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Dietary habits of Slovenian inland and coastal primiparous women and fatty acid composition of their human milk samples

Marta Jagodic[a,b], Janja Snoj Tratnik[a,b], Doris Potočnik[a,b], Darja Mazej[a], Nives Ogrinc[a,b], Milena Horvat[a,b,*]

[a] Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000, Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000, Ljubljana, Slovenia

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ABSTRACT

The quantity and composition of fatty acids (FAs) in milk can influence an infant's growth and development through maternal diet. Therefore, associations between maternal diet and FA composition in 74 mature human milk samples were investigated. This study aimed to obtain FA patterns in mature milk arising from different dietary habits in two geographically different areas in Slovenia: Koper (KP), a coastal area, and Pomurje (MS), an inland area. The results revealed statistically significant differences in the dietary intake of game, freshwater fish, and fresh and frozen seafood between the study areas. Among the mean percentages of 35 individual FAs in milk, 19 were higher in KP and 16 were higher in MS. In KP, despite the higher intake of fresh seafood, the levels of saturated and monounsaturated FAs were higher and the levels of PUFAs, ω-3, and ω-6 were lower compared to those in MS. The ω-6:ω-3 ratio did not differ significantly between the study areas. This finding was not expected and indicates a discrepancy between the measured and self-reported data - the latter lack reliable data on dietary supplements. Therefore, determination of FA profile is important as a promising biomarker of dietary intake in environmental health studies.

1. Introduction

The maternal diet and living environment are known to affect the quality of an infant's first food, namely, maternal milk via breastfeeding (Jensen, 1999; Kelishadi et al., 2012; Nishimura et al., 2013; Ruan et al., 1995). Human breast milk is an important part of the diet of infants. It contains many of nutrients that are essential for the health of new-borns (Chu, 2013; Krawinkel, 2011; WHO, 2011). The fatty acid (FA) composition of human milk has attracted much interest from the viewpoint of a child's nutrition owing to the potential health impact of certain FAs on the infant (Röllin et al., 2009; Rudge et al., 2009). Consumption of unsaturated FAs (UFAs) is considered beneficial for human health; therefore, it is important to have a sufficient amount of them in human milk. The nutraceutical components of human milk are omega-3 polyunsaturated FAs (ω-3 PUFA), which play an essential role in development in the early years of life and can improve neurological functions, and conjugated linoleic acids (CLA), which has anti-diabetic, anti-atherogenic, and anti-carcinogenic effects (Andersen et al., 2000; Birch et al., 1992; Chen et al., 1997; Grandjean and Landrigan, 2006; Innis, 2007; Jensen, 1999; Koletzko et al., 1988; Lock and Bauman, 2004; Nunes and Torres, 2010; Ruxton et al., 2004).

FAs in human milk originate from the mobilization of maternal body stores of FAs, diet, or endogenous synthesis from precursor FAs by the liver or breast tissue (Francois et al., 1998; Jensen, 1999, 1996; Sauerwald et al., 2001). Francois et al. (1998) reported that a single meal of a specific fat could significantly affect the human milk FA profile for 1–3 days (Francois et al., 1998). What, then, are the sources of FAs in human diet? Saturated FAs (SFAs) are known to mainly be present in dairy products (e.g., milk, butter, cream, and cheese) and in food containing animal fat products such as biscuits, crackers, cakes, pastries, sauces, fast food, and hamburgers. Monounsaturated FAs (MUFAs) have been used as a substitute for SFAs in nutrition, since the diet rich in MUFAs are preferred to those high in SFAs. Their main sources in human diet are butter, swine and bovine meat, mackerel, nuts (almonds, pea-, Brazil, cashew, hazel-, and pistachio nuts), seeds, olives, olive oil, and rapeseed oil. In the human diet, PUFAs are beneficial for human health, since some of them are even essential nutrients and cannot be synthesized in humans. They are present in higher amounts in vegetable oils, such as soybean and rapeseed oil. Foods rich in ω-3 PUFAs include also fatty fish (salmon, trout, herring, mackerel,

* Corresponding author. Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000, Ljubljana, Slovenia.
E-mail addresses: marta.jagodic@ijs.si (M. Jagodic), janja.tratnik@ijs.si (J. Snoj Tratnik), doris.potocnik@ijs.si (D. Potočnik), darja.mazej@ijs.si (D. Mazej), nives.ogrinc@ijs.si (N. Ogrinc), milena.horvat@ijs.si (M. Horvat).

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tuna fish, sardines, wolf-fish, and flounder), seafood (molluscs, shellfish, crustaceans, echinoderms) and fish oils. The main dietary sources of ω-6 PUFAs are vegetable oils and margarines, mayonnaise, meat, sunflower, corn oil, soybean oil, grapeseed, and sesame seeds (Chow, 2007; Francois et al., 1998; Nishimura et al., 2014; Tvrzicka et al., 2011).

