

UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Evgen BENEDIK

**PREHRANA V ČASU NOSEČNOSTI IN DOJENJA
TER MAŠČOBNO-KISLINSKA SESTAVA
HUMANEGA MLEKA**

DOKTORSKA DISERTACIJA

Ljubljana, 2015

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**NUTRITION DURING PREGNANCY AND LACTATION AND
FATTY ACID COMPOSITION OF HUMAN MILK**

DOCTORAL DISSERTATION

Ljubljana, 2015

POPRAVKI

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa Komisije za doktorski študij Univerze v Ljubljani z dne 19. 9. 2012 je bilo potrjeno, da kandidat izpolnjuje pogoje za opravljanje doktorata znanosti na Interdisciplinarnem doktorskem študijskem programu Bioznanosti, znanstveno področje prehrana. Za mentorico je bila imenovana prof. dr. Nataša Fidler Mis.

Doktorska študija je bila opravljena kot del projekta Vloga humanega mleka v razvoju črevesne mikrobiote dojenčka (št. projekta: ARRS-PROJ-J4-3606), ki je potekal na Pediatrični kliniki v Ljubljani Univerzitetnega kliničnega centra Ljubljana in Biotehniški fakulteti Univerze v Ljubljani. Praktični del študije se je v celoti izvajal na Pediatrični kliniki; klinični del študije je bil opravljen na Kliničnem oddelku za neonatologijo ter Kliničnem oddelku za gastroenterologijo, hepatologijo in nutricionistiko, medtem ko je bila analitika maščobno-kislinske sestave humanega mleka opravljena v laboratoriju Službe za specialno laboratorijsko dejavnost. Računalniška in statistična obdelava podatkov sta bili izvedeni na Pediatrični kliniki v sodelovanju z Inštitutom Jožef Stefan.

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Datum zagovora:

Podpisani izjavljam, da je disertacija rezultat lastnega raziskovalnega dela. Izjavljam, da je elektronski izvod identičen tiskanemu. Na univerzo neodplačno, neizključno, prostorsko in časovno neomejeno prenašam pravici shranitve avtorskega dela v elektronski obliki in reproduciranja ter pravico omogočanja javnega dostopa do avtorskega dela na svetovnem spletu preko Digitalne knjižnice Biotehniške fakultete.

Doktorand:
Evgen BENEDIK

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

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TD	Doktorska disertacija
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IJ	sl
JI	sl/en
AI	Zdrava prehrana v času pred in med nosečnostjo ter dojenjem pomembno vpliva na zdravje dojenčka in otroka vse v odraslo dobo pa tudi na naslednjo generacijo potomcev, kar imenujemo presnovno programiranje ali presnovni vtis. Namen doktorske disertacije je bil preveriti, ali je: I.) 4-dnevni tehtan elektronski prehranski dnevnik (e-4PD) enakovreden ali celo boljši od papirnega 4-dnevnega tehtanega prehranskega dnevnika (p-4PD); II.) ali prehrana slovenskih nosečnic in doječih mater odstopa od prehranskih priporočil; III.) ali prehranske navade, zlasti uživanje rib, ribjih izdelkov in morskih sadežev (rib/izdelkov/sadežev) ter prehranskih dopolnil, v času pred (PN; leto dni pred nosečnostjo) in med nosečnostjo (MN; od 27. do 37. tedna) ter med dojenjem (MD; od 4. do 5. tedna po porodu) vplivajo na vsebnost dolgoverižnih večkrat nenasicienih maščobnih kislin (LCP), zlasti dokozahexaenojske kislina (C22:6 n-3, DHK) v humanem mleku (HM). V študijo Moje-mleko (www.moje-mleko.si) smo v letih 2010 in 2012 vključili 174 zdravih nosečnic (prostovoljk), v glavnem iz Ljubljane in okolice. Trenutno prehrano smo spremljali z metodo p-4PD dvakrat, MN ter MD. Za ugotavljanje prehranskih navad uživanja rib/izdelkov/sadežev in prehranskih dopolnil v treh časovnih obdobjih – PN, MN in MD – smo uporabili vprašalnik o pogostosti uživanja živil (VPŽ). Analitika maščobno-kislinske sestave HM, predvsem vsebnosti DHK, je bila izvedena s pomočjo plinske kromatografije s plamenskim ionizacijskim detektorjem (GC-FID). Pridobljene podatke smo obdelali in statistično analizirali z uporabo računalniškega programa za statistično analizo SPSS 21.0 (Statistical Package for Social Sciences). Uporabili smo naslednje statistične metode: metodo opisne statistike, Spearmanov koeficient korelacije, test ANOVA, test Wilcoxon in metodo deatenuacije. V celoti je vse načrtovane aktivnosti v študiji zaključilo 152 prostovoljk. Dokazali smo, da se p-4PD lahko nadomesti z e-4PD oziroma lahko prostovoljka samostojno vodi svoj e-4PD v aplikaciji Odprte platforme za klinično prehrano (OPKP) ob predpostavki, da je računalniško dovolj usposobljena, saj smo ugotovili tudi, da sta e-4PD in računalniški program Prodi 5.9 Exper Plus, Nutri-Science, Stuttgart, Germany, 2011 (s-4PD) primerljiva med seboj. Prehranski vnos (na osnovi 4PD) slovenskih nosečnic in doječih mater ni optimalen, zaužijejo preveč nasičenih maščob, skupnega sladkorja in soli, premalo pa LCP (zlasti DHK), vitaminov (B ₉ , D in E) ter elementov v sledovih (železo, fluorid ter jod). Vnos DHK (priporočenih vsaj 200 mg DHK/dan) (na osnovi VPŽ) iz rib/izdelkov/sadežev in prehranskih dopolnil je zadosten samo MN (246 mg DHK/dan). Prehranska dopolnila z DHK, predvsem MN, signifikantno prispevajo k vsebnosti DHK v HM ($P < 0,01$). Z našo študijo smo dobili vpogled v prehranski vnos DHK s hrano in prehranskimi dopolnilmi ter v prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil v povezavi z vsebnostjo DHK v HM. Dobljeni rezultati so osvetlili problematiko prehrane pri slovenskih nosečnicah in doječih materah ter so izhodišče za ciljane ukrepe za izboljšanje prehrane na nacionalni ravni.

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AB Early nutrition plays an important role in health, not only during childhood but also through adulthood. It can even have an influence on descendants, which is called metabolic programming or metabolic imprinting. We aimed to examine whether: I.) web-based 4-day weighted dietary records (e-4PD) are equivalent to or even better than paper-based 4-day weighted dietary records (p-4PD); II.) whether Slovenian pregnant and breastfeeding women meet dietary recommendations; III.) whether dietary habits, especially consuming fish, fish products and seafood (fish/products/seafood) and dietary supplements in the time before pregnancy (PN, one year before pregnancy), during pregnancy (MN, between 27 to 37th week of pregnancy) and during lactation (MD; 4-5 weeks *post partum*) influence the content of long chain polyunsaturated fatty acids (LCP), in particular docosahexaenoic acid (C22:6 n-3, DHK) in human milk (HM). Two hundred and ninety-four healthy pregnant women (volunteers) were recruited into the "My-Milk" study (www.moje-mleko.si/en) from December 2010 to October 2012, of which 174, mainly from the Ljubljana region, participated in the clinical part of the study. The diet of the volunteers was assessed by 4PD twice; MN and MD. A Food Frequency Questionnaire (VPŽ) was used to assess habitual fish/products/seafood, as well as supplements intake, through all three time periods (PN, MN and MD). The fatty acid composition of HM (31 fatty acids), especially the content of DHK, was analyzed by gas chromatography with a flame ionization detector (GC-FID). The acquired data were processed and statistically analyzed using SPSS 21.0 (Statistical Package for Social Sciences). We used the following statistical methods: the method of descriptive statistics, Spearman's rho correlation coefficients, ANOVA test, Wilcoxon test and the residual method. All planned activities in the study were completed by 152 volunteers. We proved that p-4PD could be replaced by e-4PD or the volunteer could independently record her e-4PD in Open Platform for Clinical Nutrition (OPKP), assuming that her computer literacy is sufficient. We also found that e-4PD and the computer software Prodi 5.9 Exper Plus, Nutri-Science, Stuttgart, Germany, 2011(s-4PD) are comparable. We conclude that the diet (based on 4PD) of Slovenian pregnant and lactating women is not optimal. It is characterized by excessive intake of saturated fatty acids, total sugars and salt, and a deficient intake of LCP (especially DHK), vitamins (B₉, D, E) and trace elements (iron, fluoride and iodine). The recommended DHK intake (≥ 200 mg DHK/day) was only reached MN on account of fish/products/seafood and supplements intake together (246 mg DHK/day). Supplements with DHK, especially MN, significantly contributed to the content of DHK in HM ($P < 0.01$). Our study gives an insight into the dietary intake of DHK from food and supplements, as well as dietary habits of fish/products/seafood and supplements consumption in relation to their DHK content in HM. The results show inadequate nutrition in Slovenian pregnant and lactating women. This is the starting point for targeted actions to improve nutrition on a national level.

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OKRAJŠAVE IN SIMBOLI

% E	Delež celodnevnega energijskega vnosa
4PD1	4-dnevni tehtan prehranski dnevnik, voden v času nosečnosti (od 27. do 37. tedna)
4PD2	4-dnevni tehtan prehranski dnevnik, voden v času dojenja (od 4. do 5. tedna po porodu)
4PD	4-dnevni tehtan prehranski dnevnik
AK	Arahidonska kislina (angl. arachidonic acid)
ALK	Alfa-linolenska kislina (angl. alpha-linolenic acid)
CRM	Certificiran referenčni material (angl. certified reference material)
DHK	Dokozahexaenojska kislina (angl. docosahexaenoic acid)
e-4PD	Elektronski 4-dnevni prehranski dnevnik
EFSA	Evropska agencija za varnost hrane (angl. European food safety authority)
ENMK	Enkrat nenasičene maščobne kisline
EPK	Eikozapentaenojska kislina (angl. eicosapentaenoic acid)
FAO	Organizacija združenih narodov za prehrano in kmetijstvo (angl. Food and agriculture organisation of the United Nations)
GC-FID	Plinska kromatografija s plamensko-ionizacijskim detektorjem
HM	Humano mleko
INFOODS	Mednarodna mreža zbirk podatkov o sestavi živil (angl. International network of food data systems)
ITM	Indeks telesne mase
IVZ	Inštitut za varovanje zdravja Republike Slovenije
LA	Linolna kislina
LCP	Dolgoverižne večkrat nenasičene maščobne kisline (angl. long-chain polyunsaturated fatty acids)
LJ	Ljubljana in okolica
MB	Maribor in okolica
MD	Med dojenjem (od 4. do 5. tedna po porodu)
MEMK	Metilni estri maščobnih kislin
MetOH	Metanol
MK	Maščobna kislina
MN	Med nosečnostjo (od 27. do 37. tedna)
NG/IZ	Nova Gorica/Izola
NMK	Nasičene maščobne kisline
OPKP	Odprta platforma za klinično prehrano; spletno orodje (angl. Open platform for clinical nutrition)
p-4PD	Papirni 4-dnevni prehranski dnevnik
PN	Leto dni pred nosečnostjo
rib/izdelkov/sadežev	ribe, ribji izdelki, morski sadežev
SACN	Znanstveni svetovalni odbor za prehrano (angl. Scientific Advisory Committee on Nutrition)
S.D.	Standardna deviacija
S.E.	Standardna napaka
s-4PD	Računalniški 4-dnevni prehranski dnevnik (Prodi 5.9 Exper Plus, Nutri-Science, Stuttgart, Germany, 2011)
VNMK	Večkrat nenasičene maščobne kisline (angl. polyunsaturated fatty acids)
VPŽ1	Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil pred in med nosečnostjo
VPŽ2	Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil med dojenjem
VPŽ	Vprašalnik o pogostosti uživanja živil (angl. Food frequency questionnaire)
WHO	Svetovna zdravstvena organizacija (angl. World Health Organization)

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

Zdrava prehrana in zdrav življenjski slog v času pred nosečnostjo (za žensko in moškega), med nosečnostjo in dojenjem ter v času prvih dveh let otrokovega življenja sta pomembna dejavnika za doseganje optimalnega zdravja otroka skozi celo življenje (Sun in sod., 2013; Lillycrop in Burdge, 2015). Zdrava prehrana vpliva, ne le na zdravje dojenčka in otroka, temveč seže njen vpliv vse v odraslo dobo (zmanjšanje tveganja za razvoj prekomerne telesne mase, diabetesa, alergij, povišanega krvnega tlaka, bolezni srca in ožilja ter presnovnih bolezni) in celo na naslednjo generacijo potomcev (Koletzko in sod., 2012; Netting in sod., 2013; Vickers, 2014; Langley-Evans, 2015), kar imenujemo presnovno programiranje (angl. metabolic programming) (Lucas, 1998) ali presnovni vtis (angl. metabolic imprinting) (Waterland in Garza, 1999; Patel in Srinivasan, 2011). V času pred in med nosečnostjo ter dojenjem se razmeroma povečajo potrebe po hranilih kot tudi energiji, zato bi ženske v teh obdobjih morale v svojo prehrano vključiti čim več živil z visoko vsebnostjo esencialnih hranil (Koletzko in sod., 2013). Pestra in uravnotežena prehrana ter redni obroki noseče in doječe matere so, poleg zadostnega vnosa tekočine, izrednega pomena za zdravje matere in njenega otroka (Agostoni in sod., 2009; Koletzko in sod., 2013). S tega vidika so v letu 2007 izšla poenotena priporočila Evropske komisije in Mednarodnega društva za proučevanje maščobnih kislín in maščob, ki svetujejo, da se že v času nosečnosti, najbolje pa v prvem trimesečju, preverijo morebitne neustreznosti v prehrani. Nosečnicam, pri katerih bi ugotovili odstopanja od priporočene prehrane, naj bi zagotovili individualno prehransko svetovanje v času nosečnosti in v času dojenja (Koletzko in sod., 2007; Koletzko in sod., 2008).

Humano mleko (HM) zdravih in dobro prehranjenih mater je najustreznejša hrana in zaščita za dojenčke, saj je kompleksna mešanica hranil, rastnih faktorjev, hormonov ter bioaktivnih in drugih zaščitnih snovi (Jenness, 1979). Hranjenje dojenčka s HM se priporoča vsaj do dopolnjenega drugega leta starosti, nato pa, dokler želita doječa mati in otrok (PAHO/WHO, 2002). Izključno dojenje v prvih šestih mesecih življenja zagotovi vse dojenčkove potrebe za optimalno rast, razvoj in zdravje (Agostoni in sod., 2008).

HM vsebuje esencialni maščobni kislini, linolno (LA, C18:2 n-6) in alfa-linolensko kislino (ALK, C18:3 n-3) ter pogojno esencialne (za donošene in nedonošene dojenčke) dolgoverižne večkrat nenasičene maščobne kislíne: dokozaheksaenojsko (DHK, C22:6 n-3), eikozapentaenojsko (EPK, C20:5 n-3) in arahidonsko kislino (AK, C20:4 n-6) (angl. long-chain polyunsaturated fatty acids, LCP) (Koletzko in sod., 2001; Lapillonne, 2014; Koletzko in sod., 2014). DHK in EPK sta pomembni za zdrav razvoj otroka, predvsem v času hitre rasti in diferenciacije živčnega sistema v pozrem prenatalnem (zadnja tretjina nosečnosti) in zgodnjem postnatalnem obdobju (vse do drugega leta starosti), ko potekata hitra rast in razvoj možganov ter vida (Koletzko in sod., 2007; Ryan in sod., 2010). EPK preprečuje srčno-žilna obolenja (EFSA, 2012). LCP imajo ugoden vpliv na imunski sistem, delujejo pa tudi protivnetno (Koletzko in Rodriguez-Palmero, 1999; Koletzko in sod., 2001a; Calder, 2010; Ryan in sod., 2010; Fidler Mis in Benedik, 2011). V maternici oskrbuje zarodek z LCP zelo učinkovit transplacentalni prenosni mehanizem (Larque in sod., 2002). Po rojstvu uživajo dojeni dojenčki LCP s HM. Na maščobno-kislinsko sestavo HM vpliva prehrana v daljšem časovnem obdobju že pred in med nosečnostjo (van Houwelingen in sod., 1995; Connor in sod., 1996), ne le v času dojenja (Uauy in sod.,

1996; Fidler in sod., 2000b). Delež DHK v zrelem HM znaša od najmanj 0,06 do 0,14 ut. % pri materah, ki se prehranjujejo vegansko, in pri materah, ki živijo v celinskih državah oziroma na podeželju (Pakistan, Severna Afrika, Kanada, Nizozemska in Francija), 0,30 ut. % pri materah, ki se prehranjujejo z vegetarijansko prehrano, 0,37 ut. % pri materah, ki se prehranjujejo z mešano prehrano (omnivori), ter do 1,40 ut. % pri materah, ki žive ob morju ali na otokih in uživajo veliko morskih rib (na primer sardele, girice, orada, brancin) (Sanders, 1999; Brenna in sod., 2007). V študiji s stabilnimi izotopi je bilo ugotovljeno da 60-70 % dolgoverižnih maščobnih kislin v HM izhaja iz maščobnih rezerv matere (maščobno tkivo), 20-30 pa iz prehrane matere v krajšem časovnem obdobju (Fidler in sod., 2000b). Prehranska dopolnila, ki vsebujejo LCP, prav tako vplivajo na maščobno-kislinsko sestavo HM (Harris in sod., 1984; Makrides in sod., 1996).

Ocena prehranskega statusa je sestavljena iz prehranske ocene (ocenimo vnos energije in hranil v določenem obdobju), antropometričnih in drugih meritev (starost, telesna višina, masa, obseg glave, kožne gube, telesna gostota), določitve biomarkerjev (odražajo bodisi vnos hranil bodisi njihov vpliv) ter zdravstvenega stanja (WHO, 1963; Vorster in Hautvast, 2002).

Za proučevanje prehrane in prehranskih navad obstajajo številne metode. Neposreden, individualen pristop beleženja prehrane je najboljši način za pridobitev bolj natančnih podatkov. Poznamo metode, pri katerih beležimo podatke v krajšem časovnem obdobju, po navadi od tri do sedem dni (predvidene metode: tehtane metode ter metode ocenjene količine, na primer 4-dnevni tehtani prehranski dnevnik (4PD), metoda jedilnika prejšnjega dne oziroma prejšnjih dni (24 urni »recall«)), ter metode, pri katerih beležimo podatke o hrani, zaužiti v bližnji preteklosti ali v daljem časovnem obdobju (retrospektivne metode, metode intervjujev; na primer vprašalnik o pogostosti uživanja živil (VPŽ), ki se najpogosteje uporablja v epidemioloških študijah) (EFSA, 2009).

Namesto metode papirnega prehranskega dnevnika (p-4PD) lahko uporabljamo različne spletnne aplikacije, ki omogočajo beleženje prehranskega dnevnika neposredno v računalnik ali celo v mobilni telefon. Ena od takšnih aplikacij je Odprta platforma za klinično prehrano, OPKP (OPKP, 2015). Vedno večja težnja je, da bi se pri prehranski obravnavi pacientov uporabljale sodobne spletne tehnologije, saj tako ni treba dietetiku prepisovati prehranskega dnevnika s papirja v elektronsko obliko (e-4PD), s čimer prihrani čas in se tako lahko bolje posveti pacientu. Poleg tega pa naj bi bil prehranski dnevnik, ki ga vodi pacient, tudi natančnejši od tistega, ki ga na podlagi pacientovega p-4PD vnese v e-4PD dietetik, ker pacient v idealnem primeru izbere tisto živilo iz baze podatkov, ki jo je užival, oziroma tisto, ki je najbližje njegovemu izboru, dietetik pa si lahko izbere drugo živilo (Koroušić Seljak, 2011).

VPŽ zajema standardizirane, kvantitativne podatke o običajnih, dolgoročnih prehranjevalnih navadah posameznika in se uporablja za ugotavljanje prehrane v preteklem obdobju (Patterson in Pietinen, 2004). Poznamo tri tipe VPŽ vprašalnikov: enostavni oziroma nekvantitativni, polkvantitativni in kvantitativni. Seznam živil, ki je najpomembnejši del vprašalnika, je sestavljen tako, da zajema tista živila ali skupino živil, ki dokazano prispevajo k prehranskemu vnosu posameznega proučevanega hranila, hkrati pa prinašajo podatke o viru energije in hranil za ciljno populacijo. Pozitivna stran VPŽ je,

da so za uporabnike manj obremenjujoči, enostavni in poceni za analizo v primerjavi z, na primer, 4PD. Negativna stran pa je, da so VPŽ vprašalniki manj natančni od 4PD.

Rezultati preliminarne študije opravljene na istih slovenskih nosečnicah ($N = 69$), kot so sodelovale v naši študiji, kažejo, da je vnos vitaminov in mineralov s hrano skrb vzbujajoč, saj je kar 46,4 % preiskovank uvrščenih v skupino z revnim prehranskim statusom (zadovoljiv vnos dveh ali manj preiskovanih hranil, od skupno osmih). Poleg tega nobena od preiskovank ni doseгла priporočenega vnosa šestih ali več preiskovanih hranil. S prehranskimi dopolnili je sicer 27,5 % udeleženk pokrilo potrebe po vseh ali šestih od sedmih vitaminov in mineralov, medtem ko je bila četrtina v skupini z revnim prehranskim statusom (Puš in sod., 2013). V pilotni študiji o prehrani doječih mater v Sloveniji ($N = 40$), objavljeni leta 2003, je bil ugotovljen nezadosten prehranski vnos joda, železa, vitaminov A, D in folne kisline ter da uživajo neustrezno sestavo maščob (Širca-Čampa in sod., 2003). Slabe prehranske navade med nosečnostjo lahko vodijo do različnih zdravstvenih zapletov tako pri materi (povišan krvni tlak, debelost, nosečniški diabetes) (Borgen in sod., 2012; Gresham in sod., 2014; Schoenaker in sod., 2014; Torjusen in sod., 2014) kot pri plodu/otroku (nenormalna rast, nizka porodna masa, okvara nevralne cevi) (Moore in sod., 2004; Wu in sod., 2004; Boney in sod., 2005; Belkacemi in sod., 2010; Cetin in sod., 2010; Carmichael in sod., 2012).

