-3 PUFA dietary supplements (10%), and the variability of their responses regarding the brand, dose and frequency of use of these supplements, did not allow a clear assessment of the effects of this type of dietary supplementation. Previously, we have reported omega-3 index ˜8% in diabetic patients supplemented with omega-3 PUFA capsules (Rossmeisl et al., 2018). Therefore, the omega-3 index ˜4% in subjects who self-reported omega-3 supplementation is probably not sufficiently informative. Also, we were not able to evaluate any associations of omega-3 index with diseases, because we do not have access to the medical records.

Results of the present study substantiate the importance of direct evaluation of omega-3 PUFA in the organism, namely the use of the omega-3 index as an omega-3 PUFA intake indicator and a direct correlate of biological effects of omega-3 PUFA. Furthermore, the recent randomized, placebo-controlled VITAL trial in 25,871 men and women across the U.S. has examined whether the daily intake of dietary supplements containing vitamin D3 (2000 IU) and/or omega-3 PUFA (Omacor® fish oil, 1 g) reduces the risk of developing cancer, heart disease, and stroke in people who do not have a prior history of these illnesses (Bassuk et al., 2016; Manson et al., 2018). Although the primary outcome was negative, the analysis of a subgroup of subjects (non-Hispanic whites) revealed that people with a very low omega-3 index (total fish intake < 1.5 servings per week) benefit from the omega-3 PUFA supplementation as the risk of major cardiovascular events decreased (Manson et al., 2018). The observation that omega-3 PUFA could help at a specific combination of factors is further supported by the REDUCE-IT clinical trial where patients with elevated triacylglycerol levels had lower incidence of ischemic events when treated with EPA ester (Bhatt et al., 2018).

## 5. Conclusion

In conclusion, this was the first large-scale study of omega-3 index in the Czech Republic and we found suboptimal values of omega-3 index in the Czech population independent on the sampling region. Our idea that the urban and rural populations will have different omega-3 index due to differences in lifestyle and nutrition was not confirmed. Although fish consumption and usage of omega-3 PUFA nutritional supplements resulted in elevated omega-3 index, even higher intake of omega-3 PUFA in general is needed to achieve optimal levels of omega-3 PUFA in the organism (Harris and Von Schacky, 2004; Stark et al., 2016; von Schacky, 2014) and to fully explore their beneficial effects on health (Calder, 2013; Harris and Von Schacky, 2004; Kuda et al., 2018).

## Author contributions

Conceptualization, DH, PF, JK, OK; Data curation, PJ, NC, IS, OK; Funding acquisition, MR, DH, JK, OK; Methodology, MO, VP, PZ, PJ, DH, OK; Project administration, PJ, TM, OK; Resources, TM, DH, JK; Writing – review & editing, all authors.

## Conflicts of interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chemphyslip.2019.02.006.

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