The World Health Organisation (WHO) recommends exclusive breastfeeding for infants for the first 6 months followed by nutritionally adequate and safe complementary feeding with continued breastfeeding up to 2 years of age or beyond (WHO, 2011). This overall strategy is supported also by Slovenian national policies, laws, and programs that promote and support breastfeeding and protect the rights of working mothers breastfeeding both in healthcare institutions and in society (Bratanič, 2018). In Slovenia, information on individual durations of breastfeeding is fragmented, and there is no central system to monitor the duration of exclusive breastfeeding. Therefore, it is important to evaluate the dietary habits from pregnant and lactating woman. In 2007 and 2008, the National institute for public health and their partners performed a survey on dietary habits of Slovenian inhabitants (N = 1193), reported by the main groups of food (Gabrijelčič Blenkuš et al., 2009). Most of the participants consumed milk at least once per day (58.5%), yogurt (30.0%) and cheese (36.4%) 2–3 times per week and the rest of dairy products 1–3 times per month. Fish, seafood and eggs were mainly consumed 1–3 times per month, while different types of meat - poultry, beef and pork - were consumed once per week, respectively. However, respondents with tertiary or higher education consumed sea fish frequently (64.1%). It is also important to note, that inhabitants of eastern Slovenia consume freshwater fish more often than the inhabitants of the western part, which preferred marine over freshwater fish. The largest proportion of respondents used vegetable (64.5%) and olive oil (25.1%) for preparation of their meals. The largest proportion of respondents used butter, greaves, mayonnaise and other vegetable grease only 1–3 times per month (Gabrijelčič Blenkuš et al., 2009). Owing to the importance of the quality of human milk and its dependence on the maternal diet, the present study evaluated FA composition of mature human milk and its association with dietary habits, especially seafood intake. For this purpose, two study areas with presumed differential dietary habits were selected, one in the coast (Koper, KP) and the other inland (Pomurje, MS). We hypothesised that this would reflect on the composition of FAs determined in milk samples of the study areas residents and give a background information for potential use of FAs in milk as indicators of specific dietary habits in environmental health studies. The latter was evaluated together with stable isotopes of FAs in a separate paper (Jagodic et al., 2020). The FA composition of the colostrum in three different areas in Slovenia—-Koper, Ljubljana, and Celje (Fidler et al., 2000; Fidler and Salobir, 2000)—and in mature milk in Ljubljana (Benedik, 2015) has been examined before, thereby allowing a comparison between existing and new data for mature milk from the Koper and Pomurje areas.

2. Materials and methods

2.1. Description of study population

The study population included primiparous women (19–39 years old) who gave birth to a live born child in two different areas in Slovenia: Koper (KP), a coastal area, and Pomurje (MS), an inland area (Fig. 1). The study groups were assessed as part of a First National Human Biomonitoring study in Slovenia (HBM; 2007–2015), which has been described in detail elsewhere (Jagodic et al., 2020; Snoj Tratnik et al., 2019).

The primiparous women from KP and MS were recruited between 2011 and 2014. Biological samples (maternal blood, urine, milk, and hair) were collected between 5 and 11 weeks after delivery. Mothers collected maternal milk from multiple feeds in maximum 6 days. Through questionnaires, information about their age, residence history, family background, social factors, education, weight before pregnancy, height and weight post-partum, parity, detailed reproductive history, medical and occupational history, lifestyle, relevant paternal/pregnancy related information, information about the new-born child, and dietary information for last year (frequencies of food consumption and their sources—vegetables, fruits, nuts, milk and dairy products, eggs, poultry, game, other meat, freshwater fish, and fresh, frozen, and tinned-fish or other seafood, coffee or tea and alcoholic drinks, different dietary oils and dietary supplements) were obtained. For seafood, more detailed specifications were used: (1) seafood without any preservation processes = fresh seafood, (2) seafood that was frozen before consumption (for transport, storage, or other purposes) = frozen seafood, and (3) seafood that was canned for storage purposes = canned seafood. In this study, the term "seafood" refers to all types of sea fish and shellfish, that is, any form of sea life regarded as food by humans. Participants reported also if they were taking dietary supplements, but specific information on the supplement was not collected.

A total of 74 women in KP (36) and MS (38) signed informed consent, provided biological samples, and filled in the questionnaires as described by Jagodic et al. (2020). Recruitment and sampling in the framework of the First National HBM in Slovenia are described in detail by Snoj Tratnik et al. (2019).

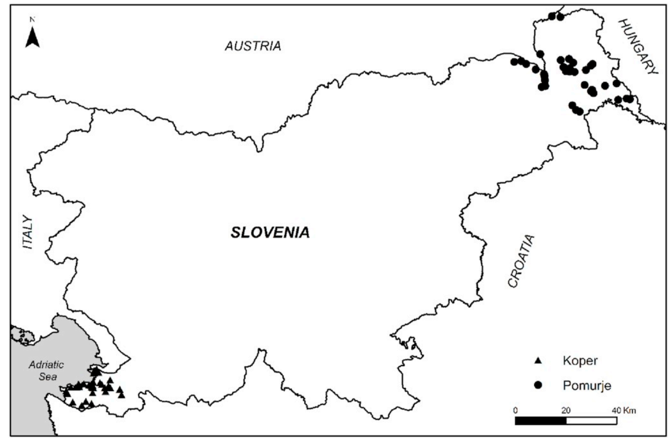

Fig. 1. Sampling areas: Koper (KP), a coastal area, and Pomurje (MS), an inland area (Jagodic et al., 2020).

2.2. Ethical considerations

First National HBM in Slovenia was approved by the Republic of Slovenia National Medical Ethics Committee (NMEC) with numbers of accordance 42/12/07 and 53/07/09. The study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants signed informed consent and all samples and accompanying data were pseudo-anonymised. Participants had a right to withdraw from the study at all stages of the study period.

2.3. Sample preparation and chemical analysis

FAs in human milk were identified and quantified by an in-situ transesterification method. Briefly, human milk triacylglycerides were extracted using the in-situ transesterification method (Park and Goins, 1994). Fatty acid methyl ester (FAME) characterization was performed using an Agilent 6890N (Network GC System) gas chromatograph with a flame ionization detector (GC-FID) equipped with a capillary column (Omegawax 320, 30 m × 0.32 mm × 0.25 µm, Supelco).