Vsebnost maščob v zrelem HM je različna in v povprečju znaša od 3,8 do 3,9 g/100 ml (Koletzko in sod., 2001a). Med dojenjem se delež (%) maščobe veča in lahko doseže celo 5,2 % (Clark in sod., 1982; Koletzko in Rodriguez-Palmero, 1999). Na maščobno-kislinsko sestavo vpliva prehrana in prehranski status med nosečnostjo in dojenjem, faza dojenja (colostrum, prehodno, zrelo mleko), razlika med dojkama, razlike med podojem, vsakodnevni ritem dojenja, genetske značilnosti, morebitne presnovne motnje matere in številni drugi dejavniki (Koletzko in Rodriguez-Palmero, 1999; Bokor in sod., 2007). Na analitsko določeno vsebnost maščob vplivata tudi čas odvzema in način zbiranja vzorcev (Clark in sod., 1982). Maščobne kisline pridejo v mlečno žlezo v obliki hilomikronov (iz prehrane), lipoproteinov nizke gostote (iz jeter) in kot kompleksi z albumini (iz maščobnega tkiva) (Demmelmaier in sod., 2001). Rezultati kažejo, da sestava in količina maščobnega tkiva mater pomembno prispevata k sestavi HM (Demmelmaier in sod., 2001). Zato so dnevna nihanja v prehranskem vnosu maščobnih kislin do neke mere presnovno uravnana – ostajajo relativno stabilna, kar bi lahko bila pomembna biološka prednost (Koletzko in Rodriguez-Palmero, 1999; Koletzko in sod., 2001b; Larque in sod., 2002). Do pred kratkim je veljalo prepričanje, da so rastlinska olja in oreščki, ki imajo visoke vsebnosti ALK (lanena semena, laneno olje, orehi, orehovo olje, semena Chia, olje oljne repice in sojino olje), dober vir DHK, predvsem za vegetarijanke, saj naj bi se v človeškem organizmu sintetizirala iz ALK. Izkazalo se je, da v ženskem telesu iz ALK nastane $< 9\%$ DHK (DeFilippis in Sperling, 2006; Williams in Burdge, 2006; Abedi in Mohammad Ali, 2014), kar je premalo za pokrivanje priporočenih dnevnih potreb po DHK. Nekatera olja iz mikroalg (tako imenovano olje enoceličnih organizmov) vsebujejo visoko vsebnost DHK in so tako primeren vir DHK za osebe, ki se prehranjujejo vegansko in vegetarijansko (Lane in sod., 2014). Prav tako je primeren vir DHK tudi ribje olje. Ribje olje naj nebi predstavljalo tveganja za zdravje, obstajajo pa pomisleki glede eventuelne prisotnosti živega srebra in drugih nečistoč (PCB in dioksini), predvsem v ribjem olju pridobljenim iz notranjih organov rib (Harris, 2004). Proizvajalci ribjega olja sicer odstranjujejo

nečistoče, a vendar nad tem trenutno ni dobrega nadzora, zaradi česar se nosečim in doječim materam priporoča raje uživanje rib z dna prehranske verige (na primer sardele, girice, orada, brancin) (Wenstrom, 2014).

Povprečna vsebnost DHK v HM v različnih delih sveta (Evropa, ZDA, Azija, Afrika) je okoli 0,32 ut. % (Brenna in sod., 2007) in je v največji meri odvisna od sestave maščobnega tkiva matere (vsebnost DHK v maščobnem tkivu matere je močno odvisna od prehranskega vnosa pred nosečnostjo (PN), predvsem pa med nosečnostjo (MN)), sledi vpliv nedavnega in trenutnega prehranskega vnosa matere (med dojenjem (MD)) ter nazadnje vpliv endogene sinteze DHK iz ALK (Fidler in sod., 2000b; Sauerwald in sod., 2000; Innis, 2014).

1.1 CILJI RAZISKOVALNEGA DELA

- I. Preverili bomo, ali je metoda e-4PD uporabna za raziskovalno delo, kot tudi, če je enakovredna ali celo boljša odp-4PD. Do zdaj uporaba e-4PD v raziskovalnem delu in klinični praksi v takem obsegu še ni bila opisana v literaturi slovenski in mednarodni literaturi.
- II. Z ovrednotenjem prehrane s pomočjo 4PD bomo pridobili podatke, na podlagi katerih bomo ocenili, ali je prehrana slovenskih nosečih in doječih mater (v nadaljevanju: prostovoljke) vključenih v študijo Moje-mleko (www.moje-mleko.si) ustrezna in ali je v skladu s priporočili.
- III. Podatki o prehranskih navadah prostovoljk iz vseh treh obdobjij (PN, MN, MD) bodo pridobljeni z VPŽ vprašalnikom (vprašalnik o pogostosti uživanja rib, ribjih izdelkov, morskih sadežev (v nadaljevanju: rib/izdelkov/sadežev) ter prehranskih dopolnil). Zanima nas ali prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil vplivajo na vsebnost LCP, zlasti DHK v HM. S tem bomo dobili prvi bolj celosten vpogled o količini zaužite DHK in o maščobni-kislinski sestavi HM pri prostovoljkah. Do zdaj teh podatkov v povezavi s prehrano še nimamo, razen za maščobno-kislinsko sestavo kolostruma in zrelega HM (Fidler in sod., 2000a; Fidler in Koletzko, 2000; Sedej, 2011).

Na podlagi dobljenih rezultatov bomo lahko opozorili ustrezne organe v državi o izvajaju ustreznih preventivnih ukrepov, kot je na primer možnost prehranskega svetovanja nosečnicam in doječim materam, kar so predlagali že Evropska komisija in Mednarodno društvo za proučevanje maščobnih kislin in maščob (Koletzko in sod., 2007; Koletzko in sod., 2008). Kot je znano, je prehrana nosečnice in doječe matere izjemno pomembna za dolgoročne učinke na razvoj in zdravje otroka vse v odraslo dobo (Lucas, 1998).

1.2 RAZISKOVALNE HIPOTEZE

- I. e-4PD je enakovreden ali celo boljši od p-4PD.
- II. Prehrana slovenskih nosečnic in doječih mater odstopa od prehranskih priporočil.
- III. Prehranske navade, zlasti uživanje rib/izdelkov/sadežev in prehranskih dopolnil PN, MN in MD, pozitivno vplivajo na vsebnost LCP, zlasti DHK v HM.

1.3 POTEK RAZISKOVALNEGA DELA

Študija Vloga materinega mleka v razvoju črevesne mikrobiote dojenčka ali krajše Moje-mleko (ARRS-RPROJ-J4-3606; nosilka prof. dr. Irena Rogelj) je potekala od decembra 2010 do oktobra 2012 v treh geografskih regijah Slovenije: 1) Osrednjeslovenska (Ljubljana in okolica (LJ)); 2) Primorska (Nova Gorica/Izola (NG/IZ)); ter 3) Štajerska (Maribor in okolica (MB)) (Priloga A). Vključevanje je potekalo predvsem v šolah za starše, ki so organizirane v lokalnem okolju.

V študijo so bile vključene zdrave nosečnice (prostovoljke), ki so imele namen dojiti svojega otroka vsaj 6 tednov in so bile pripravljene dvakrat voditi 4PD: 1. v času nosečnosti (od 27. do 37. tedna) (4PD1) ter 2. v času dojenja (od 4. do 5. tedna po porodu) (4PD2) ter izpolniti VPŽ o pogostosti uživanja rib/izdelkov/sadežev in prehranskih dopolnil za obdobje enega leta pred nosečnostjo (PN) in med nosečnostjo (MN) (VPŽ1) in prvega meseca po porodu (MD). Prostovoljke so bile naprošene mesec dni po porodu prispevati še vzorec HM. V študijo nismo vključili nosečnic, ki so bile noseče več kot 37. tednov, so imele avtoimunske kronične bolezni, akutne in kronične infekcije in povečano tveganje za prezgodnji porod. Iz študije so bile izključene tudi prostovoljke, katerih otroci so imeli hujše zdravstvene težave.

V kliničnem delu študije, ki smo jo podrobno predstavili Bogovič Matijašić in sod. (2014) (Priloga A) in je predstavljena na spletni strani www.moje-mleko.si, je sodelovalo 174 prostovoljk. Večina prostovoljk je bila iz LJ ($N = 171$) in IZ regije ($N = 3$). Prostovoljk iz NG in MB nismo vključevali v klinični del študije. Prostovoljke, ki so se odločile sodelovati v študiji, so bile povabljene na uvodno enourno predavanje na Pediatrično kliniko, UKC Ljubljana. Na predavanju smo jim bili podrobno predstavili namen in potek študije s praktičnimi primeri ter demonstracijo o vodenju p-4PD, e-4DP in izpolnjevanju VPŽ. Na uvodnem predavanju so prostovoljke prejele vsa navodila tudi v pisni obliki in kuhinjsko tehtnico (CTC, Clatronic, International GmbH), ki tehta na gram natančno. Med študijo so prostovoljke nadaljevale s svojo običajno prehrano.

Študija je bila izvedena anonimno, vsaka prostovoljka je s prijavo pridobila svojo šifro, pod katero je bila vodena med celotno študijo. Vsi podatki, pridobljeni v času študije, so bili uporabljeni izključno za namen študije in so skrbno varovani kot del medicinske dokumentacije v skladu z vsemi predpisi. Študijo je odobrila Komisija Republike Slovenije za medicinsko etiko (No. 32/07/2010; Priloga J) in je registrirana v informacijski bazi ClinicalTrials.gov (NCT01548313).

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Zbirke podatkov o sestavi živil za učinkovito prehransko obravnavo

Koroušić Seljak B., Stibilj V., Pograjc L., Fidler Mis N., Benedik E. 2013. Food composition database for effective quality nutritional care. Food Chemistry, 140, 3: 553-561

Izhodišča: Glavni cilj študije je bil preveriti metodo ocene hranil z uporabo slovenske spletnne aplikacije (OPKP; Odprta platforma za klinično prehrano, OPKP, <http://opkp.si>, Ljubljana, Slovenija). Ker je najbolj zaželena potrditev neposredna primerjava izračunanih vrednosti z vrednostmi, dobljenimi s kemijsko analizo živil, smo opravili kemijsko analizo in izračun za vzorčen niz dnevnih obrokov ($N = 20$) ter rezultate primerjali med seboj. Sekundarni cilj študije je bil oceniti uporabnost aplikacije OPKP.

Metode: Za primerjavo podatkov med analiziranimi in izračunanimi vrednostmi hranil smo uporabili Studentov t-test, upoštevajoč stopnjo zaupanja 0,01. Našli nismo nobenih statistično značilnih razlik v povprečnih vsebnostih energije, celotne prehranske vlaknine, vode, makrohranil ter izbranih mineralov in elementov v sledovih: Ca, Fe, Mg, Zn, Na, P, Cu in I. Opazili smo statistično pomembne razlike med povprečno izračunano in analitično vrednostjo selena.

Sklepi: Opazili smo izjemno, toda statistično nepomembno razliko v povprečnih vrednostih joda (-11 %). Pričujoča študija tudi kaže, da je aplikacija OPKP koristno in stroškovno učinkovito orodje tako za dietetike kot tudi uporabnike.

Dovoljenje založnika za objavo članka Koroušić Seljak in sod. (2013) v tiskani in elektronski obliki je v prilogi K.

Food Chemistry 140 (2013) 553–561



Food composition databases for effective quality nutritional care

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ABSTRACT

Background and objectives: The main aim of this study was to validate a nutrient-estimation method applied using OPEN, a Slovenian platform for clinical nutrition. As the most desirable validation is a direct comparison of the calculated values with the values obtained from a chemical analysis of the same food, we performed a chemical analysis and a calculation for a representative set of daily meals ($n = 20$) and compared the data. The secondary aim was to evaluate the usability of the OPEN for dietary assessment.

Methods: We used a Student's *t* test to observe any differences between the analysed and calculated nutrient-content data, considering the 0.01 significance level.

Results: No statistically significant differences were observed in the mean contents of energy, total dietary fibre, water, macro-nutrients, and selected essential minerals: Ca, Fe, Mg, Zn, Na, P, Cu and I. We notice statistically significant difference in the mean calculated and analytical values of selenium.

Conclusions: We noticed remarkable, but not statistically significant, difference in the mean values of iodine (~11%). The present study also indicates that the OPEN is a useful and cost-effective tool for both dieticians and patients.

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1. Introduction

The planning of institutional meals that appeal to school children, students, workers, hospital patients or military personnel, while at the same time staying within a budget, is a difficult task that may take an experienced professional some time to complete. To make this task easier and quicker, one can apply software designed to assist a human meal planner in adjusting the content of regular meals to the latest recommendations on healthy and balanced nutrition (Koroušić Seljak, 2009). In Slovenia we have designed a web application for meal planning called The Open Platform for Clinical Nutrition (OPEN, <http://opkp.si/>, accessible in November 2011).

The OPEN is aimed for meal planning, but it is also designed to calculate recipes and nutrient intakes for individuals or groups of individuals. It can apply any food composition database (FCDB) complying with EuroFIR procedures that facilitate access to and ex-

change of comparable, high quality food composition data for industry, regulators and researchers across Europe.¹

The OPEN has been used at the University Children's Hospital Ljubljana to support clinical dieticians in nutrition care process of dystrophic children and cystic fibrosis patients. The OPEN has also been applied to support research work in a clinical study "The Role of Human Milk in Development of Breast Fed Child's Intestinal Microbiota", in which 230 healthy pregnant and breast-feeding women track and analyse their nutrition (<http://www.moje-mleko.si/en/>, accessible in November 2011).

In order to prove the efficiency and correctness of a recipe-calulation procedure applied within the OPEN, we made a study (Study I) in which we evaluated the energy and nutrient contents of a sample of daily meals using analytical and calculation techniques. The major aims of the study, the results of which are presented in this paper, were to compare the energy and the

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¹ These procedures were successfully proposed to the European Committee for Standardisation (CEN) and a CEN/TC 387 project committee on Food Composition Data was launched in 2008. In August 2009, the committee produced a first working draft of a standard that is a technical specification which covers data structure of FCDBs and interchange of food composition data. Based on the usual timeframe for development of standards, standard's national implementation is anticipated by 2012.

content of selected nutrients estimated by chemical analyses and the OPEN as well as to access the comparability of the analysed and calculated nutrient-content data. Moreover, we tried to identify and eliminate systematic failures in the recipe-calculation method, possibly affecting the estimated nutrient intakes for the subjects using the OPEN.

The next study (Study II) we performed to assess the usability of the OPEN in a clinical setting was the evaluation of 4-day food diaries and the comparison of food items and quantities between the OPEN and the paper-and-pencil form. We also gathered feedback on the OPEN's user-friendliness directly from users in a form of completed questionnaire.

Both studies were approved by the Ethical Committee of the Republic of Slovenia.

2. Subjects and methods

The Study I population consisted of a group of highly physically active men (i.e., young soldiers) fully supplied with food prepared in four dining facilities for 10 days, while the Study II population consisted of 40 healthy women in the third trimester of pregnancy who recorded food intake for four consecutive days, including both weekend days, using the OPEN tool for food recording. In Study II, we also asked 132 healthy pregnant and lactating women participating in the clinical study "The Role of Human Milk in Development of Breast Fed Child's Intestinal Microbiota" to complete a questionnaire on the OPEN user-friendliness. All the subjects were volunteers.

2.1. Study I – Comparison of calculated and analysis nutrient values

First, we designed several daily meals according to the usual Slovenian food pattern for a group of men, aged 23 ± 4 years with a body-mass index of 22.5 (of average height 177 cm and weight 71.2 kg) (Pograjc, 2002). The estimated energy requirement for the subjects was approximately 15 MJ (3585 kcal) per day. We considered nutrient intakes recommended for healthy and highly physically active men (Pograjc, 2004).

2.1.1. Collection of the analytical nutrient-intake data

Then, a selection of 10 meals was prepared twice in four dining facilities at different locations by different cooks using the same recipes. The food items, however, were bought from different suppliers. Table 1a presents the energy and nutrient values of the daily meals, while Table 1b lists discussed food groups per daily meals and a subset of food items used for testing the Study I hypothesis. Each daily meal was sampled in two dining facilities, producing a composite of three samples (Fig. 1).

Next, we analysed the composite samples using analytical methods (Pograjc, Stiblji, Ščančar, & Jamnik, 2010) to obtain data on the following organic and inorganic constituents, i.e., dry matter, proteins, total fat, total dietary fibre, essential macro- and micro-elements (calcium, phosphorus, magnesium, sodium, iron, zinc and copper), essential trace elements (selenium and iodine), and physical parameters (energy and energy density).

These parameters were chosen for several reasons: (a) we were interested in any possible deficiency of some essential elements in institutional meals for highly-active men; (b) we have not found any data on the iodine intake in the daily meals of institutions; (c) selenium is an element of special concern in the nutrition of highly-active people because it contributes to the immune function as an anti-oxidant component of glutathione peroxidase and it is often found to be deficient (Phannhauser et al., 2000; Pokorn, Stiblji, Gregorić, Dermelj, & Štupar, 1998).

Table 1a
The energy and nutrient values of a daily meal.

Nutrient (unit)	Value
Energy (kJ)	14.25–15.75
Protein (g)	89–134
Fat (g)	90–134
Carbohydrates (g)	446–625
Vitamin A (µg)	1000
Vitamin B1 (mg)	1.4
Vitamin B2 (mg)	1.7
Vitamin B6 (mg)	1.8
Niacin (mg)	18
Folic acid (µg)	150
Vitamin B12 (µg)	3
Vitamin C (mg)	60
Vitamin D (mg)	7.5
Vitamin E (mg)	12
Ca (g)	1.0
P (g)	1.5
Mg (mg)	350
Fe (mg)	10
Zn (mg)	15
I (µg)	200
Cu (mg)	1.5
Se (µg)	50
NaCl (g)	<6
Breakfast	20–30% of daily energy intake
Morning snack	10–15% of daily energy intake
Lunch	35–40% of daily energy intake
Dinner	20–30% of daily energy intake

Table 1b
Food groups per daily meals and a subset of food items used for testing the Study I hypothesis.

Food group	Frequency
Milk and milk products	2×/day
Cheese spread	4×/10 days
Bread and bakery products	2×/day
Breakfast cereals	1×/10 days
Fresh fruit	2×/day
Vegetable soup	8×/10 days
Meat soup	2×/10 days
Red meat dish	13×/10 days
Red meat product (sausage, salami, pate, etc.)	8×/10 days
Poultry dish	3×/10 days
Fish dish	4×/10 days
Potato dish	8×/10 days
Rice dish	1×/10 days
Grain dish (polenta, barley, etc.)	2×/10 days
Cooked vegetable	1–2×/day
Prepared salad (green, mixed, beetroot, etc.)	2×/day
Dessert (biscuit, roll, fruit compote, pudding, etc.)	1×/day
Chocolate-hazelnut spread	2×/10 days
Herbal or fruit infusion (tea)	2×/day
Juice	1×/day
Water	Daily
Food items (daily meal 6)	Food group
pasteurised milk with 1.6% milk fat	Milk
Mixed wheat/rye bread	Bread
Chocolate-hazelnut spread	Sugar/fat product
Banana	Fruit
Beef soup with noodles	Soup
Trout fried	Fish dish
Boiled potato with parsley	Potato dish
Cooked broccoli with margarine	Vegetable dish
Cucumber salad	Prepared salad
Roll with apricot jam	Sugar product
Sandwich	Bread, meat product, cheese
Tortellini with tomato sauce	Pasta/vegetable dish
Green salad with sweet corn	Prepared salad
Herbal infusion, sweetened with sugar	Beverage (non-milk, non-alcoholic)
Water	Beverage (non-milk, non-alcoholic)

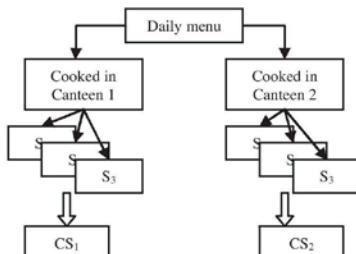


Fig. 1. Composite samples of daily meals (S – primary sample; CS – composite sample). A selection of 10 meals was cooked twice in Canteen 1 and 2 using the same recipes, but food was bought from different suppliers. Each daily meal produced a composite of three samples.

2.1.2. Calculation of nutrient intakes

We used the OPEN to calculate all the recipes as well as the daily meals used in the study, and to estimate the content of the macro-nutrients (protein, total fat, carbohydrates), nine minerals (Ca, Fe, Mg, Zn, Na, P, Cu, I and Se), water and energy in the selected daily meals.

As the recipes did not provide any specific information on the cooking salt (NaCl) content, we assumed that each daily meal had at least 11 g of added cooking salt (Hlastan Ribič, Zakotnik Maučec, Vertnik, Vergnati, & Cappuccio, 2010). In Slovenia, we normally consume cooking salt, which has an iodization level of 25 mg of potassium iodide (KI) per kg of salt.

The OPEN recipe-calculation procedure, originally recommended by INFOODS (Greenfeld & Southgate, 2003) and acknowledged by EuroFIR (Reinivuo & Laitinen, 2007), is as follows:

- (1) Collect the weight and nutrient-content data for each uncooked ingredient.
- (2) Correct the ingredient-nutrient levels for the weight of edible portions, where appropriate.
- (3) Adjust the nutrient values of the ingredients to take into account the effects of cooking
 - (a) If the data for cooked ingredients are available, use the *yield factors* to adjust from the raw to the cooked weights.
 - (b) If the data for cooked ingredients are not available, use the data for the uncooked ingredients and apply *retention factors* for the nutrient losses or gains during the cooking.
- (4) Sum the weights of the ingredients to obtain the weight of the recipe.
- (5) Sum the nutrient values of the ingredients to obtain the nutrient value of the recipe.
- (6) Use a *yield factor* to adjust the recipe weight and nutrient levels to reflect changes in fat/water contents when the whole mixture is cooked. Make additional adjustments to take account of losses (when liquid is poured off after the cooking is completed).
- (7) Determine the final values per weight (e.g., per 100 g), volume (e.g., per cup) or serving portion, as desired.

The weight of the raw dish is measured using a scale weighing to about 1 g (a less accurate scale may be used if the total weight of the ingredients is over 500 g). Herbs and spices are estimated using a scale weighing to about 10 mg.

This method takes the yield factors at the recipe level and the retention factors at the ingredient level. For the cooked ingredients

for which data are available, the yield factors for the water and fat changes are taken at the ingredient level. When salted or sugared cooking water or marinating liquid is drained after the process of cooking or marinating, respectively, the method adjusts the amount of sodium or simple sugars in the recipe by solute diffusion, as follows:

$$\text{Solute diffusion} = \frac{\text{Amount of NaCl or simple sugars}}{\frac{\text{Sum of the ingredients weights}}{\text{amount of the unvapoured cooking water}}} \quad (1)$$

This general factor is applied assuming the soluble diffusivity in most food is linearly dependent on the soluble concentration (Kaymak-Ertekin & Sultanoglu, 2000; Volpati, Michielin, Ferreira, & Petrus, 2006; Wang, Tang, & Correia, 2000).

In the study, we primarily used the Slovenian food composition data. These are maintained by the Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana. Version 2006, used in this study, contains data on approximately 40 nutrients for about 150 food items from the group of meat and meat products (Golob et al., 2006). The nutrient values were primarily obtained from analyses carried out in Slovenia, but some data were derived from the Souci-Fachmann-Kraut (SFK) FCDB (Souci, Fachmann, & Kraut, 2004).

In the Slovenian FCDB, energy-conversion factors taken from the EU directive on the identification marking of food products are used for the calculation of the energy values of foods: 17 kJ for carbohydrates (excluding dietary fibre that contribute 8 kJ/g) and protein, 37 kJ for fat, 13 kJ for organic acids and 29 kJ for ethanol. The carbohydrate values are calculated using the following method: the sum of water, protein, fat, ash, fibre and organic acids is subtracted from 100, yielding the carbohydrate value in grams. The protein values are calculated from the total Kjeldahl nitrogen content multiplied by the following factors: milk and milk products, 6.38; cereals and cereal products, 5.80; soy and soy products, 5.71; oil seeds and hard-shelled fruits, 5.30; mushrooms, 4.17; and other food products (such as meat and meat products, fish, fruit, vegetables and related products), 6.25 (AOAC, 2002) (see Tables 1a and 1b).