Individual FAs were identified and quantified by comparing their retention times with those of the standard (Supelco 37 component FAME Mix), which was analysed after every 10 samples and expressed as the weight percent of the sum of all identified FAs. In each set of samples, a blank sample was also analysed to check the quality of the data. The precision of this method, based on replicates of real samples, was up to 5%. Further, the limit of detection (LOD) was 0.004%. A more detailed description of the methodology can be found in Jagodic et al., (2020).

2.4. Statistical analysis

Non-normally distributed data were log10-tranformed. The percentage of FAs below the LOD were arbitrarily assigned the value of ½ LOD. Based on the food intake categories in the questionnaire, daily intake (meals per day) was estimated (never – 0, less than once per month – 0.02, 1–3 times per month – 0.07, once per week – 0.14, 2–4 times per week – 0.43, 5–6 times per week – 0.79, once per day – 1, more than once per day – 2.5) as described by Valent et al. (2013). Information on the type of seafood (fresh, frozen, or canned) was also included in the questionnaire. The sum of all seafood meals was calculated.

The percentage of FAs were calculated after determining individual FA as described by Jagodic et al. (2020). FAs were grouped as follows: SFA = C4:0 + C6:0 + C8:0 + C1:0:0 + iC11:0 + C11:0 + C12:0 + iC13:0 + C13:0 + iC14:0 + C14:0 + iC15:0 + aC15:0 + C15:0 + C16:0 + iC17:0 + aC17:0 + C17:0 + iC18:0 + C18:0 + C2:0:0 + C21:0 + C22:0 + C23:0 + C24:0; MUFA = C:14:1ω5 + C:15:1 + C:16:1ω9 + C:16:1ω7 + C:17:1 + C:18:1ω9 + C:18:1ω7 + C20:1ω9 + C22:1ω9; PUFA = C18:2ω6c + C18:2ω6t + C18:3ω6 + C18:3ω3 + CLAc9t11C18:2 + C20:2ω6- + C20:3ω6 + C20:3ω3 + C20:4ω6 + C20:5ω3 + C22:2ω6 + C22:4ω6 + C22:5ω6 + C22:5ω3; ω-3 FA = C18:3ω3 + C20:3ω3 + C20:5ω3 + C22:5ω3; ω-6 FA = C18:2ω6c + C18:2ω6t + C18:3ω6 + C20:2ω6 + C20:3ω6 + C20:4ω6 + C22:2ω6 + C22:4ω6 + C22:5ω6; ω-6 LCP = C22:5ω6 + C22:4ω6 + C22:2ω6 + C20:4ω6 + C20:3ω6 + C20:2ω6; ω-3 LCP = C22:5ω3 + C20:5ω3 + C20:3ω3. LPC: ω-6 LCP + ω-3 LCP; C18:2ω6 = C18:2ω6(t + c).

Statistical analyses was performed using Microsoft Excel and SPSS and STATA statistical programs. Statistical significance was defined as $p < 0.05$ and marginal significance, as $0.05 < p < 0.1$. Descriptive statistic, univariate analyses, and simple and multiple linear regression were used to examine possible associations between the FA composition and lifestyle, especially dietary habits. Univariate analyses included: Spearman's test for categorical data, Pearson's test for continuous data, analysis of variance (ANOVA), chi-square tests (Pearson's and Fischer's), and Mann-Whitney *U* test.

3. Results and discussion

3.1. General characteristics of study population

The general characteristics of study groups were obtained through questionnaires. The average maternal age was 29.2 (23.0–36.0) years for KP and 28.8 (20.0–38.0) years for MS. The respective average pre-pregnancy body mass indexes were 23.3 (17.9–35.2) kg/m^2 and 23.3 (17.7–35.1) kg/m^2. In KP, 55%, 33%, and 12% of the participating women had completed university, secondary school, and primary school, respectively. In MS, the corresponding numbers were 46%, 49%, and 5%, respectively. The average infant birth weights were 3.39 (2.3–4.4) and 3.26 (2.3–3.4) kg in KP and MS, respectively. The average infant birth lengths were 53.6 (47.0–59.0) and 50.7 (48.0–54.0) cm in KP and MS, respectively. The average infant weights on the sampling day were 4.29 (3.10–6.30) and 4.51 (2.79–5.50) kg in KP and MS, respectively. The average infant ages on the day of sampling were 1.76 (1.43–2.47) and 1.70 (1.30–2.43) months in KP and MS, respectively. Among the described characteristics, only birth length differed statistically between the areas, being higher in KP.

3.2. Dietary habits of study population

Tables S1 and S2 present the results of the food-frequency questionnaire. Statistically significant differences between the areas were observed in the daily intake of game (higher in KP), freshwater fish (lower in KP), fresh seafood (higher in KP), and frozen seafood (lower in KP) (Table S1) and in the frequency of "morning tea intake" (higher in KP), olive oil use (more in KP), and sunflower oil use (more in MS) (Table S2). The results of comparisons between KP and MS for the intake of freshwater fish, fresh seafood, and frozen seafood and the use of olive oil and sunflower oil confirmed the expected dietary differences because of geographical and agriculture factors. MS has higher local production of sunflowers than KP, and KP has higher local production of olive oil and fresh seafood than MS. The questionnaires showed no statistically significant differences between the two areas for total seafood intake (Table S1). Statistical difference was observed for fresh seafood intake, which was mainly caused by the fact that people in KP eat more fresh seafood because of its higher availability in this coastal area. Therefore, the question arises whether analyses of FAs in human milk provide better information about maternal dietary habits.