In this study we also used the SFK FCDB and the United States Department of Agriculture (USDA, 2011) FCDB, which contain data on approximately 800 food items with 260 constituents and 7500 food items with 140 constituents, respectively (see Table 2).

The weight yield and retention factors, collected by Prof. Bognár (2002) and used in the OPEN, are given for various food groups and different cooking methods. These factors are, in addition to factors provided by Bergström (1994) and McCance & Widdowson (2002), the most commonly applied by European compilers (Bell, Vásquez-Caicedo, Hartmann, & Oltersdorf, 2007). This is an advantage of the FCDB harmonisation.

2.2. Study II – Assessment of the OPEN's usability in a clinical setting

To assess the usability of the OPEN in a clinical setting, we evaluated 40 4-day food diaries. First we asked 40 women in 30.8 ± 3.9 week of pregnancy of age 30 ± 4 years with body height 166.6 ± 5.3 cm and body mass 70.8 ± 11.1 kg to track their food intakes for four consecutive days, including both weekend days. These women were of all types of education and were attending an antenatal class in Ljubljana. They recorded intakes in two ways, by using the OPEN tool for food recording and the paper-and-pencil form. The subjects were asked to weigh and record all foods consumed for 4 days. Then we performed the evaluation of the food diaries in order to compare foods and their quantities recorded by the OPEN and on the paper. We were interested in the participants' accuracy when using the computer-based tool and

Table 2

The mean, standard deviation, min and max of the calculated and analytical values for energy and nutrient contents of ten daily meals per day.

Nutrient	Calculated values Mean ± SD ^a (min–max)	Analytical values Mean ± SD (min–max)
Energy (MJ)	14.71 ± 0.39 (14.12–15.21)	15.78 ± 2.19 (12.15–19.00)
Protein (g)	125.66 ± 8.36 (112.15–141.75)	135.45 ± 17.03 (98.83–162.26)
Total fat (g)	111.11 ± 5.90 (104.58–120.18)	114.06 ± 22.83 (74.42–137.04)
Total dietary fibre (g)	52.39 ± 9.63 (41.33–74.47)	53.20 ± 12.11 (37.46–74.44)
Ca (mg)	982.90 ± 378.93 (507.00–614.00)	1090.00 ± 310.50 (531.30–1760.10)
Fe (mg)	21.90 ± 5.09 (17.70–34.50)	23.00 ± 5.30 (12.80–35.90)
Mg (mg)	503.40 ± 63.54 (408.00–606.00)	533.80 ± 106.00 (384.90–771.30)
Zn (mg)	17.46 ± 2.82 (13.60–21.90)	19.20 ± 3.70 (11.23–27.79)
Na (mg)	8504.20 ± 1280.22 (6672.00–10286.00)	9267.50 ± 1850.80 (6382.00–13284.94)
P (mg)	2215.37 ± 388.11 (1630.00–2765.00)	2509.50 ± 534.60 (1740.88–3470.84)
Cu (mg)	2.55 ± 0.19 (2.25–2.84)	2.74 ± 0.70 (1.38–4.46)
I (µg)	378.21 ± 65.72 (115.6–337.8)	459.00 ± 126 (312–702)
Se (µg)	69.33 ± 34.44 (39.30–161.40)	91.35 ± 33.45 (39.00–197.00)
Water (ml)	2950.00 ± 177.39 (2747.00–3277.00)	3136.50 ± 303.31 (2351.70–3234.40)

^a Note: SD – standard deviation.

the pencil. The comparative analysis was done for each of 3530 food items.

In order to estimate how user-friendly is the OPEN, we asked 132 subjects (pregnant and lactating women) after completing the 4-day food diary to complete a questionnaire with the following questions: (1) "How many minutes did you spend for recording 4-day food diary using the OPEN tool for food tracking and the paper?" (2) "Were you more specific in recording when using the Web application than the paper?" (3) "Did you notice computer alerts on incorrect logs?" (4) "Would you recommend the OPEN to your pregnant and lactating friends?" and (5) "Are you interested in other facilities of the Open Platform?".

2.3. Statistical analysis

2.3.1. Study I – Comparison of analytical and calculated nutrient values

In order to study the statistical differences between the matching groups of analytical and calculated energy- and nutrient-intake data for daily meals, the Student's *t* two-tailed test for paired samples (Press, Teukolsky, Vetterling, & Flannery, 2007) was applied for each of the 13 parameters. In a *t* test, which is an inferential statistical hypothesis test, the statistics have a Student's *t* distribution² if the hypothesis is true. The *t* tests were applied because the population was assumed to have a standard normal distribution for each of the parameter values.

Let us remind ourselves that the analytical data were collected on a statistically small population set of 20 composite samples (10 daily meals, with each one being prepared twice), and the calculations were made on an even smaller set of ten daily meals.

Before performing a two-sample *t* test, we applied a normality Shapiro-Wilk *w* pre-test (Royston, 1992), which is suitable for

² The Student's *t* distribution is similar to the normal distribution, i.e., it is symmetrical about 0 and bell shaped. The main difference is in the tails: the *t* distribution has larger power-law tails at infinity than the normal.

any sample size greater than three. All the parameters passed the normality test.

2.3.2. Study II – Assessment of the OPEN's user-friendliness

To study the statistical difference between the matching groups of food quantities, mean value, standard deviation and confidence interval were calculated, and the difference of means was tested by the Student's two-tailed *t* test for paired samples (Press et al., 2007). The Pearson's correlation coefficient was calculated to measure the association between food quantities collected by OPEN and the paper-and-pencil approach.

3. Results

3.1. Study I – Comparison of analytical and calculated nutrient intakes

Firstly, we collected the mean values and the standard deviations of the analytical nutrient content of 20 composite samples per daily meal as well as the calculated nutrient content of 10 daily meals per daily meal.

The results represented as the mean, the standard deviation, the minimal value and the maximal value (*n* = 20 and 10) are presented in Table 2. In addition, the mean deviations between the calculated values and the analytical values are given in Fig. 2. The Student's paired two-tailed *t* test at the significance level 0.01 showed that there were no significant differences in the calculated values and the analytical values of energy (*T* = -2.04, *P* = 0.52), total dietary fibre (*T* = 1.41, *P* = 0.18), water (*T* = 1.38, *P* = 0.18), protein (*T* = -2.47, *P* = 0.60), total fat (*T* = -1.99, *P* = 0.07), Ca (*T* = 2.84, *P* = 0.01), Fe (*T* = -0.16, *P* = 0.87), Mg (*T* = -0.21, *P* = 0.84), Zn (*T* = 0.90, *P* = 0.38), Na (*T* = -1.18, *P* = 0.25), P (*T* = 1.40, *P* = 0.18), Cu (*T* = -1.11, *P* = 0.28) and I (*T* = -0.34, *P* = 0.74), where *T* presents the calculated value of *t*. We found out a statistically significant difference in the mean calculated and analytical values of selenium (*T* = 3.29, *P* = 0.004).

Then, we performed the Shapiro-Wilk *w* test to assess the normality and the correlation and regression analysis to estimate an association of the OPEN calculation method with the analytical method. For each parameter, correlations between the calculated values and the analytical values are given in Figs. 3a–n. These figures also present the parameters of a calculated regression line α and β in a form of the equation $Y = \alpha + \beta * X$, where *X* and *Y* are calculated and analytical values of intakes, respectively, as well as the coefficient of determination R^2 that was calculated as a square of the Pearson's correlation coefficient *R* at the 0.01 level of significance. No statistically significant differences were found for the energy, macro-nutrients, total dietary fibre, water and the following elements: Ca, Fe, Mg, Zn, Na, P, Cu and I. For Se, we rejected the hypothesis that the mean difference between paired (i.e., calculated and analytical) values is zero. The median of the Pearson's correlation coefficients was 0.17, ranging from 0.03 for Mg to 0.61 for Se. Again, only for Se we rejected the hypothesis that the slope of the best-fit line is equal to zero; in other words, as the calculated value gets larger, the associated analytical value gets neither higher nor lower.

3.2. Study II – Assessment of the OPEN's user-friendliness

In the study we found out that there was no statistically significant difference at the 0.05 level among the quantities of 3530 food items recorded by 40 subjects in 4-day food diaries using the OPEN (Mean 3355.90 g; SD³ 991.90 g) and the paper-and-pencil form

³ SD – Standard deviation.

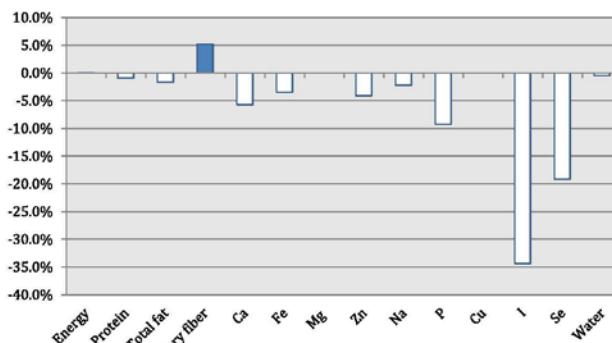


Fig. 2. Mean deviation of the calculated nutrient-content data from the analytical data.

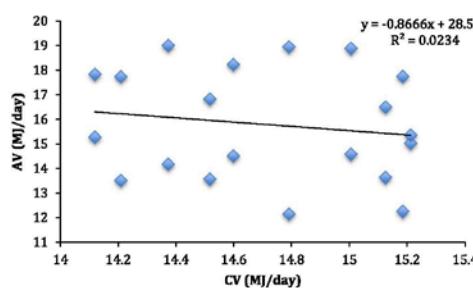


Fig. 3a. Pearson's correlation coefficients for energy intake values (CV – calculated value; AV – analytical value).

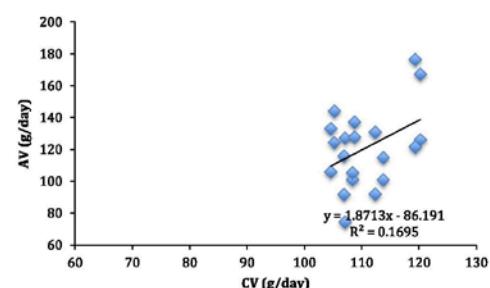


Fig. 3c. Pearson's correlation coefficients for fat intake values (CV – calculated value; AV – analytical value).

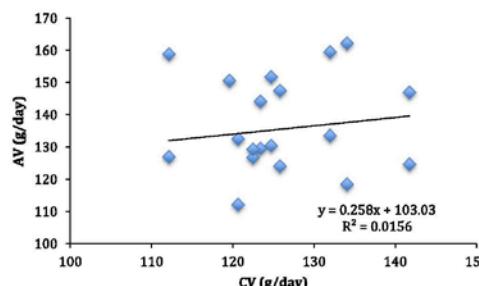


Fig. 3b. Pearson's correlation coefficients for protein intake values (CV – calculated value; AV – analytical value).

(Mean 3427.2 g; SD 958.6 g). The Pearson's correlation coefficient was 0.82 (95% CI⁴ 0.62–0.95).

Next, we collected the following information from 132 questionnaire respondents (pregnant women). In average, the respondents found food recording using the OPEN tool equally

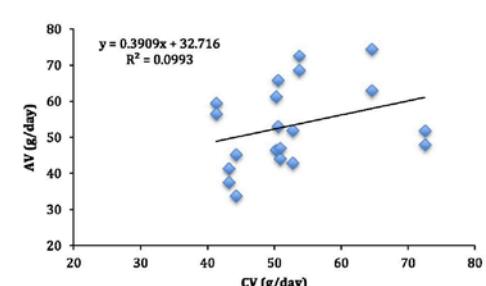


Fig. 3d. Pearson's correlation coefficients for total dietary fibre intake values (CV – calculated value; AV – analytical value).

time-demanding (Mean 32.80 min; SD 43.53 min) as the paper-and-pencil form (Mean 28.89 min; SD 4.79 min). The large SD for OPEN (43.53 min) indicates that the subjects had diverse computer literacy. For a clinical dietician the OPEN approach saves time, which can be devoted to more careful consideration of the patient. The dietician still needs to spend some time for checking the food records, but (s)he does not need to enter the whole food diary into the computer program. Only 19% of the women claimed that they

⁴ CI – Confidence interval.

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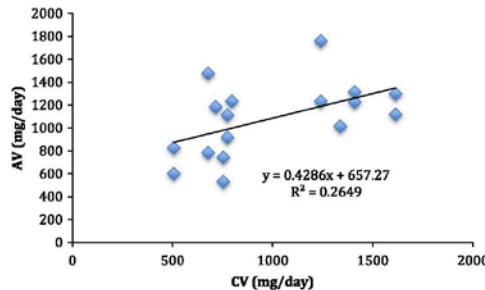


Fig. 3e. Pearson's correlation coefficients for Ca intake values (CV – calculated value; AV – analytical value).

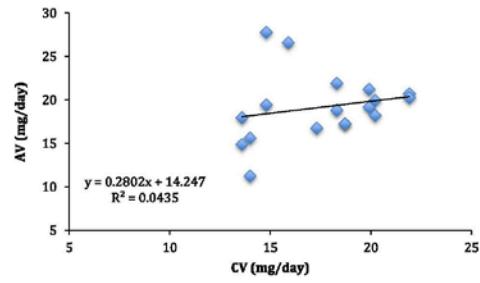


Fig. 3h. Pearson's correlation coefficients for Zn intake values (CV – calculated value; AV – analytical value).

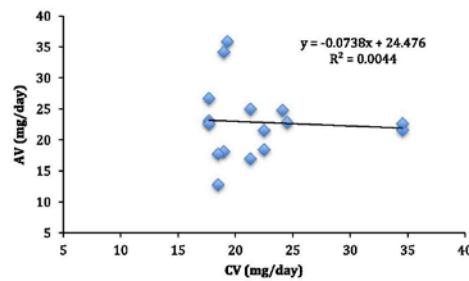


Fig. 3f. Pearson's correlation coefficients for Fe intake values (CV – calculated value; AV – analytical value).

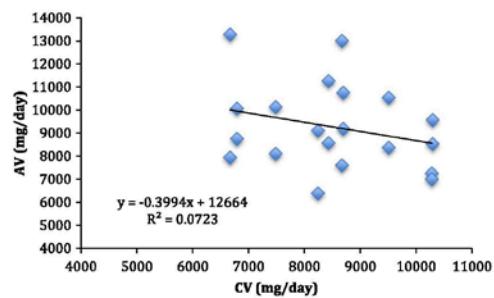


Fig. 3i. Pearson's correlation coefficients for Na intake values (CV – calculated value; AV – analytical value).

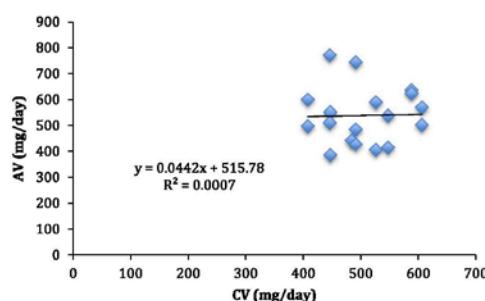


Fig. 3g. Pearson's correlation coefficients for Mg intake values (CV – calculated value; AV – analytical value).

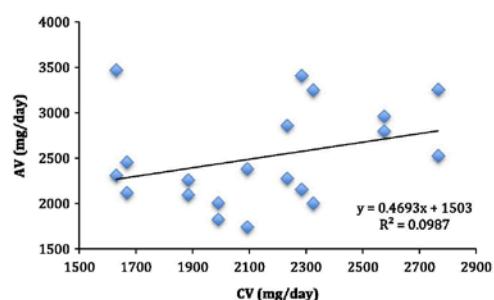


Fig. 3j. Pearson's correlation coefficients for P intake values (CV – calculated value; AV – analytical value).

were more accurate in food tracking using the paper-and-pencil form. We found out that a large percentage of the respondents (77%) were not aware enough of the alert system, which is aimed to lead the user to higher accuracy. A large part (61%) of the women would recommend the OPEN to their pregnant and lactating friends. Most of the respondents who answered "No" to the question "Are you interested in other facilities of the Open Platform?" (45%) commented that the main reason is the imminent birth of a child and lack of time.

4. Discussion

4.1. Study I – Comparison of analysed and calculated nutrient intakes

4.1.1. Macro-nutrients

In the study, we were not able to find any statistically significant differences in the mean content of the macro-nutrients (difference 0 = 2%).

Comparison with other studies: The Nutritional Study of the Andean Population of Jujuy (ENJU) compared the nutritional content of composite dishes cooked in school canteens in northwestern Argentina, determined by both chemical analyses of the samples

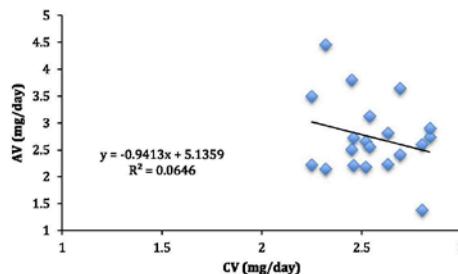


Fig. 3k. Pearson's correlation coefficients for Cu intake values (CV – calculated value; AV – analytical value).

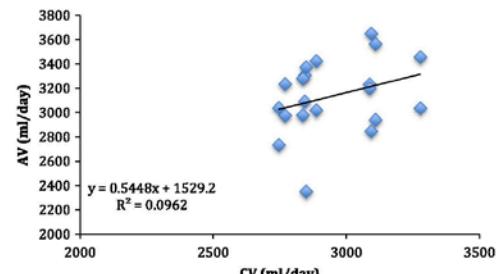


Fig. 3n. Pearson's correlation coefficients for water intake values (CV – calculated value; AV – analytical value).

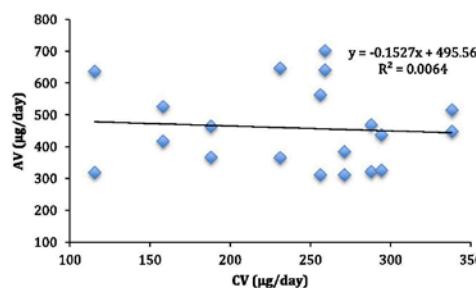


Fig. 3l. Pearson's correlation coefficients for I intake values (CV – calculated value; AV – analytical value).

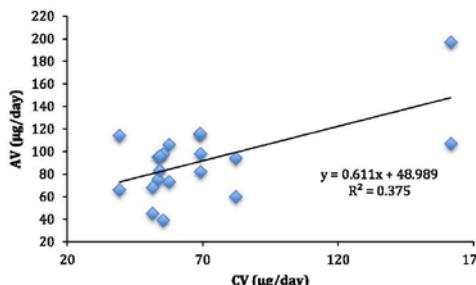


Fig. 3m. Pearson's correlation coefficients for Se intake values (CV – calculated value; AV – analytical value).

and through the use of FCDB (Romaguera, Toboada, & Peralta, 2007). The results showed that the correlation of the results for the macro-nutrient content obtained by both methods was positive and significant. No statistically significant differences between both methods were observed for the content of energy ($P = 0.008$), proteins ($P = 0.041$), lipids ($P = 0.071$) and carbohydrates ($P = 0.034$).

The Human Nutrition Information Service (HNIS) of the USDA checked the reliability of their recipe procedures by comparing the calculated and analysed values for the proximate nutrients and eight minerals, six vitamins and cholesterol in six mixed dishes (Matthews, 1988). The differences between the calculated

and analysed values for the proximate nutrients were generally less than 10%.

4.1.2. Dietary fibre

The data analysis showed that the calculated content of dietary fibre did not differ statistically significantly from the analysed content (a difference of approximately 5%). In general, the calculated values were higher than the analytical ones.

Comparison with other studies: Thorsten and Westrich (Thorsten, 1991; Westrich, 1993) presented similar results. However, their calculation methods underestimated the amount of dietary fibre by about 10%.

4.1.3. Minerals

For the minerals, no statistically significant differences were observed in the mean values (differences 1–5%, except for selenium and iodine). We noticed remarkable differences for selenium (−19%) and iodine (−11%); however, these differences were sufficient to significantly alter the correlations between calculated and analysed values only for the Se content.

Iodine. The greater part of iodine enters living organisms via the food chain. However, the intake of iodine is affected by the I content in the soil, fertilisers, drinking and irrigation water; by the use of veterinary drugs and I feed supplements; and further by the use of disinfectants and sanitizers in the dairy industry (Dermelj et al., 1990). All these influencing factors may differ between and within countries whose food-composition data are used by our application. In addition to that, there could be other reasons for higher analysed I-content values in comparison to the calculations. For example, in our study we mainly used data from the Slovenian and SFK FCDBs. As these data sources did not provide all the required data, we used missing data from the USDA FCDB, which, however, lacks data on the I content in food items. Despite iodine being essential, data on its concentration in food products and in diets are still poorly represented in most FCDBs. Iodine is, however, regarded as one of the most difficult inorganic elements to measure (Greenfield & Southgate, 2003). Another reason could be that the food was salted with a well-stored salt after the cooking procedure to reduce the sublimation of iodine (Wisnu, 2008). In general, FCDBs do not provide any information on the method of salting food prior to performing a chemical analysis.

As the Slovenian FCDB missed data on drinking water, we analysed the drinking water for iodine using samples from several sources in Slovenia, and then used the analysed value of 0.6 ng/g (Stibili, 2008). We also upgraded the Slovene FCDB with data on the I content of selected mushrooms (Dermelj et al., 1990). Nevertheless, there are still many food items that are consumed on a daily basis, such as bread and meat, that lack data in terms of the I

content. For now, only for a few types of bread and meat are these data available in the SFK and/or Slovenian FCDBs.

Selenium. The major source of selenium for humans is their food supply. However, selenium levels in foods are affected by both the content and availability of the element in the soil or feed, which may vary geographically between and within countries. Beyond that, its absorption strongly depends on the Se bioavailability, which is affected by its chemical form, dietary factors such as total protein, fat, and trace elements, and the presence of heavy metals, such as methyl mercury⁵ (Navarro-Alarcon & Cabrera-Vique, 2008). The deficiency of vitamins B₆, E, C and A may also affect the Se bioavailability, while a sufficient supply of these vitamins increases the Se anti-oxidant activity (Volk, 2006; Whitney, Cataldo, & Rolfs, 2002).

Although Se is better absorbed from plants than from animal products, fishery food is, besides meat, chicken and eggs, our main source of Se. We consume 40–50% of the recommended daily amount of Se when fish is included in a daily meal. Nevertheless, the Se content in fish may vary widely for a number of reasons. Firstly, chemical analyses are only performed on fish muscles (although the Se in water organisms mainly concentrates in organs like the liver, which are consumed with small fishes like sardines). Secondly, fish samples are taken from different geographical and/or seasonal sources. Let us give an example of the Se content in sardines (*Sardina pilchardus* (W.)) per 100 g of the edible portion (refuse 0%) taken from different data sources: 64.1 µg (the Slovene FCDB), 57.1 µg (Klapc et al., 2004), 57 µg (Navarro-Alarcon & Cabrera-Vique, 2008), 52.7 µg (the USDA FCDB, canned in oil), 49 µg (McCance and Widdowson's, canned in oil), 42.87–46.08 µg (Ventura, 2008), 35 µg (the Danish FCDB, <http://www.foodcomp.dk/>) and 13 µg (the SFK FCDB, canned in oil).