3.3. FA composition of mature milk of study population

The percentage of FAs in maternal milk in KP and MS is shown in Fig. 2 and in Table S3, which is the same as that reported by Jagodic et al. (2020). A total of 49 FAs were detected. We were not able to distinguish chromatographic peak for FAs: C22:6ω3 and C24:1ω9, which were not separated on the column used. Further in the paper, we used record "C22:6ω3/C24:1ω9" for their common peak. The mean percentages of individual FAs differed statistically significantly between the study areas for 35 FAs. Specifically, in 19 cases, the percentages were higher in KP than in MS, including for C20:5ω3 and C22:6ω3/C24:1ω9 which are typically found in fish and are required for optimal function of the visual process and brain and nervous systems (Boyer et al., 2005; Harris et al., 1984; Innis, 2007; Jensen, 1999). In the other 16 cases, the percentages were higher in MS than in KP, including for C18:2ω6, C18:3ω3, and C20:4ω6 which are important, even essential, PUFAs for the growth of humans and infants (Boyer et al., 2005; Innis, 2007; Jensen and Lapillonne, 2009; Jensen, 1999; Ruan et al., 1995; Sauerwald et al., 2001). C22:6ω3 and C20:4ω6 are known to be important PUFAs in the neuron-rich grey matter of the brain, and C22:6ω3 status is connected to the immune system (Lauritzen and Carlson, 2011). UFAs are considered beneficial to human health, as noted above. The nutraceutical components were ω − 3 PUFAs, which play essential roles in development in the early years of life and can improve neurological functions, and CLA, which has anti-diabetic, anti-atherogenic, and anti-carcinogenic effects (Lock and Bauman, 2004; Nunes and Torres, 2010).

The mean percentages of FAs in different groups differed statistically significantly between the study areas: only MUFAs had higher levels in KP than in MS, while PUFAs, ω-3 FAs, ω-6 FAs, iC13:0/C13:0, ω-6 + ω-3 FA, ω-6 LCP, ω-3 LCP, LCPs, and ω-6/ω-3 LCPs all had higher levels in MS than in KP.

Table S3 shows a comparison of the mean percentage of FAs in mature milk from KP and MS determined in the present study with reported data for some other parts of Slovenia, specifically, colostrum data from Celje, Ljubljana, and Koper (Fidler et al., 2000) and mature milk from Ljubljana (Benedik, 2015).

In the latter study, the percentage of FAs in mature milk was first evaluated according to the lactation status (colostrum *vs* mature milk). Changes in the content of certain FAs are known to depend on the time of lactation (colostrum *vs* mature milk) (Chen et al., 1997; Guesnet et al., 1993; Wu et al., 2010). Colostrum showed a higher FA percentage compared to mature milk for 82% of cases in Ljubljana (colostrum) (Fidler et al., 2000) *vs* Ljubljana (mature) (Benedik, 2015) and for 83% of cases in Koper (Fidler et al., 2000) *vs* KP (Table S3). Results from KP