In our study, we calculated the nutrient values for Se in fishes using data taken from the Slovenian FCD. In ten daily meals, fishes were served three times, once as breaded and fried anchovies, secondly as grilled trout and for the third time as canned sardines.

Comparison with other studies: The HNIS study showed differences between the calculated and analysed values for eight minerals of the order of less than 10%, except for Cu, which showed greater differences and was most often higher when chemically analysed.

The ENJU study observed statistically significant differences for the minerals. On average, the use of FCDB halved the content of Fe and Zn in the dishes compared to the analytical results.

Iodine. The study on the I content in daily diets in Poland (Kunachowicz, Stibilj, Stos, & Gosciniarek, 2000) showed similar results to ours. In all the diets studied, the analysed I-content values were higher than the calculated ones. The calculated values were 71–85% of the analytical ones. Dermelj et al. (1990) published the results of a comparative study in which the I content in standard reference materials was analysed by institutions from the USA, Canada, Finland, the Environmental Specimen Bank, KFA, Juelich, FRG and Slovenia. The mean analysed I-content value of the Slovenian diet is comparable to the results in this comparative study, while the mean calculated value is lower.

Selenium. The Swedish-Finnish study (Hakala, Knuts, Vuorinen, Hammar, & Becker, 2003) made a comparison of the nutrient-intake data calculated on the basis of two different databases, the Swedish FCDB PC-kost and the Finnish FCDB Nutrica. The results indicated a large difference (53%) between the main values of Se, which the authors explained in terms of the different legislation on Se supplementation. The investigation performed by Keck and Finley showed great variability in the Se concentrations in similar

foods and agricultural products. The total variation in the Se content was 72-fold for wheat flakes, 57-fold for wheat, and 11-fold for beef (Keck & Finley, 2006).

4.2. Study II – Assessment of the OPEN's user-friendliness

In the study, we found out that users behave in a similar way when they record foods by using a computer-based tool or the pencil. Those pregnant women that recorded OPEN food diary in an inexact way, provided us with imperfect paper food diary as well. Similar observation has been noticed by a comparative study (Touvier et al., 2011), where it was assumed that the completion of the first method may influence the completion of the second one by reducing memory bias. This study detected the reduction of logistic burden and cost similarly as we did.

How to motivate users for exact self-reporting about food and drink intake is a question that has been tackled by many R&D groups developing dietary assessment tools. Recently, a web-based tool WebDASC was developed as an intuitive, cost-effective, and engaging method to collect detailed dietary data from children (Biltoft-Jensen et al., 2012).

5. Conclusions

Although the calculated Se- and I-content values did not prove to be as accurate as the analysed ones, we need to emphasise that the calculated values for both elements suited the nutrient requirements for men under the investigation (Table 1a). In any case, it is still better to use values calculated, or even estimated, from related food items than to use a value of zero (Schakel, Buzzard, & Gebhardt, 1997).

Moreover, we did not identify any systematic failure in the recipe-calculation method that could possibly affect the estimated nutrient intakes for the subjects using the OPEN.

The study confirmed our hypothesis that the OPEN could be a reliable tool for recipe calculation with respect to macro-nutrients, selected essential minerals (Ca, Fe, Mg, Zn, Na, P and Cu), water and energy. As these calculated nutrient values are properly documented, we can regard the OPEN FCDB as being of high quality, for reasons of its completeness. However, it will be necessary to complete the data on the selenium and iodine contents in certain food products and raw materials in food-composition datasets.

In order to further increase the level of confidence in the recipe-calculation method, a study of the remaining constituents (such as vitamins, fatty acids, non-nutrients, etc.) should be performed, and this is our plan for the future.

The accuracy of food records may increase by an efficient alerting system incorporated in the software for nutrient intake estimation. There is a challenge how to perform it to be effective but not annoying for the user.

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⁵ The interaction between Se and MeHg is antagonistic as Se is recognized to decrease the MeHg toxicity when both elements are simultaneously administrated (Cabañero, Madrid, & Camara, 2007).

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2.1.2 Primerjava papirnega in elektronskega prehranskega dnevnika: Pilotna študija

Benedik E., Koroušić Seljak B., Simčič M., Rogelj I., Bratanič B., Ding EL., Orel R., Fidler Mis N. 2014. Comparison of paper- and web-based dietary records: A pilot study. Annals of Nutrition & Metabolism, 64: 156–166

Izhodišča: Papirni prehranski dnevnik se lahko nadomesti z uporabo spletnega prehranskega dnevnika tako v epidemioloških študijah kot tudi v klinični praksi, saj se s tem poenostavi in poceni logistika ter skrajša čas ovrednotenja prehranskih dnevnikov. Izvedli smo študijo, v kateri smo primerjali papirni prehranski dnevnik (p-4PD) s slovensko spletno aplikacijo prehranskega dnevnika (e-4PD; Odprta platforma za klinično prehrano, OPKP, <http://opkp.si>, Ljubljana, Slovenija).

Metode: Primerjali smo ujemanje različnih živil ($N = 1103$) iz p-4PD in e-4PD za energijo ter 48 hranilnih snovi, ki so jih vodile nosečnice prostovoljke ($N = 16$) iste štiri dni. p-4PD so bili vneseni v spletno aplikacijo prehranskega dnevnika z neodvisnim raziskovalnim dietetikom. Wilcoxonov preizkus predznačenih rangov primerja razlike v povprečnih rangih, Spearmanov koeficient korelacije rangov meri povezanost, Bland-Altmanove meje ujemanja ocenjujejo stopnjo ujemanja med dvema metodama za uporabljene parametre. Prostovoljke so izpolnile tudi kratek anketni vprašalnik o sprejemljivosti uporabe p-4PD in e-4PD.

Rezultati: Ugotovili smo visoko korelacijo med p-4PD in e-4PD. Za 45 hranil nismo našli statistično pomembnih razlik, razen za proste sladkorje ($P < 0,001$), ALK ($P = 0,041$), folno kislino ($P = 0,036$) in pantotensko kislino ($P = 0,023$). Prostovoljke so za izpolnjevanje p-4PD porabile enako časa kot za izpolnjevanje e-4PD. Večina prostovoljk (75 %) je raje izpolnjevala e-4PD.

Sklepi: Sklenili smo, da sta p-4PD in e-4PD primerljiva med seboj ter da je e-4PD priročnejši za uporabnika, hkrati pa ima bistvene logistične in stroškovne prednosti.

Dovoljenje založnika za objavo članka Benedik in sod. (2014) v tiskani in elektronski obliki je v prilogi L.

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Comparison of Paper- and Web-Based Dietary Records: A Pilot Study

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Key Words

Comparison · Paper-based dietary record · Web-based dietary record · Pregnancy

Abstract

Background/Aims: Paper-based dietary records (Paper-DR) can be replaced by web-based dietary records (Web-DR) in both epidemiological studies and clinical practice to reduce the time and logistic burden. We aimed to compare Paper-DR and Web-DR. **Methods:** We compared the matching of different food items ($n = 1,103$) from Paper-DR and Web-DR for energy and 48 nutrients among 16 pregnant volunteers, with DR for the same individuals matched for the same 4 days. Paper-DR were coded into the web-based version (referred to as Paper-Web-DR) independently by the same research dietitian. The Wilcoxon signed-rank test comparing mean rank differences, Spearman's ρ to measure associations and Bland-Altman limits of agreement to evaluate the level of agreement between the two dietary methods across the range of parameters were used. Volunteers also completed an evaluation questionnaire regarding the user acceptability of Paper-DR and Web-DR. **Results:** A high correlation between Paper-DR and Web-DR was noted. There were statistically insignificant differences among 45 nutrients, except for free sugars ($p < 0.001$), α -linolenic acid ($p = 0.041$), folate ($p = 0.036$) and pantothenic acid ($p = 0.023$).

Volunteers found the Paper-DR equally time-consuming as the Web-DR. The majority of the volunteers (75%) preferred the Web-DR. **Conclusions:** Paper-DR and Web-DR were comparable across a range of nutritional parameters, with a few exceptions. The Web-DR was more convenient for the majority and has substantial logistic and cost advantages.

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Introduction

Traditional self-monitoring of dietary intake involves manually logging on paper the amount of all food and drinks consumed over a certain period of time [1]. The duration most often used in the literature is 3 or 4 days of dietary recording (2 or 3 weekdays and 1 weekend day), which has previously given acceptable and reliable data [2] and resulted in a relatively low dropout [3].

With the advancement of information and communication technologies, the assessment of dietary intake has received increased attention for large-scale population nutrition research [4–10]. Despite the availability of novel tools, the usual method of self-monitoring continues to be paper-based dietary records (Paper-DR), which are time-consuming, tedious and inconvenient for study volunteers as well as for dietitians [11, 12].

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In the study presented in this paper, we aimed to examine whether 4-day Paper-DR and a web-based version (Web-DR) kept by the same pregnant women (volunteers) yield similar nutritional intake estimates [energy, 18 macronutrients, sums of fatty acids (saturated, monounsaturated, n-6, n-3 and n-6 + n-3), alcohol and 24 micronutrients] on a food item basis. We also tested the user-friendliness of the web-based tool compared to the paper-based version.

Methods

Study Design

This pilot study is part of the Slovenian research project entitled 'The role of human milk in development of a breast-fed child's intestinal microbiota', abbreviated to My-Milk (www.moje-mleko.si/en) [13]. Briefly, the My-Milk study aims to elucidate the role of microbiota and the fatty acid composition of a mother's milk in the development of intestinal microbiota and the overall health status of a newborn infant.

Volunteers were included in the study if they were willing to participate and keep Paper-DR and also optional Web-DR (at home for the same 4 days, i.e. 3 weekdays and 1 weekend day) and to complete the evaluation form. Volunteers for this pilot study were recruited from January until April 2011 at the Gynecological Clinic, University Medical Center Ljubljana, while attending the 'School for Parents' program. Participants came mainly from Ljubljana (the capital city). The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Ljubljana (No. 32/07/2010) and is registered at ClinicalTrials.gov (NCT01548313).

Study Population

Volunteers received 20 min of oral instruction from a research dietitian and a computer expert as well as written instructions on how to keep Paper-DR (recorded first) and optionally Web-DR (entered at the end of each recording day, after the Paper-DR were recorded) for those who showed interest in recording Paper-DR as well as Web-DR simultaneously. We provided them with kitchen scales with 1-gram resolution (CTC, Clatronic® International GmbH). We asked them not to make any dietary changes during the trial. Basic anthropometrical measurements (age, week of pregnancy, body height and body mass prior to pregnancy), sociodemographic data (marital status, number of children, level of education, place of residence) and lifestyle data (smoking, alcohol, physical activities) were recorded for each volunteer. Body mass was measured with a certified medical scale to the nearest 0.1 kg and body height to the nearest 0.5 cm (Seca digital scale 769, Germany). Volunteers' data were coded, and all information was kept confidential.

By the end of April 2011, 54 volunteers had been included in the My-Milk study. Of these, 16 were willing to complete both the Paper-DR and Web-DR. Participants had a mean (SD) age of 31 (4.0) years, were in the 29th (5th) week of gestation and had a mean pre-gravid body mass index of 21.4 (2.5). All participants were married or living in a cohabiting relationship; 12 (75%) had no previous children and 4 (25%) had 1 child. Overall, the participants were

fairly well educated [postgraduate: 3 (19%); tertiary: 9 (56%); secondary: 4 (25%); primary: 0 (0%)]; all were employed, all were non-smokers and all were active in sports. During pregnancy, 11 of the volunteers (69%) drank an alcoholic beverage at least once a month. Nine of the volunteers (56%) were from urban areas of residence.

Methodology of the Comparative Study

We asked the volunteers to record the intake of all foods, drinks and food supplements consumed throughout 4 consecutive days (from Sunday to Wednesday) and to complete an evaluation form on the user acceptability of the Paper-DR and Web-DR at the end of the fourth day.

We coded each food item for each of the 4 days separately (e.g. if the first food item listed in the DR was food 'A' it was given the code number 1, and the code was the same for both Paper-Web-DR and Web-DR even if the food item was recorded more than once on the same day; on the next day the same food item received a different code number, according to the order listed in the DR; total of 1,103 food items). For comparison between Paper-DR and Web-DR, 49 parameters (energy, carbohydrate, total sugars, free sugars, starches, fiber, fat, myristic acid, palmitic acid, stearic acid, sum of saturated fatty acids, oleic acid, sum of monounsaturated fatty acids, linoleic acid, arachidonic acid, sum of n-6 fatty acids, α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, sum of n-3 fatty acids, sum of n-6 and n-3 fatty acids, cholesterol, protein, water, alcohol, folate, niacin, pantothenic acid, retinol equivalents, riboflavin, thiamine, vitamin B₁₂, vitamin B₆, vitamin C, vitamin D, vitamin E, biotin, calcium, magnesium, phosphorus, potassium, sodium, chloride, iron, copper, fluoride, iodine, manganese and zinc) were calculated for all food items ($n = 1,103$) using the Open Platform for Clinical Nutrition (OPEN) [14]. Standard recipes were used for the calculation of nutrient content and food items in prepared dishes.

The list of observed nutrients was selected on the basis of a previous study on nutrient intake of Slovenian adolescents [15] and on the basis of interest for the My-Milk study (i.e. linoleic acid, arachidonic acid, α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) [13].

Paper-DR

For Paper-DR, 5 pages were provided, including one page of instructions with an example for one daily dietary record. Detailed information regarding (1) time of consumption, (2) quantities in grams per milliliter or, exceptionally, also in household measures (such as cup, tablespoon, teaspoon, cup of coffee, slice of bread) and (3) foods with brand name when appropriate and the type of preparation was requested. The DR were checked when received by the same research dietitian who gained experience in dietary assessment through previous clinical work with patients. The dietitian entered the Paper-DR into OPEN (hereinafter referred as Paper-Web-DR) for comparison with the Web-DR and compared the correctness of the entries between the paper and web versions twice.

Web-DR

For the purposes of this study, the participating pregnant women used OPEN [14], which is a web-based tool for dietary intake assessment and diet planning. OPEN has been described in more detail elsewhere [16]. Briefly, the application can apply any food composition database (FCDB) complying with European Food Information Resource procedures, including BS EN 16104:2012 (Food data.

Structure and interchange format), which facilitates access to and exchange of comparable, high-quality food composition data. In the Slovenian version of OPEN that was applied in this study, we used food composition data from Slovenian [17, 18] food composition tables. Missing food composition data were mainly borrowed from other European FCDBs [19]. For a very few food items, such as exotic fruits and spices imported to Slovenia from abroad, we applied American data [20]. To calculate food composition data for traditional and frequently consumed Slovenian dishes, OPEN applies a recipe calculation procedure, recommended by the International Network of Food Data Systems [21] and recognized by EuroFIR [22], which we evaluated previously [23]. By default, OPEN refers to international dietary recommendations, which can be modified by the dietitian/nutritionist to suit the needs of individuals.

Each day of the study, after completing the paper version (Paper-DR), volunteers also entered their food intake into Web-DR by means of OPEN. A search engine incorporated into OPEN helps to find food items by key words. If a given food item is not found, the user can add it manually by clicking 'add an unknown food item' or 'add a recipe'. Computer experts regularly update the OPEN FCDB with new entries. In order to enhance the accuracy of the reported food intake, OPEN has special integrated computer alerts which circle in red the box in which the food item is entered. An alert occurs when the entered food item has too high or too low an amount (more or less than the food unit defined for each food group) or when the entered food item has the specific allergen to which the user specified that she or he is allergic. However, OPEN has no special alerts or reminders for possible addition of, for example, cocoa powder or sugar added to a cup of milk, but the user could choose the right food item based on a picture or a short description of the composition of the food item listed based on a key word. As OPEN also presents the meal in a graphical form of food pyramid, the user can observe an error in the entry. The data included for each food item were amount consumed and date and time of consumption. After a meal had been entered, OPEN stored the information. Volunteers could go back to make corrections to any previously entered data by entering their username and password. Web-DR completed by the volunteers were instantly available for the research dietitians to process. In addition, OPEN has more standardized reporting, less missing data on portions, improved time efficiency and reduced opportunity for data entry errors compared to the paper version.

The Evaluation Form

The evaluation form queried the user-friendliness and attractiveness of OPEN. Volunteers were asked how many minutes they spent recording Paper-DR and Web-DR; whether they were more specific in recording when using Paper-DR or Web-DR; whether they noticed computer alerts on incorrect logs; whether they would recommend OPEN to their friends, and whether they were interested in the other facilities of OPEN.

Statistical Analysis

The sample size was smaller than 2,000 elements (the number of investigated food items was 1,103), so we applied the Shapiro-Wilk normality test to determine whether or not the data set was modeled by a normal distribution. The data set of observed food items did not have a normal distribution, so Spearman's ρ was used to measure associations between nutrient intakes calculated from Paper-Web-DR and Web-DR. For paired comparisons within each individual parameter among all 49 parameters between Pa-

per-Web-DR and Web-DR, the Wilcoxon signed-rank test was used. For all analyses, the significance level was two-sided and set at 0.050. Spearman's ρ was considered significant at a level of 0.001. Bland-Altman limits of agreement (with 95% CIs) were used to evaluate the level of agreement between the two dietary methods across the range of intakes. The difference in means between the two methods was plotted against the average of the two methods for each parameter (energy, micro- and macronutrients). The frequency distribution method was used for the evaluation form querying the user-friendliness and attractiveness of OPEN. Statistical analyses were performed using the statistical software SPSS version 21 (2012, SPSS Inc., Chicago, Ill., USA).

Results

Comparison of Paper-Web-DR with Web-DR

We first calculated Spearman's ρ for the 49 parameters to check the correlation between the 1,103 food items from 128 daily sums from Paper-Web-DR and Web-DR, recorded by 16 volunteers over 4 days (16 volunteers \times 4 days = 64 daily sums for Paper-Web-DR vs. 64 daily sums for Web-DR).

Then we applied the Wilcoxon nonparametric test to check the null hypothesis; the median difference between Paper-Web-DR and Web-DR was zero.

Additionally, we calculated Spearman's ρ and performed the Wilcoxon test for each volunteer separately. The results of the reported intakes with Paper-Web-DR and Web-DR for each of the 49 parameters on their own are shown in tables 1 and 2. The Wilcoxon signed-rank test showed that the null hypothesis could not be rejected for most of the observed parameters (45 out of 49), with the exception of 4 parameters, namely free sugars ($p < 0.001$), α -linolenic acid ($p = 0.041$), folate ($p = 0.036$) and pantothenic acid ($p = 0.023$). The Spearman's ρ for nutrients ranged from 0.63 for eicosapentaenoic acid to 0.96 for carbohydrates (tables 1, 2). To illustrate the limits of agreement between the two methods, we plotted Bland-Altman scatter plots for food intakes for energy, macronutrients and micronutrients (fig. 1, outliers not shown). For all 49 parameters, the Bland-Altman scatter plots have the shape of an 'arrow', which indicates significant differences in nutrient intake of all 1,103 food items between the Paper-DR and Web-DR, which was expected. This phenomenon could be illustrated with iron intake from a food item low in iron (an apple; smaller intake differences) and a food item high in iron (pork liver; higher intake differences).

User Acceptability

All volunteers returned the evaluation form. They found dietary recording using Paper-DR to be as time-

Table 1. Comparison of energy and macronutrient intake of all food items ($n = 1,103$) calculated from 4-day Paper-DR entered into Web-DR by a research dietitian (Paper-Web-DR) versus 4-day Web-DR entered by the volunteers (Web-DR)

Parameter	Paper-Web-DR			Web-DR			Wilcoxon rank-sum test p value	Mean Spearman's ρ
	mean	median	SD	mean	median	SD		
Energy, kcal	119.96	79.24	156.92	126.62	79.37	176.52	0.059	0.94
Carbohydrate, g	15.55	8.23	23.39	16.24	7.90	26.68	0.226	0.96
Total sugars ^a , g	6.04	1.74	9.11	6.17	1.64	9.36	0.519	0.89
Free sugars ^b , g	0.57	0.00	3.39	0.82	0.00	4.26	0.001	0.69
Starches, g	5.45	0.00	17.80	5.43	0.00	17.57	0.762	0.83
Fiber, g	1.56	0.40	2.62	1.63	0.41	3.20	0.409	0.90
Fat, g	4.20	1.07	7.66	4.60	1.14	10.41	0.112	0.92
Myristic acid, g	0.17	0.00	0.45	0.20	0.00	0.93	0.966	0.87
Palmitic acid, g	0.68	0.09	1.32	0.79	0.08	2.46	0.473	0.86
Stearic acid, g	0.29	0.02	0.61	0.33	0.01	0.99	0.483	0.87
Σ Saturated fatty acids, g	1.53	0.21	3.33	1.70	0.2	5.57	0.908	0.87
Oleic acid, g	0.81	0.00	1.97	0.85	0.00	2.60	0.702	0.79
Σ Monounsaturated fatty acids, g	1.00	0.09	2.21	1.04	0.09	2.40	0.300	0.82
Linoleic acid, g	0.58	0.08	1.70	0.56	0.08	1.35	0.744	0.84
Arachidonic acid, g	0.01	0.00	0.03	0.01	0.00	0.03	0.620	0.83
Σ n-6 fatty acids, g	0.35	0.00	1.33	0.34	0.00	1.11	0.629	0.79
α -Linolenic acid, g	0.10	0.02	0.32	0.09	0.02	0.30	0.041	0.84
Eicosapentaenoic acid, mg	0.40	0.00	0.08	0.30	0.00	0.08	0.812	0.63
Docosahexaenoic acid, mg	0.30	0.00	0.04	0.30	0.00	0.04	0.895	0.69
Σ n-3 fatty acids, g	0.07	0.00	0.25	0.07	0.00	0.24	0.122	0.81
Σ (n-6) + (n-3) fatty acids, g	0.59	0.10	1.67	0.56	0.10	1.29	0.911	0.83
Cholesterol, mg	14.94	0.00	48.17	15.09	0.00	55.88	0.088	0.86
Protein, g	4.93	2.01	8.19	5.03	2.00	8.33	0.848	0.95
Water ^c , g	160.89	59.23	346.14	157.69	59.12	346.40	0.501	0.90
Alcohol, g	0.01	0.00	0.11	0.02	0.00	0.25	0.944	0.71

For p values, the significance level is 0.050; null hypothesis: equal means. All Spearman's ρ are significant at the 0.001 level.

^a Total sugars (all monosaccharides and disaccharides): free sugars plus sugars naturally present in foods (e.g. lactose in milk, fructose in fruits).

^b Free sugars: all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and fruit juices [37].

^c Total water from beverages and solid foods.

demanding as with Web-DR (average time: 18 ± 16 vs. 19 ± 8 min). Three of the volunteers (19%) were more specific and consistent in dietary recording using Web-DR than with Paper-DR; 7 (44%) also noted the computer alerts on incorrect logs; 12 (75%) would recommend Web-DR to their friends, and 12 (75%) showed interest in other facilities of OPEN.