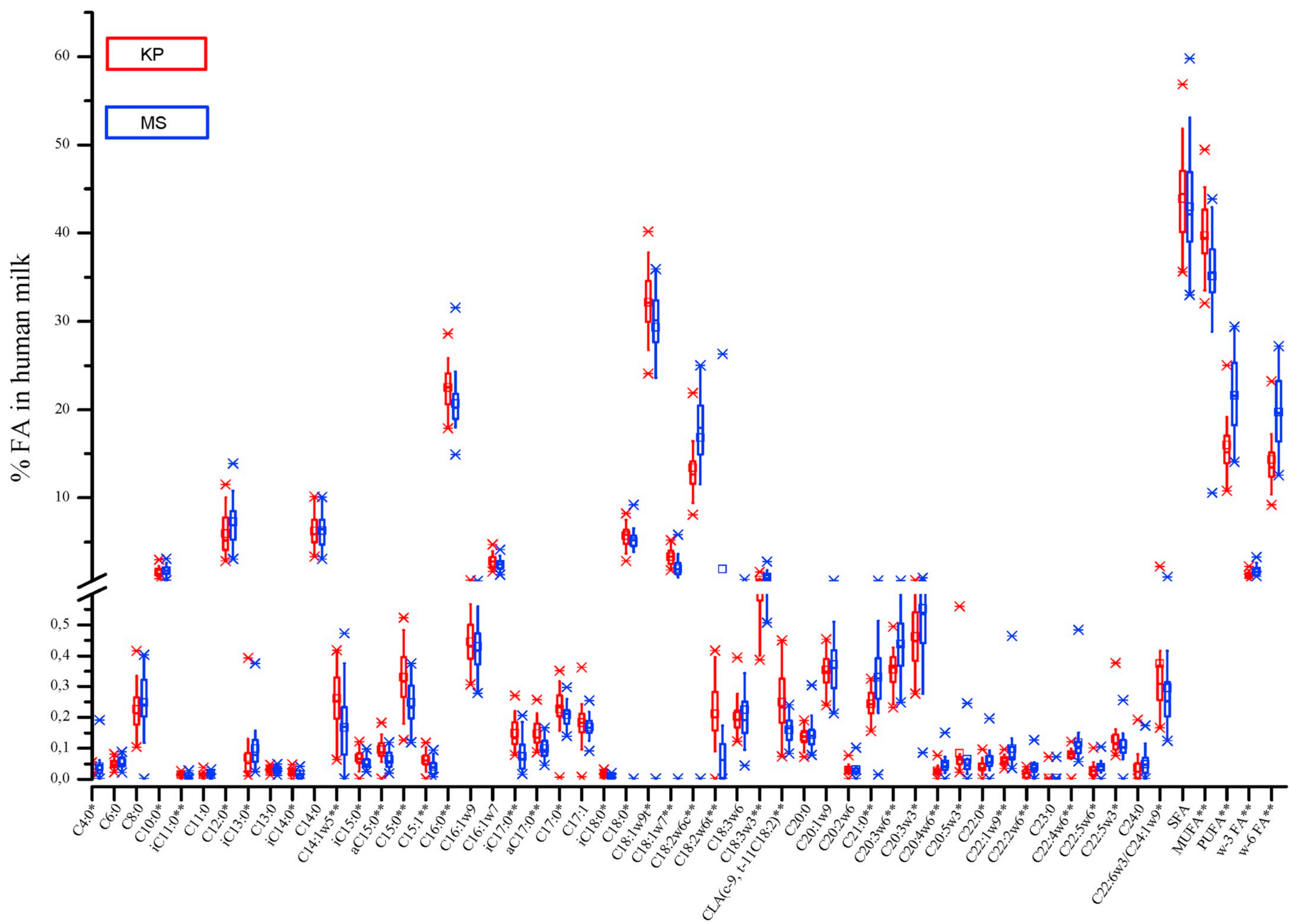

Fig. 2. FA composition of maternal milk and comparison between studied areas - coastal (KP) and inland (MS) (* two groups- KP *vs* MS are statistically significantly different ($p < 0.05$), ** two groups- KP *vs* MS are statistically significantly different ($p < 0.01$), Mann-Whitney U test) (Jagodic et al., 2020).

showed higher percentages of C18:3ω6, C20:3ω3, and SFAs in mature milk (Table S3) *vs* colostrum (Fidler et al., 2000). One reason could be the smaller number of SFAs detected in studies of the colostrum (Fidler et al., 2000). Previous studies (Chen et al., 1997; Wu et al., 2010) reported that the total amount of SFAs increases during the lactation period; the same was observed in the present study despite the lower percentage of individual SFAs in Slovenia compared to France (Guesnet et al., 1993). However, this was not necessarily observed for all individual FAs in the present study and the study by Chen et al. (1997).

Comparing the amount of MUFA in colostrum (Fidler et al., 2000) and mature milk (present study, Table S3), the levels were higher in colostrum than in mature milk, as observed for both ω-6 and ω-3 LCP and their ratio (ω-6 LCP:ω-3 LCP). This observation is in accordance with the literature (Chen et al., 1997; Guesnet et al., 1993). Similarly was observed also in the case of percentage of 20:4ω6 (Table S3) and is in accordance with the study by Chen et al. (1997).

A comparison of the percentages of 28 FAs (reported in both studies) in mature milk between different areas: KP, MS (Table S3) and Ljubljana (Benedik, 2015); revealed a lower percentage of FAs in Ljubljana *vs* KP for 13 FAs and in Ljubljana *vs* MS for 17 FAs. The data of percentage of FAs in KP and MS, as a sum or separately, were compared with literature information for Italy, a neighbouring country (Serra et al., 1997). SFAs in mature milk in Slovenia, MS (43.0%) and KP (43.8%) were similar to those in Italy (45.5%). MUFAs were lower in Slovenia, especially in MS (39.9% in KP and 35.1% in MS *vs* 42.7% in Italy). PUFAs were higher in Slovenia than Italy in (15.9% in KP and 21.6% in MS *vs* 11.8% in Italy). C18:2ω6 was higher in KP than in Italy (13.6% in KP *vs* 9.8% in Italy). Further, C18:3ω3 was higher in KP than in Italy (0.71% in KP *vs* 0.36% in Italy) (Serra et al., 1997). Although the diet of residents of the KP area, which is close to the Italian border, is considered to be similar to Italian's with frequent fresh seafood consumption, there was not much similarity of FAs % in milk between Italy and KP region, which contradicts the abovementioned general belief. Another comparison was made with a European population using the review by Koletzko et al. (1992). FA levels in mature milk from KP and MS mostly fit within the European population ranges reported in the reviewed studies for mature human milk (Koletzko et al., 1992). Difference was obtained for MS in the cases of C18:2ω6 (18.8% *vs* 6.9–16.4%) and sum of ω-6 and ω-3 PUFAs (21.5% *vs* 8.5–19.6%), which had higher percentages than reported for European population (Koletzko et al., 1992). Further, higher values for the 18:2ω6:18:3ω3 ratio were seen in both KP (20.7% *vs* 8.6–16.9%) and MS (21.3% *vs* 8.6–16.9%) compared to literature (Koletzko et al., 1992). A study by Nishimura et al. (2013) of women living far from coastal areas in Brazil indicated lower percentages of SFAs (42.7% *vs* 43.4%) and MUFAs (29.2% *vs* 37.4%) and higher percentages of ω-3 (2.1% *vs* 1.6%) and ω-6 PUFA (21.9% *vs* 17.1%) compared to Slovenia (2013). Further, Chen et al. (1995) reported that in Slovenia, total SFA (43.4% *vs* 38.5%), ω-6 PUFA (18.9% *vs* 11.4%), and ω-3 LCP (0.7% *vs* 0.3%) were higher but total ω-6 LCP (0.63% *vs* 0.83%) was lower compared to Canada; ω-3 PUFA was higher in MS (1.7% *vs* 1.5%) and lower in KP (1.4% *vs* 1.5%) compared to Canada.

3.4. Relationships between FAs and dietary habits

3.4.1. Daily intake of different foods

Studies suggested that maternal diet is a key factor influencing the FA composition of human milk. Studies of FAs with tracer techniques using stable isotopes showed that most PUFAs in maternal milk are not derived directly from the maternal diet but from tissue stores (Jensen, 1999; Kelishadi et al., 2012; Nishimura et al., 2014; Ruan et al., 1995; Sauerwald et al., 2001). However, long-term dietary intake affects the FA composition in tissue stores; therefore, the dietary habits remained the key factor (Sauerwald et al., 2001) and were considered accordingly.