Discussion

Various instruments are used to assess nutrient intake and food consumption, each with advantages and disadvantages [8]. As with any new mode of assessment, particularly those that are technology based, understanding

the sampling biases introduced and respondent reactions is important [6]. Typically, people do not fill out DR because of lack of time and the inconvenience of taking a pad of paper with them wherever they go to eat. It makes more sense to use DR that are supported by devices that are integrated into the daily lives of people (e.g. computer, tablet, smartphone, personal digital assistant).

To the best of our knowledge, this is the first study to compare two self-administered methods (Paper-DR vs. Web-DR) for DR completed by the same persons. The comparison of self-administrated Paper-DR and Web-DR or personal digital assistant dietary records has already been described in the literature, but with the limitation that the DR were not completed by the same volunteers [5, 11, 24, 25]. There are a few studies in which

Table 2. Comparison of micronutrient (vitamins, minerals and trace elements) intake of all food items ($n = 1,103$) calculated from 4-day Paper-DR entered into Web-DR by a research dietitian (Paper-Web-DR) versus 4-day Web-DR entered by the volunteers (Web-DR)

Parameter	Paper-Web-DR			Web-DR			Wilcoxon rank-sum test p value	Mean Spearman's ρ
	mean	median	SD	mean	median	SD		
Vitamins								
Biotin, µg	2.41	0.00	4.50	2.26	0.00	4.33	0.084	0.82
Folate, µg	22.99	8.61	40.81	23.25	8.00	47.91	0.036	0.88
Niacin, mg	1,886.90	368.28	3,673.25	1,889.83	319.50	3,645.83	0.372	0.88
Pantothenic acid, mg	0.38	0.18	0.62	0.38	0.16	0.71	0.023	0.86
Retinol equivalents, mg	84.66	2.04	1,265.33	89.53	2.1	1,339.31	0.602	0.87
Riboflavin, mg	0.11	0.05	0.17	0.12	0.05	0.18	0.264	0.84
Thiamine, mg	0.09	0.04	0.14	0.09	0.04	0.16	0.066	0.86
Vitamin B ₁₂ , µg	0.22	0.00	0.57	0.24	0.00	0.67	0.764	0.88
Vitamin B ₆ , mg	0.11	0.05	0.19	0.11	0.05	0.21	0.532	0.86
Vitamin C, mg	9.31	0.30	25.17	8.91	0.30	24.15	0.732	0.89
Vitamin D, µg	0.10	0.00	0.43	0.11	0.00	0.47	0.278	0.84
Vitamin E, mg	0.67	0.15	1.60	0.69	0.13	1.53	0.993	0.85
Minerals								
Calcium, mg	63.59	18.00	121.29	65.21	18.09	151.41	0.346	0.86
Magnesium, mg	23.27	13.80	33.44	23.33	12.91	36.00	0.520	0.85
Phosphorus, mg	83.98	34.00	120.06	84.31	34.00	130.39	0.820	0.87
Potassium, mg	205.20	124.50	272.24	200.64	112.50	286.38	0.465	0.85
Sodium, mg	129.07	18.60	263.51	132.24	15.00	318.86	0.167	0.82
Chloride, mg	208.54	47.25	403.86	215.79	50.00	492.00	0.184	0.83
Trace elements								
Iron, mg	0.87	0.33	1.48	0.88	0.31	1.59	0.341	0.87
Copper, mg	98.94	49.40	179.46	101.13	43.20	228.08	0.214	0.84
Fluoride, mg	13.74	1.60	24.75	13.18	0.94	25.62	0.441	0.78
Iodine, µg	5.40	0.81	14.86	5.21	0.61	15.91	0.780	0.76
Manganese, mg	814.14	48.36	3,743.50	706.47	42.5	3,033.27	0.963	0.86
Zinc, mg	0.58	0.21	0.92	0.60	0.20	1.07	0.734	0.83

For p values, the significance level is 0.050; null hypothesis: equal means. All Spearman's ρ are significant at the 0.001 level.

different paper-based questionnaires (e.g. food frequency, anthropometric and sociodemographic questionnaires) have been compared to web-based questionnaires by the same volunteers [26–30].

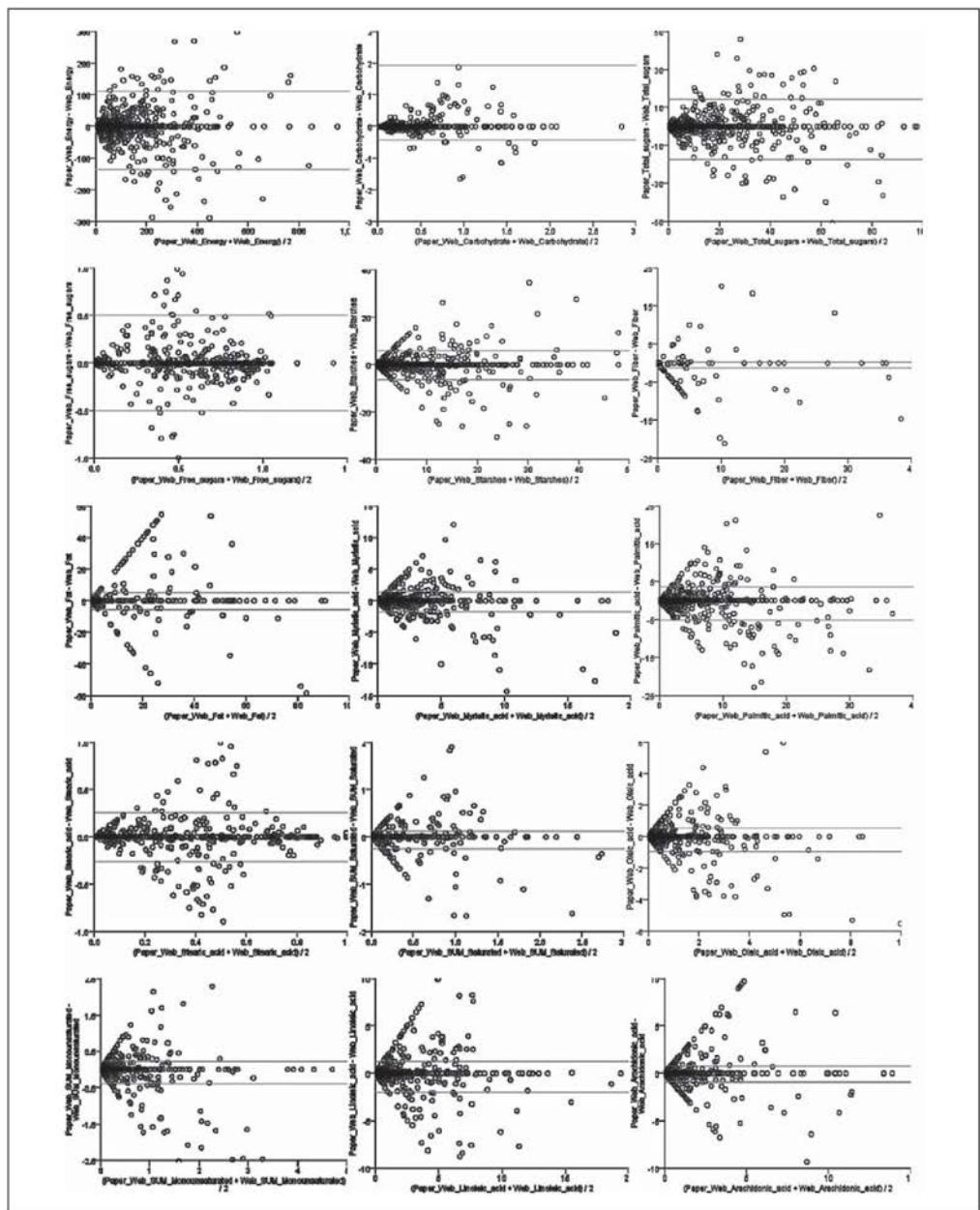
Advantages of Web-DR

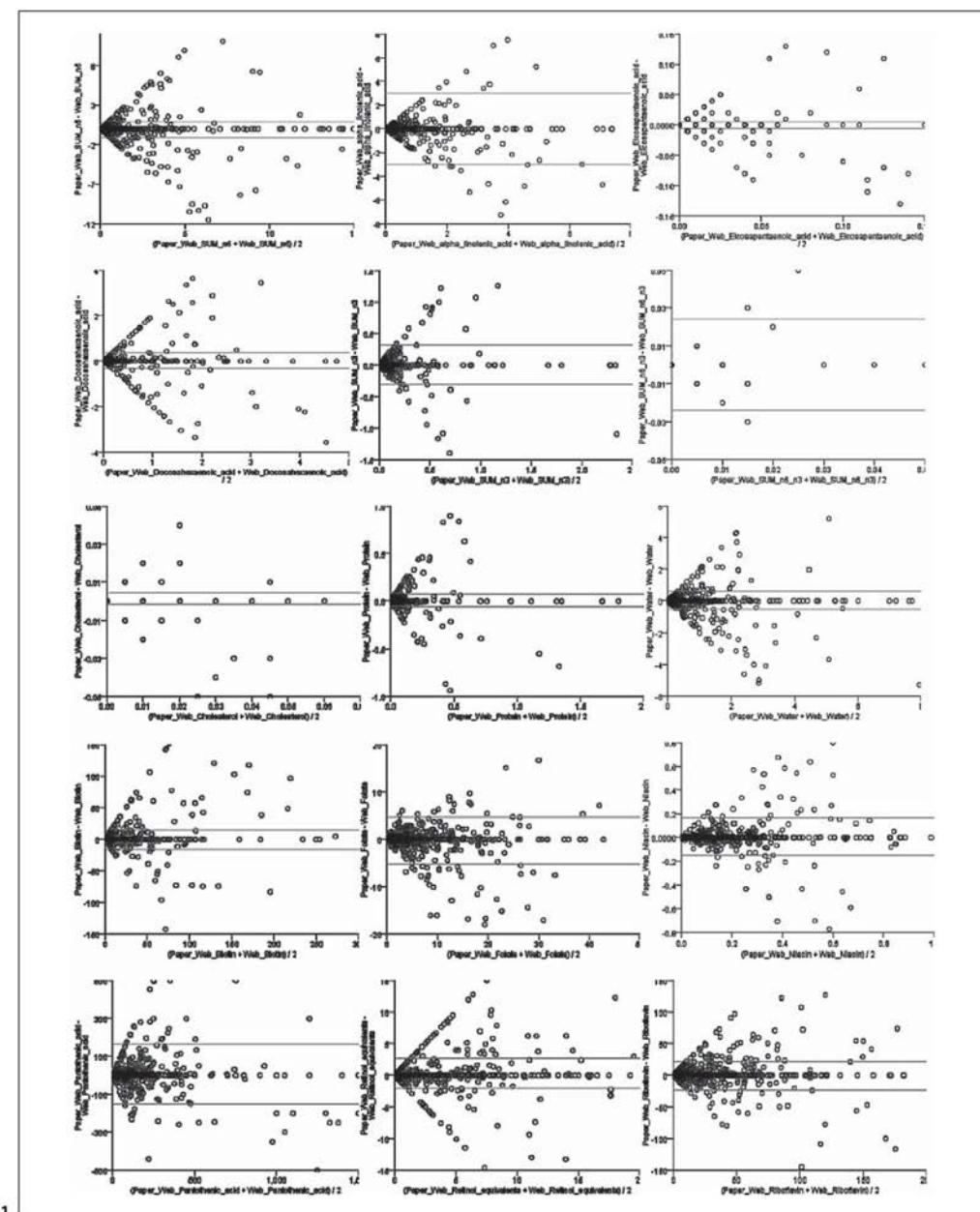
In the study presented in this paper, we calculated and compared the assessment of dietary intake using Paper-DR (Paper-Web-DR) as the gold standard [31] versus the novel Web-DR completed by the volunteers. There was no statistical difference between the overall matching of Paper-Web-DR versus Web-DR. Of the 49 parameters for both Paper-Web-DR and Web-DR, 41 parameters were highly correlated (Spearman's $\rho > 0.8$), while 8 parameters were ranked from 0.63 to 0.79 (tables 1, 2). Acceptance of the new method (Web-DR) was high ($n = 12$; 75%).

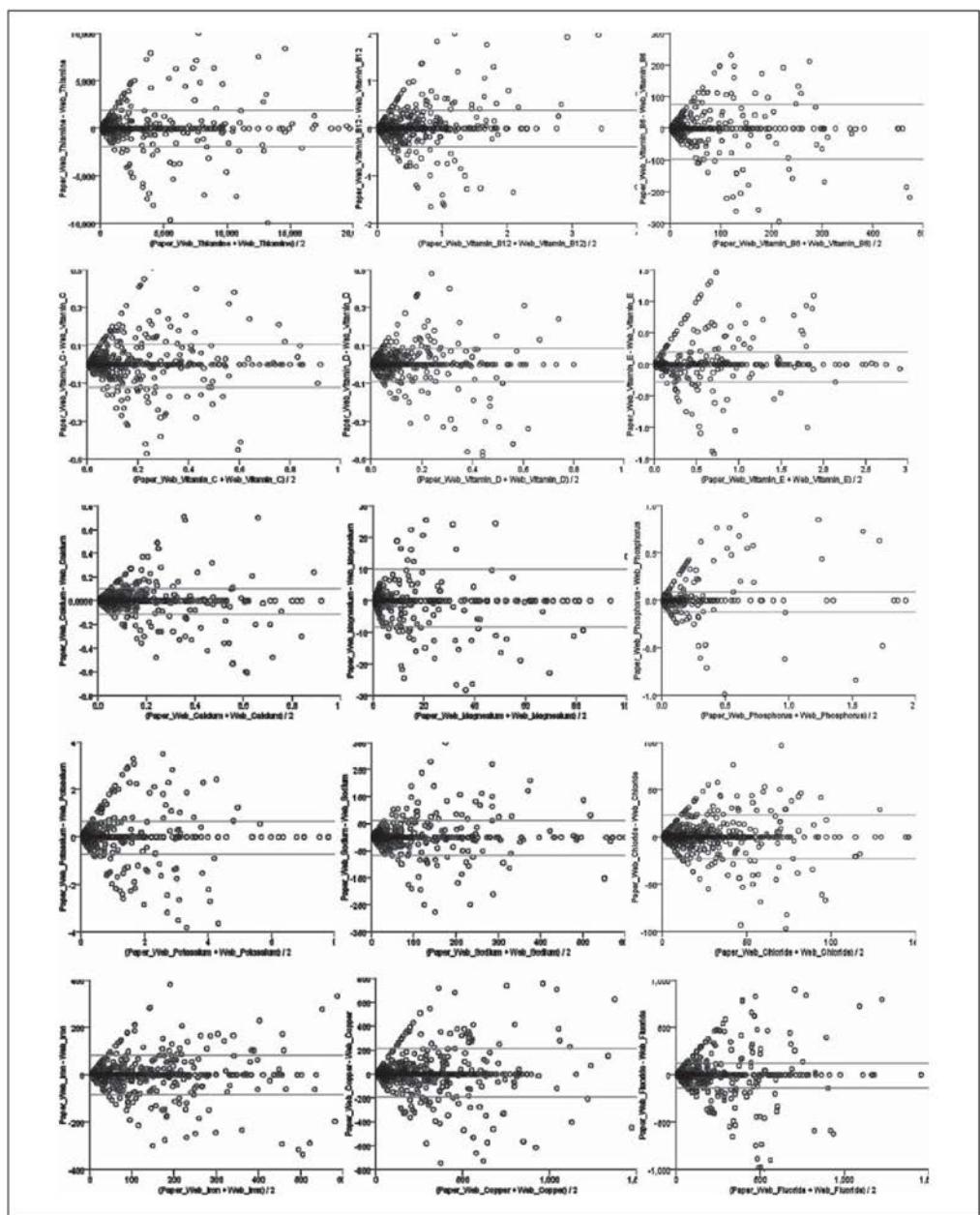
We used the Bland-Altman method to assess the bias and limits of agreement and showed that the estimates of energy and nutrient intakes obtained by the two methods were comparable. The distribution of points within the limits of agreement suggested that the Paper-Web-DR and Web-DR methods were comparable, although the mean of the differences indicated that the Paper-Web-DR slightly underestimated energy and carbohydrate. Limits of agreement ranged from 76 to 120% for energy and from 58 to 123% for carbohydrate. In the study by Ambrosini et al. [32], limits of agreement between 50 and 200% were

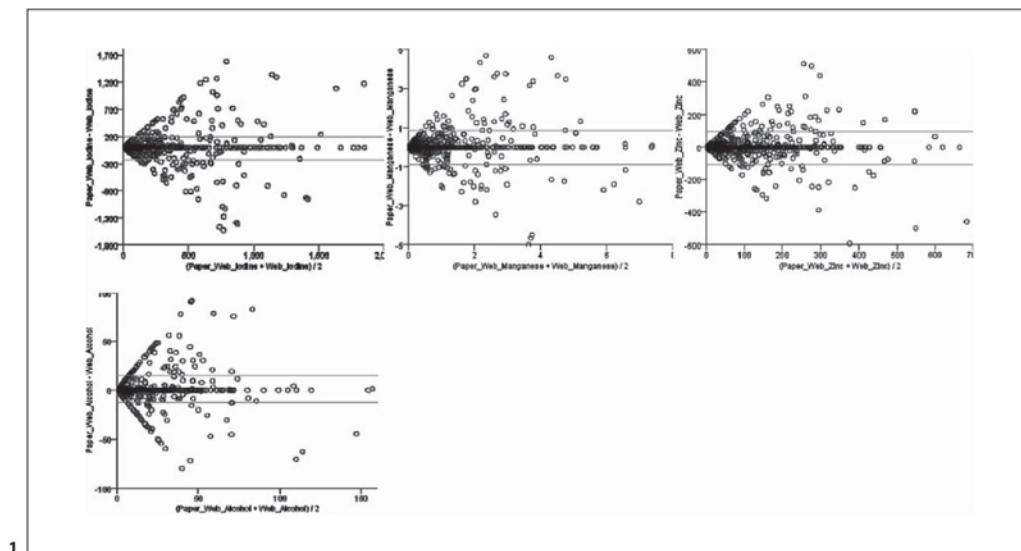
Fig. 1. Bland-Altman plots of agreement between the two methods (Paper-Web-DR vs. Web-DR) for all 49 parameters (for better visualization, outliers are not shown).

(For figure see next pages.)









considered suitable and clinically acceptable. Limits of agreement equal to 50 and 200% indicate that 95% of all participants' Paper-Web-DR estimates were between half and 2 times that of their Web-DR estimates. In addition, limits of agreement from our study were narrower than those reported by Ambrosini et al. [32] and MacIntyre et al. [33] but wider than those reported by Jackson et al. [34]. However, we think that the limits of agreement from our study are clinically acceptable and that the developed Web-DR could be applied in a larger study.

In the study, it was shown that Web-DR (using OPEN) can save time for the research dietitian, because (s)he does not need to enter the DR into the computer program but spends only 10–15 min checking the DR. The dietitian can invest the rest of the saved time in the patient's education.

In addition, OPEN has less missing data on portion sizes and reduces the opportunity for data entry errors. Some volunteers were imprecise when describing meat dishes in Paper-DR; the type of meat used and how it was prepared were not specified. For example, in some cases volunteers wrote on Paper-DR only chicken meat, without specifying whether it was white or dark meat and how it was prepared, although we had asked them to include this information. These kinds of mistake were not present when entering Web-DR because of help from the pro-

gram and as the DR had already been recorded on paper during the same day.

In a few cases, food type selection was a problem as the volunteers selected a food type in Web-DR, whereas the research dietitian selected the food type based on Paper-DR; for example, volunteers chose full grain bread type A in Web-DR, while the research dietitian chose full grain bread type B based on Paper-DR. When the exact food was not available in the OPEN FCDB, the closest substitute was used (sometimes volunteers selected a different substitute to that chosen by the research dietitian). In cases of complex food items (e.g. goulash, soups, salads), volunteers were instructed to write the recipe first on paper and then at the end of the day in OPEN, meaning that they could provide detailed information.

This study showed that Web-DR improves the volunteer's compliance compared to traditional Paper-DR. In general, the volunteers accepted the application well.

Lessons Learned

The study's null hypothesis was rejected by the Wilcoxon test for free sugars ($p < 0.001$), α -linolenic acid ($p = 0.041$), folate ($p = 0.036$) and pantothenic acid ($p = 0.023$). In most cases, these differences were due to mistakenly entered food units in Web-DR by the volunteers

(e.g. instead of grams on Paper-DR they used decagrams in Web-DR), which can be improved in the future by providing educational instructions on digital entry forms. In the case of free sugars, the differences were mainly due to lack of data from the manufacturers and consequently in the OPEN database. Exploring the reasons for the difference in folate intake between Web-DR and Paper-DR, we found that in the OPEN FCDB, data for folate content were stored either with the identifier (tag name) FOLFD or FOL, while only data with the identifier FOL were considered in the analysis.

After conducting the study, we realized some of its limitations. Concerning the design of the My-Milk study, the DR were first written on Paper-DR and at the end of the day into Web-DR, which might be a source of systematic error. To minimize the possible error due to the order of recording DR, in the future such studies should be conducted randomly, with half of the volunteers keeping Paper-DR first and the other half the Web-DR first.

Imprecision was noted with some of the volunteers ($n = 4$; 25%) when using Paper-DR and Web-DR in relation to food quantities (the quantity of selected food was not the same in Paper-DR and Web-DR; for example, the household measure 'slice of bread' selected in Web-DR and exact grams in Paper-DR). Nineteen percent of volunteers ($n = 3$) did not weigh all food items and/or beverages but used household measures, e.g. tablespoon, teaspoon, cup of coffee, slice of bread, despite instructions not to do so. These volunteers also stood out in relation to other observed parameters. Since completion of this study, we have integrated a tool in OPEN that enables or disables the use of household measures.

Lack of interest and/or reducing memory bias on the part of volunteers when completing the second version of DR (Web-DR) might have affected their entries, as also reported previously [35]. Most volunteers participating in this study who answered 'No' to the last question ('Are you interested in other facilities of OPEN?') commented that the main reason was the imminent birth of a child and lack of time. Low computer literacy of some volunteers was also noted.

Technical Limitations

During the study we also identified a few technical limitations with Web-DR. The first limitation was a slower internet connection speed, which decreases the user-friendliness of OPEN; consequently, users searching for foods need to wait longer than expected for the food list

to appear on the screen. However, this limitation is expected to diminish in the near future when high-speed internet will be brought to the general population. In the Probst and Tapsell [36] study, it was reported that spelling errors and errors in the identification of specific foods can also cause problems, especially with self-administered web-based dietary records. There was the same problem in our case, especially in relation to some local traditional foods that have different names for the same items across Slovenia (e.g. lard) and some newly adopted international words (i.e. pizza, ketchup). This second technical limitation has also now been considered in OPEN by introducing composed food names for such dishes.

It was shown that Web-DR (using OPEN) can provide information of equal or superior quality to Paper-DR, with substantial logistic and cost advantages for researchers and clinical dietitians. Paper-DR remain the gold standard for assessing individual dietary intake for those with low computer literacy, while Web-DR are more convenient for the majority. Further studies are needed to investigate the acceptance and validity of Web-DR among less educated volunteers and volunteers who are not pretrained in filling out DR. Nevertheless, the use of advanced technology in DR recording shows great promise.