Table S4 shows correlations between the FA compositions in milk samples of our study participants and daily intake of different foods, which was reported by the participants as explained in Sections 2.1 and 2.4.

Vegetable intake correlated positively and significantly with the percentage of C6:0 (Table S4). In general, a vegan/vegetarian diet is rich in C18:2ω6 and C18:3ω3 (Finley et al., 1985; Sanders and Reddy, 1992; Sauerwald et al., 2001), and vegetables are rich in C12:0, C14:0, C16:0, C18:1, C18:0, C18:2, and C18:3 with C16:0, C18:1, and C18:2 predominating (Bruchner and Peng, 2007). Increased consumption of MUFAs and PUFAs is known to be related to decreased levels of SFAs and a favourable PUFAs:SFAs ratio; many vegetables have a favourable ω-3:ω-6 ratio (Bruchner and Peng, 2007). However, the results (Table S4) did not show statistically significant correlations between vegetable intake and the predominating FAs mentioned above.

Higher fruit intake resulted in higher percentage of C20:5ω3, lower percentage of C22:5ω6, and lower ω-6:ω-3 FA ratio (Table S4). No significant correlations were seen with higher fruit intake and PUFAs or MUFAs in breast milk (Table S4). However, the questionnaire data in our study did not include different types or parts of fruits. Fruits show large interspecies differences as well as large differences within different parts of the fruit (mesocarp, kernels/seeds, and nuts or products from them). However, fruits and vegetables both generally contain more UFAs compared to SFAs (Kamel and Kakuda, 2007).

Intake of nuts positively and significantly correlated with the percentages of iC15:0, aC15:0, C15:0, C15:1, iC17:0, and aC17:0 and negatively correlated with the percentages of C20:3ω3, C22:4ω6, C22:5ω6, and sum of percentages of ω-3 LCP and all LCPs in breast milk (Table S4). Kamel and Kakuda (2007) reported that most nuts contain more than 50% fat; most of these are UFAs, with high percentages of C18:2ω6 and C18:1. In disagreement with our study the results did not show correlation with those two (C18:2ω6 and C18:1).

Intake of milk positively and significantly correlated with the percentages of C16:1ω7 and C20:2ω6 and negatively correlated with the percentages of C20:3ω3 and LCP in breast milk (Table S4). Previous studies suggested that the FA profile of milk and dairy products depends on the diet of cows. The concentrations of SFAs in milk are usually lower in summer compared to winter because of the higher intake of PUFAs from fresh forage during the summer (Chilliard et al., 2007; Collomb et al., 2008). Palmquist and Jansen (2007) reported that in milk and butter, the three major constituents in SFAs, MUFAs, and PUFAs are C16:0, C18:0, and C14:0; C18:1ω9, C16:1ω7, and C14:1ω5; and C18:2ω6, C18:2t, and C18:3ω3, respectively. Our result did not show significant correlations with the group of SFAs, MUFAs or PUFAs, but in agreement with the literature (Palmquist and Jansen (2007)) significant correlation was observed for C16:1ω7.

Higher intake of eggs resulted in higher percentages of iC14:0, iC15:0, aC15:0, C15:0, C15:1, aC17:0, and CLA and higher (iC15:0 + aC15:0):C15:0 ratio in breast milk. Poultry positively and significantly correlated with the percentages of C20:3ω3, C22:4ω6, and C22:5ω6 and the sum of ω-3 LCP and LCP and correlated negatively with C18:1ω7 (Table S4). Cantor et al. (2007) reported that of the FAs in egg, 36%, 48%, and 16% are SFAs, MUFAs, and PUFAs, respectively. The predominant FAs in egg are C16:0, C18:1, and C18:2. For light chicken muscle, the lipids comprise 37% SFAs, 33% MUFAs, and 31% PUFAs with the main representatives being C16:0, C18:0, C18:1, C16:1, C18:2, C20:4ω6, and C22:6ω3, and for dark muscle without skin, the lipids comprise 31% SFAs, 38% MUFAs, and 31% PUFAs with a similar FA profile to that of light chicken muscle. In comparison to chicken muscle, turkey muscle has less SFAs and PUFAs but more MUFAs (Cantor et al., 2007). Our results (Table S4) did not show any significant correlations between the predominant FAs in poultry and egg as reported by Cantor et al. (2007) with FAs in milk samples and their daily intake factors.

The intake of game correlated positively with the percentages of iC11:0, iC14:0, aC15:0, C15:0, aC17:0, C17:1, iC18:0, C18:2ω6t, and CLA and negatively with the percentages of C18:2ω6(t + c), sum of PUFA, ω-6 FA, ω-6:ω-3 FA ratio, and sum of ω-6 and ω-3 FA (Table S4). The intake of other meats only correlated negatively with the percentage of iC17:0 (Table S4). FAs in meat usually have medium to long chain lengths. Of the FAs in meat, 40%, 40%, and 2%–25% are SFAs, MUFAs, and PUFAs, respectively, with C18:1ω9 being the major FA (Wood et al., 2007). In contrast to the literature (Wood et al., 2007), our result did not show significant correlation between the daily intake of any meat and C18:1ω9 in breast milk.

Intake of freshwater fish correlated positively with the percentages of C4:0, C18:2ω6c, C18:2ω6(c + t), sum of PUFA, ω-6 FA, ω-6:ω-3 FA ratio, and sum of ω-6 and ω-3 FA and correlated negatively with the percentages of C15:1, iC17:0, aC17:0, C18:1ω9, C18:1ω7, C18:2ω6t, CLA, C22:6ω3/C24:1ω9, and sum of MUFA (Table S4). The results showed different correlations for each studied type of seafood with the composition of stable isotopes of FAs. Intake of fresh seafood correlated positively with higher percentages of C14:1ω5, iC15:0, aC15:0, C15:1, iC17:0, aC17:0, C18:1ω9, C18:1ω7, C18:1ω6t, C18:3ω6, CLA, C20:5ω3, C22:5ω3, and sum of MUFA (Table S4), while negatively with the percentages of C4:0, C8:0, C10:0, C11:0, C12:0, iC13:0, C18:2ω6(c + t), C20:3ω3, C22:2ω6, C22:4ω6, C22:5ω6, sum of PUFA, ω-6 FA, ω-6:ω-3 FA ratio, sum of ω-6 and ω-3 FA, ω-6 LCP, LCP, and C18:2ω6:C18:3ω3 ratio (Table S4).

Frozen seafood intake correlated positively only with the percentage of iC13:0; while canned seafood intake correlated positively only with the percentage of C18:3ω6 (Table S4) and negatively with the percentages of C22:1ω9 and C22:2ω6 (Table S4).

All seafood intake correlated positively with the percentages of iC15:0, aC15:0, C15:1, iC17:0, aC17:0, C18:1ω7, CLA, and sum of MUFA., while negatively with the percentages of C4:0, C18:2ω6(c + t), C22:2ω6, C22:4ω6, C22:5ω3, sum of PUFA, ω-6 FA, ω-6:ω-3 FA ratio, sum of ω-6 and ω-3 FA, ω-6 LCP and C18:2ω6:C18:3ω3 ratio (Table S4).

Fish and fish oil are known to be good sources of FAs such as ω-3 FA (especially 20:5ω3 and 22:6ω3) (Ackman, 2007; Boyer et al., 2005; Harris et al., 1984; Innis, 2007). Francois et al. (1998) showed that 20:5ω3 and 22:6ω3 were significantly higher after consuming menhaden oil and remained elevated in human milk longer than other FAs. They also noted that when lactating mothers consume fish regularly, their milk contains greater amounts of 20:5ω3 and 22:6ω3 for a longer period of time than the milk of mothers who eat fish only occasionally (Francois et al., 1998). Further, Benedik et al. observed that 22:6ω3 was influenced by fish intake before/during pregnancy and during lactation and with 22:6ω3 supplement intake during pregnancy and lactation (Benedik, 2015; Benedik et al., 2014). Ackman reported that some freshwater fish have FAs similar to those of seawater fish, including important ones like C20:5ω3 and C22:6ω3 FAs (Ackman, 2007). From the abovementioned literature we would expect that more ω-3 FA (especially 20:5ω3 and 22:6ω3) would be associated with higher daily intake of seafood, but results showed positive correlations only in case of fresh seafood and C18:3ω6, CLA, C20:5ω3 and C22:5ω3. Of course, it should be noted here, that 22:6ω3 was not determined separately, which means we could not distinguish it from C24:1ω9 to check the association with seafood.

Coffee and tea intake correlated positively only with the percentage of C23:0, while negatively with the percentage of C11:0 and sum of SFA. Furthermore, alcohol intake correlated negatively with the percentage of C20:1ω9 (Table S4). The latter was expected, because alcohol, tea, and coffee have low percentages of fats on a weighed or a volume basis. The main FAs in typical red wines are C16:0, C14:0, and C12:0 (Chang and Chow, 2007; Kamel and Kakuda, 2007), which did not show any significant correlations with our data.

3.4.2. Use of different oils and supplements in diet

Because certain differences in FA contents (KP *vs* MS) were not fully explainable in the discussion above, we also focused on the consumption of different oils (some of which were locally produced) and dietary supplements. In MS, participants might be consuming more locally produced food based on animal and plant fats (e.g. corn oil and sunflower oil rich in C18:2ω6 and canola oil rich in C18:3ω3) (Francois et al., 1998; Jensen, 1999). In accordance with this, higher consumption of sunflower oil (using *vs* not using) was reported in mothers from MS than from KP (55% *vs* 19%) (Table S2), but unfortunately, not all types of animal and plant fats and oils were specified to check further associations (see Table S2). Another reason for the differences between the study areas could be the intake of food supplements, although the difference in self-reported intake was not statistically significant between MS and KP (81% *vs* 75%) (Table S2) and could be questionable owing to the use of self-reported questionnaires.

The use of omega-based dietary supplements, other types of dietary supplements, olive oil/sunflower oil, butter, margarine, or other types of oil were examined using a Mann-Whitney *U* test to identify any connections between our experimental results and the self-reported information; the results are presented in Table S5.

The use of olive oil was associated with C18:1, C18:2, and C18:3, specifically with the percentages of C18:1ω9, C18:1ω7, C18:2ω6c, C18:2ω6t, C18:2ω6(c + t), C18:3ω3, and CLA as well as with those of C4:0, C12:0, iC14:0, C14:1ω5, iC15:0, aC15:0, C15:0, C15:1, iC17:0, aC17:0, C17:0, C18:1ω9, C18:1ω7, C18:2ω6c, C18:2ω6t, C18:2ω6(c + t), C18:3ω3, CLA, C21:0, C22:0, C22:1ω9, C22:2ω6, C22:5ω6, C24:0, sum of SFA, PUFA, ω-3 FA, ω-6:ω-3 FA ratio, iC13:0/C13:0, ω-6 LCP, and ω-3 LCP in milk samples (Table S5). From the literature, the FAs with highest percentages in olive oils are C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, and C20:0, with C18:1 having the highest percentage (White, 2007).