Finally, our study participants belonged to middle and high education and occupational categories; caution is thus required when extrapolating our results to the general population and especially to people of low socioeconomic status and a low level of education.

The study was limited to comparing the matching of parameters in food items, while the matching of total daily dietary intake of energy and nutrients will be published later.

To conclude, the present pilot study clearly demonstrated that for most nutrients there was no statistical difference between the overall matching of Paper-Web-DR versus Web-DR. Also, the new method (Web-DR) was more convenient for the majority of participants and has substantial logistic and cost advantages.

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Disclosure Statement

There are no conflicts of interest.

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2.1.3 Primerjava spletnne aplikacije (e-4PD) in računalniškega programa (s-4PD) za ovrednotenje prehranskih dnevnikov

Benedik E., Koroušić Seljak B., Hribar M., Rogelj I., Bratanič B., Orel R., Fidler Mis N. 2015. Comparison of a web-based dietary assessment tool with software for the evaluation of dietary records. Slovenian Journal of Public Health, 54, 2: 91–97

Izhodišča in metode: V klinični praksi po navadi uporabljamo za ovrednotenje prehranskih dnevnikov računalniško podporo bodisi v obliki računalniškega programa bodisi spletnne aplikacije. Namen študije je bil predstaviti rezultate primerjave e-4PD in s-4PD. p-4PD, ki so ga vodile prostovoljke (N = 63), je izkušeni klinični dietetik vnesel v e-4PD in s-4PD.

Rezultati: Izračunani energijski vnos ter izračunan vnos 45 makro- in mikrohranil s pomočjo e-4PD in s-4PD smo statistično primerjali z neparametričnim Spearmanovim koeficientom ($> 0,600$). Ugotovili smo visoko korelacijo ($> 0,800$) med metodama za energijski vnos ter 11 hranil (ogljikovi hidrati, skupne maščobe, beljakovine, voda, kalij, kalcij, fosfor, skupna prehranska vlaknina, vitamin C, folna in stearinska kislina), zmerno (od 0,600 do 0,799) za 18 hranil, šibko (od 0,400 do 0,599) za 11 hranil, medtem ko za pet hranil ni bilo korelacije (AK, niacin, ALK, fluorid, skupni sladkorji).

Zaključki: Rezultati ovrednotenja prehranskih dnevnikov z e-4PD in s-4PD so v visoki korelaciji za energijsko vrednost in vsebnost makrohranil.

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COMPARISON OF A WEB-BASED DIETARY ASSESSMENT TOOL WITH SOFTWARE FOR THE EVALUATION OF DIETARY RECORDS

PRIMERJAVA SPLETNE APLIKACIJE IN RAČUNALNIŠKEGA PROGRAMA ZA OVREDNOTENJE PREHRANSKIH DNEVNIKOV

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ABSTRACT

Keywords:

web-based dietary assessment tools, dietary records, comparison, pregnant women

Background. Dietary assessment in clinical practice is performed by means of computer support, either in the form of a web-based tool or software. The aim of the paper is to present the results of the comparison of a Slovenian web-based tool with German software for the evaluation of four-day weighted paper-and-pencil-based dietary records (paper-DRs) in pregnant women.

Methods. A volunteer group of pregnant women (n=63) completed paper-DRs. These records were entered by an experienced research dietitian into a web-based application (Open Platform for Clinical Nutrition, OPEN, <http://opkp.si/en>, Ljubljana, Slovenia) and software application (Prodi 5.7 Expert plus, Nutri-Science, Stuttgart, Germany, 2011). The results for calculated energy intake, as well as 45 macro- and micronutrient intakes, were statistically compared by using the non-parametric Spearman's rank correlation coefficient. The cut-off for Spearman's rho was set at >0.600.

Results. 12 nutritional parameters (energy, carbohydrates, fat, protein, water, potassium, calcium, phosphorus, dietary fiber, vitamin C, folic acid, and stearic acid) were in high correlation (>0.800), 18 in moderate (0.600-0.799), 11 in weak correlation (0.400-0.599), while 5 (arachidonic acid, niacin, alpha-linolenic acid, fluoride, total sugars) did not show any statistical correlation.

Conclusion. Comparison of the results of the evaluation of dietary records using a web-based dietary assessment tool with those using software shows that there is a high correlation for energy and macronutrient content.

IZVLEČEK

Ključne besede:

spletne aplikacije za ovrednotenje prehranskih dnevnikov, primerjava, nosečnice

Izhodišča. V klinični praksi za ovrednotenje prehranskih dnevnikov običajno uporabljamo računalniško podporo, bodisi v obliki računalniškega programa ali spletne aplikacije. Namen članka je predstaviti rezultate primerjave nemškega računalniškega programa in slovenske spletne aplikacije za ovrednotenje prehranskega vnosha na osnovi metode štiridnevnega papirnega tehtanega prehranskega dnevnika (papirni PD), ki so ga vodile nosečnice.

Metode. Skupina nosečnic prostovoljek (n=63) je vodila papirni PD. Izkušeni klinični dijetetik je vnesel dnevниke v spletno aplikacijo (Odperta platforma za klinično prehrano, OPKP, <http://opkp.si>, Ljubljana, Slovenia) in računalniški program (Prodi 5.7 Expert Plus, Nutri-Science, Stuttgart, Germany, 2011). Rezultate za izračunani energijski vnos ter vnos 45 makro- in mikrohranil s pomočjo aplikacije in programa smo statistično primerjali z neparametričnim Spearmanovim koeficientom (>0,600).

Rezultati. Visoko korelacijo (>0,800) med metodama smo ugotovili za 12 hranil (energija, ogljikovi hidrati, skupne maščobe, beljakovine, voda, kalij, kalcij, fosfor, skupna prehranska vlaknina, vitamin C, folna kislina in stearinska kislina), zmerno (0,600-0,799) za 18 hranil, šibko (0,400-0,599) za 11 hranil, medtem ko za 5 hranil ni bilo korelacije (arachidonska kislina, niacin, alfa-linolenska kislina, fluor, skupni sladkorji).

Zaključki. Rezultati ovrednotenja prehranskih dnevnikov s spletno aplikacijo in računalniškim programom so v visoki korelaciji za energijsko vrednost in vsebnost makrohranil.

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1 INTRODUCTION

Dietary records are an important tool for estimating food and nutrient intakes in different groups of the population. In addition to dietary records, there are other methods of dietary assessment (food frequency questionnaires, 24-hour recall method), but paper dietary recording (paper-DR) has proven to be the best and most accurate way of evaluating food and nutrient intake (1-5). In the dietary record approach, each respondent must describe the foods and amounts consumed, including the name of the food (brand name, if possible), preparation methods, recipes for food mixtures and portion sizes consumed over a certain period of time (6-7). The amounts consumed can be measured either by using a scale or estimated by household measures or models, pictures or without visual aids (8). Ideally, the recording is done at the time of eating in order to avoid reliance on memory. A recording period of more than seven consecutive days is usually unsatisfactory, because of respondent fatigue or non-compliance (8). The duration most often used in the literature is three or four days of dietary recording (two or three weekdays and one weekend day), which has previously given acceptable and reliable data, and caused relatively low dropout (9-10).

There is an increase in computer support tools (such as software, web-based applications or mobile applications) available for both the general population and nutritional experts, which have received an increasing attention for large-scale population nutrition research (7, 11-17). The goal of computer support for the general population is to facilitate and simplify recording, as well as to be able to access the results quickly. Computer support tools allow end-users to enter food intake and receive feedback relating to energy and nutrient intake. The collected dietary data can be processed and calculated in place, or exported by a research dietitian for data analysis. Despite the availability of novel tools, the usual method of self-monitoring continues to be the paper-DR, which is time consuming, tedious and inconvenient for study volunteers, as well as for the research dietitians (11, 18).

We aimed to compare matching results of four-day paper-DRs kept by 63 pregnant women (hereinafter referred as volunteers), entered by an experienced research dietitian into the web-based application Open Platform for Clinical Nutrition (OPEN; hereinafter referred as web-DR) and the software Prodi 5.7 Expert plus, Nutri-Science, Stuttgart, Germany, 2011 (Prodi; hereinafter referred as SW-DR). Our objective was to examine whether web-DR and SW-DR yield similar results of energy and nutritional intake estimates for 45 macro- and micronutrients.

2 METHODS

2.1 Study Design

This pilot study is a part of the Slovenian research project entitled 'The role of human milk in development of a breast fed child's intestinal microbiota' or 'My-Milk,' in short, which has been described elsewhere (available at: www.moje-mleko.si/en) (19). Briefly, the 'My-Milk' study aims to elucidate the role of microbiota and the fatty acid composition of mother's milk in the development of intestinal microbiota and the overall health status of a newborn infant.

Within this pilot study, we aimed to determine whether web-DR is equivalent to SW-DR, which would substantially reduce logistical and cost burdens in clinical practice, since the web-DR could be recorded directly by the volunteer/user/patient and only checked by a dietitian. Volunteers were included in the study if they were healthy and willing to participate by keeping a paper-DR at home throughout four consecutive days, including one weekend day (from Sunday to Wednesday), because of the protocol of 'My-Milk' study.

They were recruited from January until May 2011, at the Gynaecological Clinic, University Medical Centre Ljubljana, while attending the 'School for Parents.' The volunteers came mainly from Ljubljana (the capital of Slovenia) and its surrounding areas. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Ljubljana, Slovenia (No. 32/07/2010), and is registered at ClinicalTrials.gov (NCT01548313).

The volunteers received 15 minutes of oral instruction from a research dietitian, as well as written instructions on how to keep a paper-DR. We provided them with a kitchen scale, with 1 g resolution (CTC, Clatronic® International GmbH), and asked them not to make any dietary changes during the trial. We recorded the basic anthropometrical measurements (age, week of pregnancy, body height and pre-pregnancy body mass for each volunteer) and basic socio-demographic data (level of education and employment status). Body mass was measured with a certified medical scale to the nearest 0.1 kg and body height to the nearest 0.5 cm (Seca digital scale 769, Germany). The volunteers' data were coded and all information was kept confidential.

2.2 Study Population

By the end of May 2011, 65 volunteers had been approached for study recruitment; two of them withdrew from the study because of lack of interest. In total, 63 volunteers completed the paper-DR. Their average age was 30.4 (± 4.0) years, they were in the 30.7th (± 4) week of pregnancy and had a pre-pregnancy body mass index of 25.3 (± 3.6) kg/m². The majority of the volunteers were better educated (postgraduate: 13 (21%); tertiary: 42

(67%); secondary: 8 (13%), primary: 0 (0%), and all were employed.

2.3 Methodology of the Comparative Study

We asked the volunteers to record the intake of all foods, drinks and food supplements consumed over four consecutive days; from Sunday to Wednesday.

The sum values of recording for all four days together for web-DR ($n=63$) and SW-DR ($n=63$) were compared for energy and 45 macro- and micronutrients (hereinafter referred to as 46 parameters) (Table 1).

We selected the list of observed nutrients on the basis of the previous study comparing nutrient intake of Slovenian adolescents (20) and, additionally, on the basis of nutrients that are of special interest in the 'My-Milk' study (i.e., fatty acids: linoleic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) (19). For dishes specified in the diaries, we used Slovenian traditional recipes and frequently used recipes to identify the ingredients.

2.3.1 Paper-DR

The paper-DR had five pages, including one page of instructions with an example of one daily dietary record. Detailed information regarding the: a) time of consumption, b) quantities in grams/milliliters or, exceptionally, also in household measures (such as cup, tablespoon, teaspoon, cup of coffee, slice of bread, etc.), c) foods with brand names when appropriate, and the type of preparation were requested. The paper-DRs were checked when received by the experienced research dietitian.

The research dietitian entered the paper-DR into the web-DR and SW-DR and checked the entries twice.

2.3.2 Web-DR (OPEN)

OPEN is the first Slovenian web-based tool for assessment of dietary intake, as well as for diet planning, and it has been described in more detail elsewhere (21-22). Briefly, it consists of data from Slovenian (23-24), European (25) and, to a limited extent, also American (26) food composition tables. To support its use in different countries and languages, OPEN allows translation of the user interface into any language, as well as the use of any food composition dataset that complies with Food data structure and format standard (BS EN 16104:2012). To calculate food composition data for traditional and frequently consumed Slovenian dishes, OPEN applied a recipe-calculation procedure, originally recommended by INFOODS (27) and recognized by EuroFIR (28). In order

to prove the efficiency and correctness of the recipe-calculation procedure applied within OPEN, the energy and nutrient contents of composite samples of daily meals (each sampled four times) were compared by using both analytical and calculation techniques (29, 30). The data included for each food item from paper-DRs were: the amount consumed, the date and time of consumption. After a meal had been entered by the research dietitian, OPEN stored the information.

2.3.3 SW-DR (Prodi)

Prodi is German software for nutritional counseling and nutritional therapy available in German and English language. It supports meal planning and calculation, as well as documentation of the consultancy. Foods and their ingredients are readily available, calculated and compared.

In this pilot study, we used Prodi 5.7 Expert plus Nutri-Science, Stuttgart, Germany, 2011, which contains the database of approximately 14,800 foods from the Bundeslebensmittelschlüssel 3.01 (BLS 3.01) database, Fachmann-Kraut-Nährwerttabellen (FKN, Stuttgart, 2005) database, and industrial products and dietetic foods.

2.4 Statistical Analysis

We applied the Shapiro-Wilk normality test to determine whether or not the dataset was modeled with a normal distribution. The dataset of observed parameters did not have a normal distribution, so non-parametric Spearman's rho coefficients were used to measure the correlation of results of nutrient intake calculated by OPEN and Prodi. We defined acceptable correlation as being 0.600 or more. Statistical analyses were performed using the statistical software SPSS ver. 21 (SPSS Inc, Chicago, IL, 2012).

3 RESULTS

Our data show that there was no systematic error in entering. For all 126 DRs (63 in web-DR and 63 in SW-DR), we first calculated the average, SD and median values. We then calculated Spearman's rho correlation coefficients for 46 parameters to check the correlation between web-DR and SW-DR.

In the Table 1 the average and median daily nutrition content for 46 nutritional parameters from paper-DRs ($n=63$) entered into web-DR and SW-DR. Figure 1 shows the Spearman's rho correlation coefficients for 46 nutritional parameters. The Spearman's correlation coefficient for parameters ranged from -0.05 for total sugars to 0.95 for water.

Table 1. Averages and medians of daily nutrient content for all parameters calculated from 63 four-day paper-based dietary records (paper-DR) entered into web-based dietary records (web-DR) and software-based dietary records (SW-DR).

	Average(SD)		Median	
	web-DR	SW-DR	web-DR	SW-DR
Energy [kcal]	2017.21(386.53)	1994.89(363.02)	2094.13	2025.50
[kJ]	8350.63(1618.03)	8350.63(1519.58)	8766.00	8478.75
Carbohydrates [g]	263.14(56.91)	243.59(49.47)	270.79	241.75
Total sugar [g]	114.77(32.45)	0.61(2.70)	111.95	0.00
Starch [g]	78.35(30.79)	116.00(30.35)	79.76	117.50
Dietary fiber [g]	23.64(8.07)	28.15(9.39)	22.63	27.80
Fats [g]	71.50(18.30)	74.20(19.23)	68.97	74.25
SFA* [g]	25.38(6.94)	25.72(8.43)	24.92	25.25
Myristic acid [g]	2.92(1.11)	3.30(1.09)	2.80	3.33
Palmitic acid [g]	12.62(3.40)	13.14(3.67)	12.28	13.10
Stearic acid [g]	5.65(1.69)	6.05(2.09)	5.62	5.80
MUFA** [g]	18.72(5.36)	22.99(7.88)	18.05	21.75
Oleic acid [g]	13.35(4.89)	21.75(6.88)	12.57	21.13
PUFA*** [g]	11.26(3.24)	12.84(5.90)	10.45	11.40
Linoleic acid [g]	10.72(3.37)	11.40(5.53)	10.09	9.15
Alpha-Linolenic acid [g]	1.48(0.63)	1.06(0.34)	1.27	1.00
Arachidonic acid [g]	0.10(0.06)	0.17(0.16)	0.08	0.10
Eicosapentaenoic acid [g]	0.04(0.07)	0.05(0.08)	0.01	0.00
Docosahexaenoic acid [g]	0.13(0.26)	0.17(0.16)	0.03	0.10
Cholesterol [mg]	253.41(102.34)	254.68(103.62)	230.08	231.50
Proteins [g]	78.64(16.90)	79.02(17.95)	78.30	79.10
Water [g]	2928.89(1355.09)	2758.93(872.28)	2707.06	2808.50
Alcohol [g]	0.57(1.09)	0.64(1.17)	0.06	0.15
Vitamins:				
Biotin [µg]	35.97(11.15)	52.08(20.42)	34.76	49.75
Folic acid [µg]	388.64(106.78)	298.59(109.33)	377.37	277.25
Niacin [µg]	30698.05(8505.74)	27343.77(7964.66)	30861.95	26568.50
Pantothenic acid [mg]	6.13(2.04)	5.94(2.09)	5.68	5.40
Vitamin A [mg]	0.76(1.34)	0.41(0.20)	0.52	0.36
Riboflavin [mg]	1.91(0.48)	1.65(0.49)	1.91	1.62
Thiamine [mg]	1.47(0.38)	1.36(0.53)	1.42	1.22
Vitamin B ₁₂ [µg]	3.90(1.26)	5.23(2.08)	3.87	4.83
Vitamin B ₆ [mg]	1.89(0.48)	1.81(0.48)	1.86	1.82
Vitamin C [mg]	173.69(93.75)	189.07(74.99)	156.09	171.25
Vitamin D [µg]	2.58(2.64)	2.50(3.07)	1.73	1.75
Vitamin E [mg]	11.96(4.18)	15.10(6.01)	11.00	14.68
Minerals:				
Calcium [mg]	1106.75(508.97)	1084.81(320.02)	1042.99	1088.25
Magnesium [mg]	571.99(1100.57)	386.16(102.35)	363.15	376.50
Phosphorus [mg]	1349.86(305.69)	1475.62(352.82)	1334.92	1437.00

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	Average(SD)		Median	
	web-DR	SW-DR	web-DR	SW-DR
Potassium [mg]	3349.91(783.74)	3557.52(922.98)	3294.96	3418.00
Sodium [mg]	3130.55(2416.03)	2343.81(872.59)	2474.43	2178.25
Chloride [mg]	4934.61(3759.24)	3951.81(1386.93)	4074.61	3595.75
Trace elements:				
Iron [mg]	14.06(3.79)	14.28(3.71)	14.07	13.98
Copper [µg]	1684.52(440.59)	2472.60(595.37)	1721.94	2546.25
Fluoride [µg]	297.19(255.45)	906.85(341.91)	241.35	857.50
Iodine [µg]	132.65(117.95)	171.22(62.78)	98.48	161.00
Manganese [µg]	5194.11(3363.54)	4738.35(1730.32)	4348.27	4438.50
Zinc [mg]	9.64(2.34)	12.31(2.88)	9.96	12.25

* Sum of saturated fatty acids

** Sum of monounsaturated fatty acids

*** Sum of polyunsaturated fatty acids

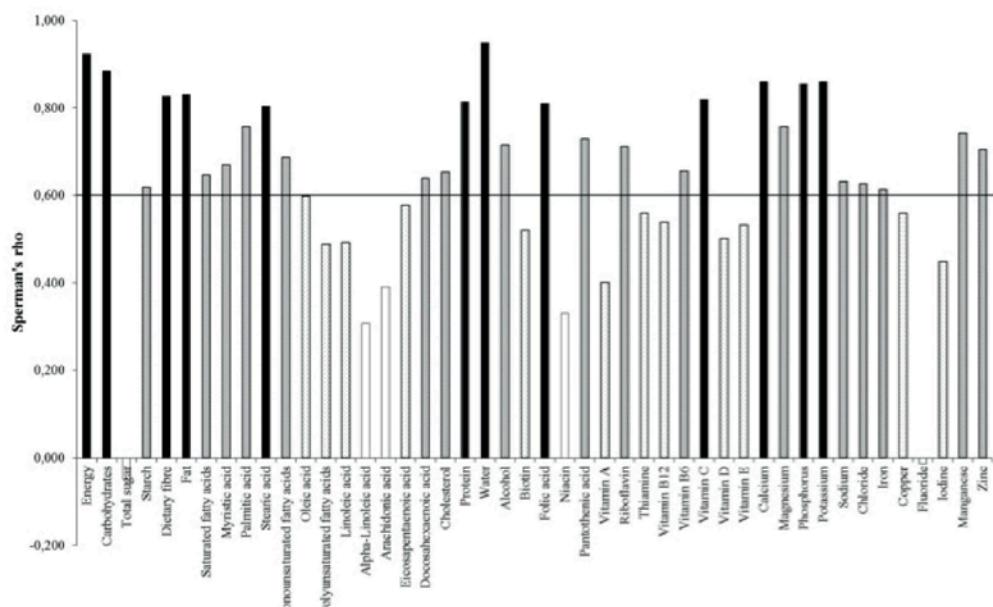


Figure 1. Spearman's rho correlation coefficients for 46 parameters calculated from 63 four-day dietary records recorded by 63 volunteers and entered into web- and software-based dietary records by a dietitian (cut-off for Spearman's rho as strongly positive correlation was set at >0.800 (black columns), as medium significant at 0.600-0.799 (grey columns), as weak at 0.400-0.599 (spotted columns) and no correlation (white columns)). Spearman's rho coefficients for all studied correlations are significant at the 0.05 level.

4 DISCUSSION

To the best of our knowledge, this is the first study to compare two self-administered methods (web-DR versus SW-DR) completed by the same persons and entered into both applications by the same research dietitian. We have already compared the assessment of dietary intake using paper-DR as the gold standard (1) versus the novel web-DR completed by the same volunteers (31). There was no difference between total matching of paper-DR versus web-DR. The next step was to compare the two most frequently used dietary record softwares in clinical practice in Slovenia; OPKP (Web-DR) and Prodi (SW-DR).

Average ranges (median) are not different between the two methods (Table 1). The basic parameters in nutrition assessment (i.e., energy, carbohydrates, proteins, fat and water) were highly correlated between the methods (>0.800) (Figure 1). As expected, some of the parameters, such as arachidonic acid, niacin, alpha-linolenic acid, fluoride and total sugars, did not correlate. In our opinion, the reason for the discrepancy with arachidonic acid, niacin and alpha-linolenic acid was mainly a lack of compositional data for branded food items. Namely, foods rich in these nutrients are meat and meat products, fish and fish products, eggs and egg products, nuts and nut products, and grain-based products. In OPEN, the Slovenian food composition data for meat, fish and their products were used. Since meat of Slovenian origin accounts for the largest share of meat consumed in this country, a comparison of compositional data on Slovenian meat with data from the literature was made, showing a wide variation, particularly for the total fat content, fatty acid composition and cholesterol content (24).

In the case of fluoride and total sugars, the differences were due to different food composition databases (mainly there is no data for total sugar in Prodi). The lack of correlation could also be due to human error but this is less likely, because all paper-DRs were entered into the web-DR and SW-DR by the same research dietitian and they were checked twice.

Values for total saturated, monounsaturated, and polyunsaturated fatty acids may include individual fatty acids not reported; therefore, the sum of their values may exceed the sum of the individual fatty acids. In rare cases, the sum of the individual fatty acids may exceed the sum of the values given for the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). These differences are generally caused by rounding and should be relatively small.

Various instruments are used nowadays to assess nutrient intake and food consumption, each with advantages and disadvantages (16). However, it makes more sense to use a DR that is supported by devices that are integrated into

the daily lives of people (computer, tablet, smartphone, etc.), as has already been described in the literature (14, 18, 32, 33).

Our study also had some other limitations. Firstly, not participants themselves, but the research dietitian entered the paper-DR into the web-DR and SW-DR. It would be interesting to analyze the matching of dietary assessment with both methods, conducted by the same volunteers.

Secondly, when the exact food was not available in the OPEN food composition database, the closest substitute was used (the research dietetic sometimes selected a different substitute in OPKP to that chosen in Prodi).

Thirdly, some technical limitations with web-DR were observed/reported, such as a slower internet connection speed, which can decrease the user-friendliness of OPEN; users, consequently, had to wait longer than expected for the food list to appear on the screen. In the Probst and Tapsell study (34), it was reported that spelling errors and errors in the identification of specific foods can also cause problems, especially with self-administered web-based dietary records, but not in SW-DR. There was the same problem in our case, especially in relation to some local traditional foods that have different names for the same items across Slovenia (e.g., lard), or some newly adopted international words (e.g., pizza, ketchup).

5 CONCLUSION

Our study shows that web-DR (OPEN) provides dietary intake data information of equal or superior quality to that of SW-DR (Prodi), mainly because it is based on Slovenian food composition data, which are integrated in OPEN. The use of advanced technology in DR recording has shown and continues to show great promise. We have shown that either one of the nutritional dietary record softwares can be used in clinical practice.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

The study protocol was approved by the Slovene National Medical Ethics Committee (No. 32/07/2010).

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2.2 OSTALO POVEZOVALNO ZNANSTVENO DELO

2.2.1 Sociodemografski, zdravstveni in antropometrični podatki nosečnic in doječih mater vključenih v študijo Moje-mleko

Prostovoljke, vključene v študijo, so odgovarjale na vprašanja, vezana na sociodemografske, zdravstvene in antropometrične podatke, ki so pomembni pri pravilnem vrednotenju in interpretaciji podatkov o prehranskem vnosu, prehranskih navadah in tudi vsebnosti maščobnih kislin v HM.

V študijo smo od decembra 2010 do oktobra 2012 vključili 294 nosečnic iz treh slovenskih regij: LJ, IZ/NG in MB. V kliničnem delu študije (Priloga A) je sodelovalo 174 prostovoljk iz ljubljanske in izolske regije z okolico. V celoti je vse načrtovane aktivnosti v študiji zaključilo 152 prostovoljk.

Anketna vprašalnika za prostovoljke

Sociodemografske podatke, kot so zakonski stan, število otrok v družini, stopnja izobrazbe in kraj bivanja, ter podatke o življenjskem slogu prostovoljk, kot so kajenje, uživanje alkohola in fizične aktivnosti, kot tudi o splošnem zdravstvenem stanju prostovoljke smo pridobili s pomočjo dveh vprašalnikov: prvič so ga izpolnjevale v zadnjem trimesečju nosečnosti (Priloga E) in drugič štiri tedne po porodu (Priloga F).

Antropometrične meritve

Te meritve so bile opravljene pri nosečnicah in doječih materah na Pediatrični kliniki v Ljubljani. Meritve so izvajali za to strokovno usposobljeni raziskovalci Biotehniške fakultete, Oddelka za biologijo, Katedre za fiziologijo, antropologijo in etologijo, Skupine za antropologijo. Pridobili smo podatke o starosti, višini in masi prostovoljke pred zanositvijo. Izmerili smo telesno maso, obseg zapestja, obseg sproščene in pokrčene nadlakti, dolžino nadlakti, dolžino stegna, obseg stegna, obseg goleni, velikost kožnih gub na tricepsu (angl. triceps), bicepsu (angl. biceps), pod lopatico (angl. subscapular), nad črevnico (angl. suprailiac), na sprednjem delu stegna (angl. front thigh) in na notranji strani goleni (medial calf). Meritve so bile opravljene dvakrat in sicer v zadnjem trimesečju nosečnosti (prvi obisk na Pediatrični kliniki) in štiri tedne po porodu (drugi obisk na Pediatrični kliniki).

Telesno višino smo merili s certificirano medicinsko tehnicno na 0,5 cm natančno in telesno maso na 0,1 kg natančno (Seca digitalna tehnicna 769, Nemčija). Obsege smo merili z neraztegljivim tankim merilnim trakom (Fiber glass tape, Kitajska). Kožne gube smo merili s Harpenden kaliperjem (John Bull, Velika Britanija). Iz pridobljenih podatkov smo izračunali indeks telesne mase (ITM, ki je razmerje med telesno maso v kilogramih in kvadratom telesne višine v metrih). ITM uporabljamo za osnovno oceno posameznikove prehranjejenosti (prenizka telesna masa: ITM < 18,5; normalna telesna masa: ITM od 18,5 do 25,0; povišana telesna masa: ITM > 25,0) (WHO, 2013). Na osnovi izmerjenih kožnih

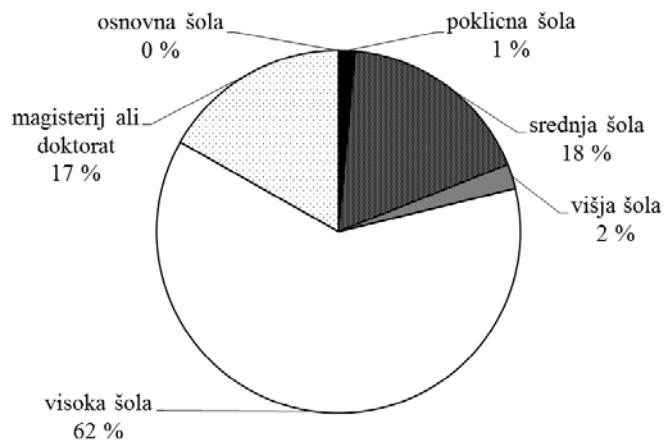
gub smo izračunali gostoto telesa (g/cm^3) (Durnin in Womersly, 1974), iz katere pa smo izračunali delež maščobnega tkiva (%) (Siri, 1993) pri nosečnicah (Robič in sod., 2013) in doječih materah.

Statistična analiza

Pridobljene podatke smo obdelali in statistično analizirali z uporabo računalniškega programa Excel Microsoft Office 2010. Uporabili smo statistično metodo opisne statistike.

Povprečna starost prostovoljk (srednja vrednost \pm S.D.) je $30,6 \pm 4,3$ leta (razpon starosti od 20 do 44 let). Večina prostovoljk (73 %) prihaja iz mestnega okolja, 27 % s podeželja. 96 % prostovoljk živi skupaj s svojimi partnerji; od tega jih 54 % živi v zunajzakonski skupnosti, 44 % je poročenih. Za 60 % prostovoljk je bila to prva nosečnost. Večina prostovoljk, ki so pred nosečnostjo kadile, je to med nosečnostjo in dojenjem opustilo, 2 % prostovoljk pa se temu ni odpovedalo. V prvem mesecu življenja je bilo izključno dojenih 73 % dojenčkov, v prvih treh mesecih pa 68 %. Do prvega leta starosti se je delno dojilo še 42 % dojenčkov.

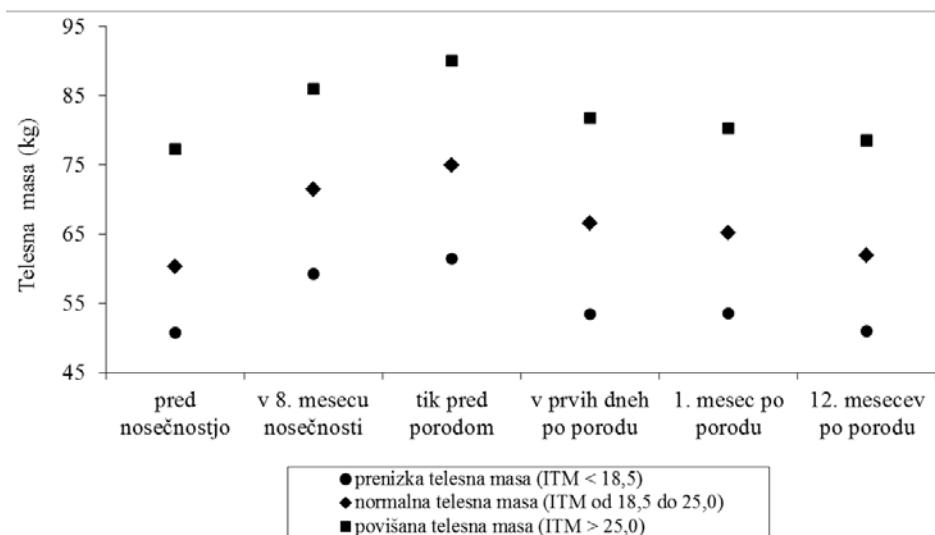
Slika 1 prikazuje izobrazbeno strukturo prostovoljk. Večina (62 %) ima visokošolsko ali univerzitetno izobrazbo. Nobena prostovoljka ni imela samo osnovnošolske izobrazbe.



Slika 1: Izobrazbena struktura prostovoljk

Figure 1: Educational structure of the volunteers

Slika 2 prikazuje povprečne vrednosti telesne mase prostovoljk v študiji za vsako skupino posebej (prenizka, normalna ali povišana), šest različnih obdobjij, pred nosečnostjo do 12 mesecev po porodu.



Slika 2: Povprečne vrednosti telesnih mas sodelujočih prostovoljk za vse tri skupine (prenizka: N = 5; normalna: N = 115 in povišana telesna masa: N = 24) za šest različnih obdobjij.

Figure 2: Average body mass of participants for all three groups (low: N=5; normal: N=115 and overweight body mass: N=24) for six periods (before pregnancy, last trimester of pregnancy, before birth, in first days after birth, one month after birth and twelve month after birth).

Izračunan delež maščobnega tkiva (srednja vrednost \pm S.D.) pri nosečnicah znaša $32,5 \pm 4,6\%$, pri materah 4 tedne po porodu pa $30,2 \pm 4,8\%$.

2.2.2 Prehrana slovenskih nosečnic in doječih mater

2.2.2.1 Uvod

Nacionalnih podatkov o prehranskem vnosu nosečnic in doječih mater, predvsem iz Srednje Evrope, je malo, vključno s Slovenijo. Želeli smo pridobiti podatke o prehrani slovenskih nosečnic in doječih mater, kar smo izvedli s pomočjo metode 4PD. Skupaj smo proučevali uživanje 41 prehranskih parametrov: energijski vnos, skupne ogljikove hidrate, skupne sladkorje, škrob, prehransko vlaknino, maščobe, nasičene maščobne kisline (NMK), enkrat nenasičene maščobne kisline (ENMK), večkrat nenasičene maščobne kisline (VNMK), vsoto n-3 maščobnih kislin, vsoto n-6 maščobnih kislin, EPK, DHK, holesterol, beljakovine, alkohol, vodo, vitamine (biotin, folna kislina, niacin, pantotenska kislina, retinol ekvivalent, riboflavin, tiamin, vitamin B₁₂, vitamin B₆, vitamin C, vitamin D, vitamin E), minerale (kalcij, magnezij, fosfor, kalij, natrij, klorid) in elemente v sledovih (železo, baker, fluorid, jod, mangan in cink).

2.2.2.2 Metode dela

Metoda prehranskega dnevnika (4PD)

Trenutno prehrano smo spremajali z metodo 4PD (Priloga B) dvakrat, in sicer MN (od 27. do 37. tedna) ter MD (od 4. do 5. tedna po porodu). Metoda 4PD je natančneje opisana v poglavjih 2.1.2 in 2.1.3. Na kratko; gre za 4PD, ki so ga prostovoljke vodile štiri dni (en dan med vikendom – nedelja in tri zaporedne dni v tednu – ponedeljek, torek, sreda). Natančno so tehtale ali beležile vso hrano in pičajočo, preden so jo zaužile; stehtati in opisati je bilo treba tudi morebitne ostanke hrane. Beležile so tudi uživanje prehranskih dopolnil, ki pa jih nismo upoštevali pri analizi prehranskega dnevnika, saj vseh prehranskih dopolnil do dneva, ko smo opravili analize prehranskega dnevnika, še niso bila vsa vključena v OPKP. Analiza prehranskega dnevnika bi bila tako nepopolna. Podrobnejše smo prehranska dopolnila obravnavali v okviru VPŽ1 in VPŽ2 vprašalnika (poglavje: 2.2.3). Če prostovoljki ni uspelo stehtati hrane in pičače, je bila dovoljena uporaba domačih mer oziroma gospodinjskih enot (kozarec, skodelica, zajemalka, jedilna žlica, čajna žlička, ščepec, kos kruha in podobno). Prostovljkam nismo pripravili predavanja o zdravi prehrani, nasprotno, opozorili smo jih, naj med vodenjem 4PD ne spreminja svojih prehranskih navad, saj je bil namen ugotoviti dejansko stanje njihove prehrane. Prosili smo jih, če jim je čas dopuščal, da so vzporedno, poleg p-4PD, vodile še e-4PD (podrobnejše opisano v poglavju 2.1.2; Benedik in sod., 2014).

Spletna aplikacija Odprta platforma za klinično prehrano (OPKP)

Za analizo prehranskih dnevnikov smo uporabili spletno aplikacijo OPKP na podlagi ugotovitve, da sta si e-4PD in s-4PD enakovredna (poglavje 2.1.3; Benedik in sod., 2015). Na kratko, OPKP omogoča beleženje in analizo prehranskega dnevnika v spletnem okolju. Za izračun podatkov o sestavi jedi uporablja standardizirani postopek, ki ga je določila mednarodna organizacija FAO INFOODS in potrdila evropska mreža EuroFIR. Uporabnik, v naši študiji raziskovalni dietetik, v OPKP vnaša zapise prehranskih dnevnikov tako, da si izbere točno določeno živilo iz baze živil ali navede sestavine in postopek priprave, aplikacija pa nato samodejno izračuna energijsko in hranilno vrednost jedi.

Referenčne vrednosti in priporočila

Vnos energije in hranil smo primerjali z Referenčnimi vrednostmi za vnos hranil (Referenčne vrednosti ..., 2004; German Nutrition Society, 2012), priporočili WHO/FAO (WHO/FAO, 2003; WHO, 2015) ter priporočilom Evropske komisije in Mednarodnega društva za proučevanje maščobnih kislin in maščob (Koletzko in sod., 2007; Koletzko in sod., 2008).

Statistična analiza

Pridobljene podatke smo obdelali in statistično analizirali z uporabo dveh računalniških programov za statistično analizo; SPSS 21.0 (Statistical Package for Social Sciences) in statističnim paketom R (R development core team, 2013). Uporabili smo metodo opisne statistike (srednja vrednost, standardni odklon), t-test za odvisne vzorce (parni t-test) za iskanje razlik v prehranskem vnosu med nosečnicami in doječimi materami, ter t-test za en vzorec, s katerim smo iskali razlike v primerjavi s priporočili. Vnos hranil je bil s pomočjo rezidualne metode (Willett in Stampfer, 1986) standardiziran glede na energijski vnos.

2.2.2.3 Rezultati

Dnevni vnos energije, makrohranil, DHK in EPK iz prehrane v primerjavi s priporočili so predstavljeni v preglednici 1. Nosečnice in doječe matere zaužijejo 33 in 34 % energije iz maščob, 52 in 51 % iz ogljikovih hidratov in 15 % v obeh obdobjih iz beljakovin. Deleži energije iz NMK, ENMK in VNMK v prehrani so 11,5; 8,6 in 4,8 % (pri nosečnicah) ter 12,1; 8,1 in 4,5 % (pri doječih materah). Vnos EPK iz prehrane je znašal 35 ± 90 mg/dan MN in 43 ± 89 mg/dan MD, vnos DHK pa 92 ± 152 mg/dan MN in 108 ± 199 mg/dan MD. Vnos holesterola pri nosečnicah znaša 274 ± 366 mg/dan, pri doječih materah pa 295 ± 318 mg/dan. Vnos vode iz hrane in pihače (voda, čaj, kava, 100 % sadni sokovi, nektarji, gazirane pihače, alkoholne pihače) je pri nosečnicah znašal 3233 ± 1085 ml/dan, pri doječih materah pa 3349 ± 1503 ml/dan.

Preglednica 1: Vnos energije, makrohranil, DHK in EPK iz prehrane pri slovenskih nosečnicah in doječih materah pridobljen na podlagi 4-dnevnega tehtanega prehranskega dnevnika (4PD) v primerjavi s priporočili (WHO/FAO, 2003; Referenčne vrednosti ..., 2004; Koletzko in sod., 2007; EFSA, 2012; IARD, 2015; WHO, 2015).

Table 1: Dietary intake of energy, macronutrients, DHK and EPA in Slovenian pregnant and lactating women obtained from the 4-day weighted dietary protocol (4PD) and compared to the recommendations (WHO/FAO, 2003; Referenčne vrednosti ..., 2004; Koletzko et al., 2007; EFSA, 2012; IARD, 2015; WHO, 2015).

	Prostovoljke		Priporočila	
	Nosečnice (N = 155)	Doječe matere (N = 140)	Nosečnice	Doječe matere
Energijski vnos (kcal/dan) (MJ/dan)	$2074 \pm 377\#$ $8,7 \pm 1,6\#$	$2126 \pm 501\#$ $8,9 \pm 2,1\#$	$2555^{a,b}$ $10,6^{a,b}$	$2935^{a,b}$ $12,2^{a,b}$
Ogljikovi hidrati (g/dan) (% E)*	271 ± 27 $52 \pm 5\#^{a,d}$	269 ± 34 $51 \pm 6\#^b$	— $> 50^a/55-75^d$	— $> 50^a/55-75^d$
Skupni sladkorji (g/dan) ^{c*} (% E)*	119 ± 24 23 ± 6	104 ± 28 19 ± 5	— —	— —
Škrob (g/dan) (% E)	76 ± 29 15 ± 6	83 ± 35 16 ± 7	— —	— —
Prehranska vlaknina (g/dan)* (% E)* (g/MJ) ^{g*}	25 ± 7 $2,4 \pm 0,7$ $3,0 \pm 1,1$	$22 \pm 8\#$ $2,1 \pm 0,7$ $2,7 \pm 1,2\#$	30^d — $3,0^a$	30^d — $3,0^a$

se nadaljuje

nadaljevanje

	Prostovoljke		Priporočila	
	Nosečnice (N = 155)	Doječe matere (N = 140)	Nosečnice	Doječe matere
Maščoba (g/dan)*	76 ± 12	80 ± 15	—	—
(% E)*	33 ± 5# ^{a,f,d}	34 ± 6# ^{a,f,d}	30 ^a , f/30–35 ^d	30 ^a , f/30–35 ^d
NMK (g/dan)*	27 ± 6	28 ± 9	—	—
(% E)	11,5 ± 2,5#	12,1 ± 3,6#	< 10 ^{a, d}	< 10 ^{a, d}
ENMK (g/dan)	20 ± 6	19 ± 6	—	—
(% E)	8,6 ± 2,6#	8,1 ± 2,7#	≥ 10 ^a	≥ 10 ^a
VNMK (g/dan)	11 ± 4	11 ± 4	—	—
(% E)	4,8 ± 1,6# ^{a,d}	4,5 ± 1,7# ^{a,d}	7–10 ^a /6–10 ^d	7–10 ^a /6–10 ^d
Σ n-3 (mg/dan)	1,0 ± 0,6	1,1 ± 1,1	—	—
(% E)	0,5 ± 0,3# ^d	0,5 ± 0,5# ^d	0,5 ^a /1–2 ^d	0,5 ^a /1–2 ^d
Σ n-6 (mg/dan)	5,9 ± 3,7	5,3 ± 3,3	—	—
(% E)*	2,6 ± 1,6# ^d	2,3 ± 1,4# ^{a,d}	2,5 ^a /5–8 ^d	2,5 ^a /5–8 ^d
EPK (mg/dan)	35 ± 90	43 ± 89	— ^e	— ^e
DHK (mg/dan)	92 ± 152#	108 ± 199#	≥ 200 ^{f, e}	≥ 200 ^{f, e}
Holesterol (mg/dan)	274 ± 366	295 ± 318	< 300 ^{a, d}	< 300 ^{a, d}
Beljakovine (g/dan)*	78 ± 11#	82 ± 14#	58 ^a	63 ^a
(% E)*	15 ± 2#	15 ± 3#	10–15 ^d	10–15 ^d
(g/kg telesne mase/dan) ^{g,*}	1,1 ± 0,2	1,2 ± 0,3	—	—
Alkohol (ml/dan)	0,5 ± 1,2#	0,8 ± 1,5#	0 ⁱ	0 ⁱ
(% E)	0,2 ± 0,3#	0,3 ± 0,5#	0 ⁱ	0 ⁱ
Voda (ml/dan) ^h	3233 ± 1085#	3349 ± 1503#	2700 ^a	3100 ^a

Vrednosti so podane kot srednje vrednosti ± S.D.

% E = delež celodnevnega energijskega vnosa (uporabljene nedeatenuirane vrednosti izračunane na podlagi splošnega Atwater energijskega pretvornika (v kcal/g): ogljikovi hidrati = 4, prehranska vlaknina = 2, maščoba = 9, alkohol = 7 (FAO, 2003)). Zaradi pomanjkljivih podatkov o sestavi živil in pijač v OPKP, predvsem zaradi skupnih sladkorjev, vsota deleža energije pri ogljikovih hidratih ne predstavlja 52 % ampak 40,4 %.

* P < 0,05 med nosečnicami in doječimi materami. # P < 0,05 v primerjavi s priporočili. ^a upoštevana srednja vrednost priporočil.

NMK = nasičene maščobne kisline; ENMK = enkrat nenasičene maščobne kisline; VNMK = večkrat nenasičene maščobne kisline; EPK = eikozapentaenojska kislina; DHK = dokozaheksaojska kislina. – = ni priporočil (WHO/FAO, 2003; Referenčne vrednosti ..., 2004).

^aPriporočila za starostno skupino 25 do < 51 let; energijski vrednosti je v času nosečnosti dodanih dodatnih 255 kcal/dan (1,1 MJ/dan) in v prvih štirih mesecev dojenja dodatnih 635 kcal/dan (2,7 MJ/dan) (Referenčne vrednosti ..., 2004).

^bPovprečne dnevne potrebe, izračunane na podlagi bazalnega metabolizma posameznika z normalno telesno maso, pomnoženo s stopnjo fizične aktivnosti; 1,75 (Referenčne vrednosti ..., 2004).

^cSkupni sladkor = prosti sladkor (monosaharidi in disaharidi dodani v hrano s strani proizvajalca/kuharja/potrošnika in naravno prisotni sladkorji v medu, sirupih in sadnih sokovih + naravno prisotni sladkorji (laktoza v mleku, sladkorji v sadju) (WHO, 2015).

^dSACN priporočila (SACN, 2015).

^eEFSA priporočila sicer navajajo priporočen dnevni vnos EPK + DHK za odrasle (250 mg/dan) + dodatnih od 100 do 200 mg DHK/dan za nosečnice in doječe matere, vendar ne navajajo priporočenega skupnega celodnevnega vnosa posebej za EPK in DHK (EFSA, 2012).