The use of sunflower oil was associated with the percentages of C12:0, C18:1ω9, C18:1ω7, C18:2ω6c, C18:2ω6t, C18:2ω6(c + t), and CLA as well as with those of C8:0, C11:0, C12:0, C15:1, iC17:0, aC17:0, C21:0, C20:3ω6, C20:3ω3, C22:0, C22:2ω6, C22:5ω6, C22:5ω3, sum of PUFA, ω-3 FA, ω-6:ω-3 FA ratio, ω-6 LCP, ω-3 LCP, and C18:2ω6:C18:3ω3 ratio (Table S5). Sunflower oil has the highest percentage of C18:2, followed by C18:1, C16:0, C18:0, C12:0, C18:3, C20:0, C14:0 and C16:1 (White, 2007).

In our study, the use of butter was associated with the percentages of C4:0, C12:0, C15:0, C16:0, iC17:0, aC17:0, C18:1ω9, C18:2ω6(c + t), and CLA as well as with those of iC15:0, aC15:0, C22:1ω9, C22:2ω6, sum of PUFA, ω-3 FA, ω-6:ω-3 FA ratio, and ω-6 LCP (Table S5). White (2007) reported FA percentages higher than 0.1% in butter fat, with C18:1 and C16:0 being the highest, followed by C18:0, C14:0, C4:0, C12:0, C18:2, C6:0, C10:0, C16:1, C15:0, C8:0, C14:1, C17:0, C18:3, C20:1, C17:1, C20:4, C11:0, and C13:0.

The use of margarine was associated with the percentages of C16:1ω9, C17:1, C18:2ω6c, C18:2ω6(c + t), sum of PUFA, ω-3 FA, ω-6:ω-3 FA ratio, iC13:0:C13:0 ratio, and ω-6 LCP (Table S5). The use of other types of oil was associated with the percentages of only C6:0 and iC13:0 (Table S5). Trans-FAs account for less than 5% of total FAs in butter, whereas they can account for up to 60% of FAs in margarine (Dhaka et al., 2011). However, in our study, no differences were seen in specific trans-FAs between butter and margarine intake (Table S5).

3.4.3. Frequency of different meals during the day

The frequency of different meals during the day (descriptive statistics is given in Table S2) had different influences on FA levels (Table S6). The frequency of lunch did not differ between participants, so no correlations were determined. The frequency of breakfast and dinner did not correlate statistically significant with the percentages of any FAs. In mothers who reported having 'morning tea' and 'afternoon tea' more often, the levels of iC11:0, iC14:0, C14:1ω5, aC15:0, C15:0, C16:0, C18:2ω6t, and CLA were higher and those of C18:2ω6c, C18:2ω6(c + t), C22:1ω9, sum of PUFA, ω-6 FA, ω-6, and ω-3 FA were lower. Further, the 'morning tea' frequency correlated positively with the percentages of iC17:0, aC17:0, C18:0, and sum of MUFA. The results suggested that mothers had eaten certain food items which had higher proportions of these FAs (iC11:0, iC14:0, C14:1ω5, aC15:0, C15:0, C16:0, C18:2ω6t, CLA, iC17:0, aC17:0, C18:0, and MUFA) during the morning and afternoon teas. 'Morning tea' correlated negatively only with C18:3ω3 and 'afternoon tea' correlated negatively with only C22:2ω6 and C24:0.

3.5. Limitations and strengths of the study

The major limitation of the present study is that the samples of milk were primarily not collected for the purpose of this particular study. Collection of samples including the sampling areas was designed within the First National HBM program with objectives to estimate overall exposure of Slovenian inhabitant to different environmental pollutants. There were 12 study areas in the First National HBM, among which two were selected for the purpose of the present study – coastal and inland. Consequently, the sample size was low and probably not entirely representative for the respective areas. Furthermore, the questionnaire was not especially designed for the FAs evaluation in breast milk. However, the study gives a good starting point for evaluating dietary habits using advanced biomarker technique because it has an advantage of two distinct geographic areas with residents having distinct dietary habits as described above.

4. Conclusions

The study presents results of FAs in mature human milk from two geographically distinct areas, KP (coastal area) and MS (inland area), in Slovenia. Differences were expected in the dietary habits and frequencies of consumption of some local food items. The food frequency questionnaire data showed that the study areas (inland *vs* coastal) differed statistically significantly in terms of the intake of game (lower in inland area), freshwater fish (higher in inland area), fresh seafood (lower in inland area), and frozen seafood (higher in inland area). The results of the FA composition of human milk showed that the mean percentages of 35 individual FAs differed statistically significantly between the study areas: they were higher in KP for 19 FAs and in MS for 16 FAs. Despite the higher intake of fresh seafood in KP, the levels of SFAs and MUFAs were higher in KP and the levels of PUFAs, ω-3, and ω-6 were lower in KP compared to those in MS. This finding was not expected and indicates a discrepancy between the measured and self-reported data. However, the study confirmed that the dietary habits of Slovenian inland and coastal primiparous women influenced the FAs composition of their milk samples. Because the self-reported data on food intake is difficult to fill in and has high uncertainty, this study should serve as a good starting point toward establishing more precise biomarkers of dietary intake.

CRediT authorship contribution statement

Marta Jagodic: Writing - original draft, Formal analysis, Investigation, Methodology, Validation. **Janja Snoj Tratnik:** Writing - review & editing. **Doris Potočnik:** Formal analysis, Investigation, Methodology, Validation. **Darja Mazej:** Conceptualization, Writing -

review & editing. **Nives Ogrinc:** Writing - review & editing. **Milena Horvat:** Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111299.

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