^fPriporočilo Evropske komisije in Mednarodnega društva za proučevanje maščobnih kislín in maščob (Koletzko in sod., 2007; Koletzko in sod., 2008).

^gUporabljene deatenuirane vrednosti.

^hSkupna voda iz pijač in hrane.

ⁱICAP priporočilo (IARD, 2015).

Dnevni vnos mikrohranil (12 vitaminov, 6 mineralov in 6 elementov v sledovih) je predstavljen v preglednici 2.

Preglednica 2: Vnos izbranih vitaminov, mineralov in elementov v sledovih pri slovenskih nosečnicah in doječih materah pridobljen na podlagi 4-dnevnega tehtanega prehranskega dnevnika (4PD) v primerjavi s priporočili (WHO/FAO, 2003; Referenčne vrednosti ..., 2004; German Nutrition Society, 2012).

Table 2: Intake of selected vitamins, minerals and trace elements in Slovenian pregnant and lactating women obtained from the 4-day weighted dietary protocol (4PD) and compared to the recommendations (WHO/FAO, 2003; Referenčne vrednosti ..., 2004; German Nutrition Society, 2012).

	Prostovoljke		Priporočila ^{a, b, c}	
	Nosečnice (N = 155)	Doječe matere (N = 140)	Nosečnice	Doječe matere
Vitamini				
A (retinol ekvivalent) (mg/dan) ^d	1,3 ± 1,0#	1,2 ± 0,8#	1,1	1,5
B ₁ (tiamin) (mg/dan)	1,6 ± 0,5#	1,5 ± 0,5#	1,2	1,4
B ₂ (riboflavin) (mg/dan)	2,1 ± 0,6#	2,0 ± 0,6#	1,5	1,6
B ₃ (niacin) (mg niacin ekvivalenta/dan)	31,5 ± 9,2#	29,4 ± 10,4#	15	17
B ₅ (pantotenska kislina) (mg/dan)*	6,6 ± 2,6#	5,8 ± 2,5	6	6
B ₆ (mg/dan)	2,0 ± 0,7	1,8 ± 0,7	1,9	1,9
B ₉ (folna kislina) (µg ekvivalenta folne kisline/dan)*	386 ± 118#	339 ± 99#	600	600
B ₁₂ (µg/dan)	4,0 ± 2,0#	4,5 ± 4,2	3,5	4,0
C (mg/dan)*	162 ± 86#	102 ± 90#	110	150
D (µg/dan)*	2,0 ± 2,0#	2,5 ± 2,5#	20,0 ^c	20,0 ^c
E (mg/dan)*	12,8 ± 5,3	10,2 ± 4,3#	13	17
H (biotin) (µg/dan)	38,1 ± 31,2#	33,8 ± 29,3#	30–60	30–60
Minerali				
Kalcij (mg/dan)*	1096 ± 248#	1019 ± 358	1000	1000
Magnezij (mg/dan)*	385 ± 107#	344 ± 98#	310	390
Fosfor (mg/dan)	1332 ± 229#	1282 ± 244#	800	900
Kalij (mg/dan)*	3320 ± 669#	2878 ± 668#	2000	2000
Natrij (mg/dan)	2684 ± 897# ^o	2801 ± 972# ^o	550	550
			< 2000 ^b	< 2000 ^b
Klorid (mg/dan)	4182 ± 1345#	4385 ± 1481#	830	830
Elementi v sledovih				
Železo (mg/dan)	14,5 ± 7,4#	15,2 ± 7,0#	30	20
Baker (mg/dan)*	1,7 ± 0,4#	1,6 ± 0,5#	1,0–1,5	1,0–1,5
Fluorid (mg/dan)*	0,3 ± 0,3#	0,2 ± 0,1#	3,1	3,1
Jod (µg/dan)	101,1 ± 44,1#	93,3 ± 49,1#	200–230	200–260
Mangan (mg/dan)	4,9 ± 3,7#	4,7 ± 2,8#	2,0–5,0	2,0–5,0
Cink (mg/dan)	10,0 ± 2,9	9,5 ± 2,3#	10,0	11,0

Vrednosti so podane kot srednje vrednosti ± S.D.

* P < 0,05 med nosečnicami in doječimi materami. # P < 0,05 v primerjavi s priporočili. V primeru ko priporočilo podaja razpon, je upoštevana srednja vrednost priporočil. V primeru da gre za več različnih priporočil za eno hranilo, je bilo za vsako priporočilo izračunan t-test.

^aPriporočila za starostno skupino 25 do < 51 let (Referenčne vrednosti ..., 2004).

^bWHO/FAO priporočila (WHO/FAO, 2003). Zgornja dovoljena količina glede na WHO/FAO priporočila; ustreza < 5 g NaCl/dan (NaCl (g) = Na (g) x 2,54).

^cNove referenčne vrednosti za vitamin D (German Nutrition Society, 2012; Benedik in Fidler Mis, 2013).

^dRetinol ekvivalent = vitamin A + α-karoten (1 mg retinol ekvivalenta = 12 mg α-karotena) + β-karoten (1 mg retinol ekvivalenta = 6 mg β-karotena) + γ-karoten (1 mg retinol ekvivalenta = 12 mg γ-karotena) (Referenčne vrednosti ..., 2004).

2.2.3 Prehranske navade slovenskih nosečnic in doječih mater

2.2.3.1 Uvod

Za vpogled v prehranske navade prostovoljk glede uživanja rib/izdelkov/sadežev in prehranskih dopolnil smo uporabili VPŽ in sicer v treh časovnih obdobjih: PN in MN (VPŽ1) ter MD (VPŽ2). VPŽ je ena izmed najprimernejših metod za ocenjevanje prehranskih navad v epidemioloških študijah saj poda podatek o povprečju vnosa določenega živila v daljšem časovnem obdobju (EFSA, 2009).

2.2.3.2 Metode dela

Metoda pogostosti uživanja posameznih živil

VPŽ1 in VPŽ2, ki smo ju uporabili, smo priredili na podlagi dveh vprašalnikov z Norveške, kjer so prav tako proučevali uživanje rib/izdelkov/sadežev in prehranskih dopolnil v času MN in MD (Birgisdottir in sod., 2008; Norwegian Institute of Public Health, 2011). Omenjene skupine živil predstavljajo namreč največji prehranski vir DHK. Živila z visoko vsebnostjo ALK, kot so rastlinska olja in semena, niso zajeta v VPŽ, ker se le majhen del ALK v organizmu pretvori v DHK (DeFilippis in Sperling, 2006; Williams in Burdge, 2006; Abedi in Mohammad Ali, 2014). VPŽ, ki smo ga uporabili v naši študiji je semikvantitativni, sestavljen iz 31 vprašanj o pogostosti uživanja posameznih živil, razvrščenih v štiri skupine: morske ribe, sladkovodne ribe, morski sadeži in drugo. Sledila so vprašanja o načinu priprave živil (uživanje surovih, prekajenih, pečenih, dušenih ali ocvrtilih rib) in vnosu ribjih izdelkov (konzerve, paštete, namazi) (Prilogi C in D). Prostovoljke so za vsako posamezno skupino in vrsto živila glede na način priprave označile pogostost uživanja (9 frekvenc: nikoli, enkrat letno, dvakrat letno, enkrat mesečno, od dva- do trikrat mesečno, enkrat tedensko, dvakrat tedensko, trikrat tedensko, \geq štirikrat tedensko) ter velikost porcije (mala = 100 g; srednja = 125 g; velika = 175 g). Pri vprašanju o vnosu ribjih izdelkov je bilo, poleg označbe pogostosti uživanja, treba navesti še vrsto zaužitega ribjega izdelka in zaužito količino (v g/obrok). Vprašalnik je za lažjo predstavo vseboval slikovni prikaz standardnih velikosti porcij zaužitega obroka. Vprašalnik je vseboval tudi poglavje o prehranskih dopolnilih, kjer so prostovoljke poimensko navedle katera prehranska dopolnila vsa uživajo, kako pogosto in v kakšni količini. Vprašanja so bila postavljena za vsako preiskovano obdobje posebej: VPŽ1: PN (za čas enega leta pred nosečnotjo) in MN (za čas prvih osmih mesecev nosečnosti); VPŽ2: MD (za čas prvega meseca po porodu). Vprašanja so si bila enaka, le vprašanj o vnosu prehranskih dopolnil ni bilo na vprašalniku PN.

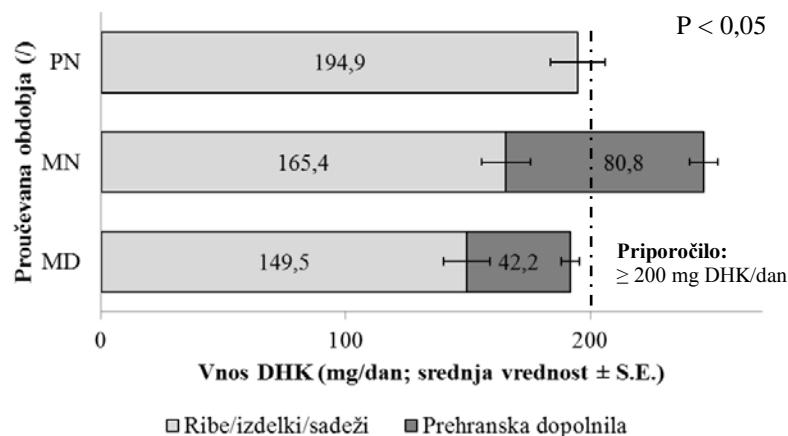
S pomočjo uporabe spletnne aplikacije OPKP smo iz vprašalnikov izračunali količino zaužite DHK (mg/dan) z ribami/izdelki/sadeži in s prehranskimi dopolnili.

Statistična analiza

Podatke smo obdelali in statistično analizirali z uporabo računalniškega programa Excel Microsoft Office 2010 in računalniškega programa za statistično analizo SPSS 21.0 (Statistical Package for Social Sciences). Uporabili smo metodo opisne statistike (srednja vrednost, standardni odklon).

2.2.3.3 Rezultati

Prostovoljke so PN, MN in MD v povprečju zaužile 29, 25 in 26 g(rib/izdelkov/sadežev)/dan. Od vseh rib (skupno 100 %), v vseh treh obdobjih skupaj (PN, MN, MD), so najpogosteje uživale tuno (20 %), lososa (15 %), postrv (13 %), orado (12 %) in osliča (12 %). Med morskimi sadeži so najpogosteje uživale lignje (53 %), kozice (33 %) in školjke (9 %). Tiste prostovoljke, ki so navedle, da uživajo tuno, je bila ta večinoma iz konzerve (75 %), posledično so tunine konzerve predstavljale glaven vir DHK (22 % glede na celoten vnos DHK iz rib/izdelkov/sadežev). Med ribami glaven vir DHK predstavlja losos (15 %), med morskimi sadeži pa lignji (6 %). Uživanja prehranskih dopolnil z DHK v obdobju PN nismo beležili. Dopolnila uživa MN 35,9 %, MD pa 21,4 % prostovoljk. Slika 3 prikazuje vnos DHK (mg DHK/dan; povprečje \pm S.E.) iz rib/izdelkov/sadežev in prehranskih dopolnil za tri proučevana obdobja; PN ($194,9 \pm 11,2$ mg DHK/dan iz rib/izdelkov/sadežev; vnos iz prehranskih dopolnil ni znan), MN (246 ± 8 mg DHK/dan, od tega $165,4 \pm 10,0$ mg DHK dan iz rib/izdelkov/sadežev in $80,8 \pm 5,8$ mg DHK/dan iz prehranskih dopolnil) ter MD (191 ± 7 mg DHK/dan, od tega $149,5 \pm 9,4$ mg DHK/dan iz rib/izdelkov/sadežev ter $42,2 \pm 3,8$ mg DHK/dan iz prehranskih dopolnil). Vnos DHK iz rib/izdelkov/sadežev niti iz prehranskih dopolnil ni bil v korelaciji niti z izobrazbo, geografsko regijo niti s starostjo prostovoljk.



Slika 3: Vnos DHK (mg DHK/day; srednja vrednost \pm S.E.) PN (v času pred nosečnostjo), MN (v času med nosečnostjo), MD (v času med dojenjem) iz rib/izdelkov/sadežev ter prehranskih dopolnil (razen PN) z DHK ($P < 0,05$).

Figure 3: DHK intake (mg DHK/day; mean \pm S.E.) PN (before pregnancy), MN (during pregnancy) and MD (during first month of lactation) from fish, seafood and fish products, and food supplements (except PN) containing DHK ($P < 0.05$).

2.2.4 Vsebnost DHK v humanem mleku v odvisnosti od prehranskega vnosa

2.2.4.1 Uvod

DHK je pogojno esencialne za donošene in nedonošene dojenčke, je pomemben gradnik celičnih membran, predvsem živčnih celic (nevronov). Tako jo je največ v možganih in očesni mrežnici. DHK je tudi izhodna molekula (prekurzor) za nastanek eikozanoidov, ki imajo vrsto pomembnih bioloških funkcij v organizmu (regulirajo spanje, ugodno vplivajo na učenje, živčne celice ipd.) (Cetin in sod., 2009; Grieger in Clifton, 2015). DHK prehaja preko placente v plod (možgane) predvsem v drugi polovici nosečnosti in preko HM ozziroma hrane v prvih dveh letih življenja, kar vpliva na rast in razvoj otroka (nevrološki razvoj, razvoj vida) (McCann in Ames, 2005).

Ženske v rodni dobi si morajo prizadevati za zadosten vnos DHK, ki naj bi bil vsaj 200 mg DHK/dan, kar dosežejo z eno do dvema porcijama morskih rib na teden, vključno z eno porcijo mastnih rib (Koletzko in sod., 2007; Koletzko in sod., 2008). Poleg tega predstavljajo vir DHK tudi ribje olje in prehranska dopolnila z DHK, predvsem v primeru, da nosečnica ozziroma mati ne uživa dovolj morskih rib (De Giuseppe in sod., 2014). Obstaja več različnih priporočil glede uživanja DHK za noseče in doječe matere (od < 100 mg DHK/dan do > 500 mg DHK/dan), ki so podrobnejše predstavljene v FAO/WHO (2014). Zadostna oskrba z LCP, zlasti z DHK, je pomembna za razvoj otroka predvsem v času hitre rasti in diferenciacije živčnega sistema v pozrem prenatalnem (zadnja tretjina nosečnosti) in zgodnjem postnatalnem obdobju (vse do drugega leta starosti), ko poteka hitra rast in razvoj možganov ter vida (Delgado-Noguera in sod., 2010; Ryan in sod., 2010; Morse, 2012; Koletzko in sod., 2014).

HM predstavlja najboljši vir DHK za dojenčka, čeprav vsebnost DHK v HM znotraj in med populacijami zelo variira (Smit in sod., 2002; Brenna in sod., 2007). Povprečna vsebnost DHK v HM, določena na podlagi 84 študij iz različnih delov sveta (Evrope, ZDA, Azije, Afrike), v kateri je skupno sodelovalo 2974 prostovoljk, je 0,32 ut. % (Brenna in sod., 2007). Vsebnost DHK v HM je odvisna v največji meri od materinih maščobnih zalog, ki jih je pridobila v času PN, predvsem pa v času MN (sestava maščobnih zalog je odvisna od prehrane v daljšem časovnem obdobju), sledi vpliv prehrane MD in na koncu vpliv endogene sinteze DHK iz ALA (Sauerwald in sod., 2000; Lassek in Gaulin, 2011; Lauritzen in Carlson, 2011; Innis, 2014).

V okviru te študije smo določili vsebnost DHK v zrelem HM ter preverili vpliv prehranskega vnosa DHK iz rib/izdelkov/sadežev in prehranskih dopolnil, ki smo ga določili z VPŽ1 in VPŽ2 (poglavlje 2.2.3) za vsa tri obdobja: PN, MN in MD.

2.2.4.2 Vzorec in metode dela

Vzorec HM

Doječim materam smo 4 tedne po porodu (pri drugem pregledu) na dan 5 (četrtek), dan za tem ko so končale z vodenjem drugega (zadnjega) 4DP2, ob obisku na Pediatrični kliniki v Ljubljani odvzeli vzorec zrelega HM (alikvot na začetku (1 ml) in koncu dojenja (1 ml) za pridobitev reprezentativnega vzorca HM) za analitiko maščobno-kislinske sestave. Pred tem je bilo področje areole in bradavice očiščeno s fiziološko raztopino, HM pa si je prostovoljka izbrizgala ročno (Priloga G). Vzorce smo prepihalo z dušikom in jih do analize skladiščili v zamrzovalni omari pri minus 80 °C.

Analitika maščobno-kislinske sestave HM

Ta je bil izvedena v Službi za specialno laboratorijsko diagnostiko Pediatrične klinike s pomočjo plinske kromatografije s plamenškim ionizacijskim detektorjem (GC-FID). Tuk pred analizo smo vzorce odtalili pri sobni temperaturi in jih v vodni kopeli temperirali pri 38 °C 10 minut. Alikvot mleka smo uporabili za *in situ* transesterifikacijo maščob (Park in Goins, 1994; Fidler Mis in Hren, 2003). Maščobe smo ekstrahirali z dodajanjem metilenklorida in 0,5 N natrijevega hidroksida, pripravljenega v metanolu, tik pred uporabo. Dodali smo tudi interni standard (heneikozanojska kislina; C21:0). Sledilo je segrevanje za 10 minut pri 90 °C in hlajenje do sobne temperature. Metilne estre maščobnih kislin (MEMK) smo pridobili z dodajanjem 14 % borovega trifluorida v metanolu s segrevanjem za 10 minut pri 90 °C in ohlajanju (Glej: Protokol priprave MEMK). Ekstraktu MEMK smo dodali destilirano vodo in heksan. MEMK smo analizirali z GC-FID (Agilent Technologies 7980), opremljenim s sistemom za samodejno odvzemanje tekočih vzorcev (Agilent Technologies 7683). Ločitev je potekala na kapilarni koloni Agilent J&W HP-88 (100 m x 0,25 mm x 0,20 µm). Maščobno-kislinsko sestavo MEMK smo določevali s primerjavo retencijskih časov s standardnimi vzorci (Interni standardi, Priloga H). Kvantificirali smo jih kot utežne odstotke (ut. %) vseh maščobnih kislin s pomočjo določitve faktorjev odziva (angl. response factors) in deležev površin (uporabljen Supelco 37 FAME Mix standard). Vsi vzorci so bili analizirani v paralelkah. Natančnost, zanesljivost in ponovljivost analitske metode so bile preverjene z 10 zaporednimi analizami standarda FAME 37 Mix istega lota znotraj treh dni. FAME 38 Mix je bil uporabljen tudi vsako 10 analizo vzorcev za sprotno preverjanje identificiranih površin in retencijskih časov. Za oceno zanesljivosti analitskega postopka je bil uporabljen referenčni material mlečne maščobe (Anhydrous Milk Fat; CRM-164), certificiran leta 1993.

Protokol priprave MEMK (prirejeno po Park in Goins, 1994):

- 1) Zamrznjeno HM segrevamo v termobloku na 38 °C/10 min. Pred nadaljevanjem vzorec dobro premešamo z obračanjem (20x).
- 2) V epruvete z navojem in teflonskim zamaškom odpipetiramo (v dveh paralelkah):
 - + 2,4 ml sveže 0,5 M NaOH v MetOH (2g NaOH/100 ml CH₃OH)
 - + 240 µl CH₂Cl₂
 - + 60 µl internega standarda C21:0 (2 mg/ml)
 - + 360 µl homogenega HM
 - + prepihamo z N₂ (5 s), dobro zapremo in premešamo na vortex-u (10 s).
- 3) Sledi segrevanje pri 90 °C/10 min v vodni kopeli. Vsebina postane rjave barve zaradi Maillardove reakcije.
- 4) Epruvete hitro ohladimo v ledeni kopeli na sobno temperaturo, dodamo:
 - + 2,4 ml hladnega 14 % BF₃ v MetOH
 - + rahlo prepihamo z N₂ (5 s) in premešamo na vortex-u (~3 s).
- 5) Epruvete ponovno segrevamo pri 90 °C/10 min v vodni kopeli.
- 6) Epruvete ponovno ohladimo v ledeni kopeli na sobno temperaturo in dodamo:
 - + 2,4 ml destilirane vode
 - + 1 ml heksana
 - + prepihamo z N₂ (~3 s).
- 7) Nastale MEMK ekstrahiramo z močnim stresanjem 1 minuto na vortex-u, pri čemer se metilni estri izločijo v heksansko fazo.
- 8) Sledi centrifugiranje (10 min pri 2000 x g).
- 9) Po centrifugiranju prenesemo zgornjo heksansko fazo v zatemnjene viale in še zadnjič rahlo prepihamo z N₂ (3 s).
- 10) Tako pripravljene vzorce shranimo v zamrzovalnik na – 20 °C do pričetka analize.
- 11) Pred analizo vzorce predhodno termostatiramo na sobno T in jih rahlo premešamo.

Kromatografski pogoji pri analitiki HM

Temperatura injektorja: 250 °C

Temperatura detektorja: 260 °C

Temperaturni program kolone:

konstantno 65 °C, 4 min

segrevanje od 65 °C do 150 °C, 20 °C/min, 0 min

segrevanje od 150 °C do 185 °C, 2,5 °C/min, 0 min

segrevanje od 185 °C do 195 °C, 0,5 °C/min, 2 min

segrevanje od 195 °C do 220 °C, 1 °C/min, 15 min

segrevanje od 220 °C do 235 °C, 20 °C/min, 10 min

konstantno 235 °C, 2 min

Injiciranje: Split, 50 : 1, injicirani volumen 1 µl HM

Pretoki plinov:
 N_2 – 25 ml/min,
sintetični zrak – 400 ml/min,
 H_2 – 30 ml/min in
He: konstantno 0,3 ml/min, 15 min
povečanje pretoka iz 0,30 ml/min na 0,38 ml/min, 6 min
povečanje pretoka iz 0,38 ml/min na 0,56 ml/min, 74 min

Aparature in reagenti

Aparature

- plinski kromatograf (Agilent Technologies 7980, Kitajska), opremljenim s sistemom za samodejno odvzemanje tekočih vzorcev (Agilent Technologies 7683, Kitajska);
- kapilarna kolona (Agilent J&W HP-88; 100 m x 0,25 mm x 0,20 μm);
- analitska tehnica (Sartorius BP 210S, Goettingen, Nemčija);
- vodna kopel (Wasserbad Memmert WB7, Memmert GmbH + Co. KG, Schwabach);
- univerzalna centrifuga (Eppendorf centrifuge 5702 R, Hamburg, Nemčija);
- sistem za destilacijo vode (Branstead NANOpure, Iowa, ZDA);
- termoblok (Metal block thermostat VLM 1.0 HT, VLM GmbH, Leopoldshöhe, Nemčija);
- ultrazvočna kopel (Transsonic 460/H, Elma GmbH & Co KG, Singen, Nemčija);
- stresalnik (Vibromix 10, Tehtnica, Železniki, Slovenija);
- hladilna omara (Gorenje RK62FSY2, Gorenje d.d., Velenje, Slovenija);
- zamrzovalna omara (Gorenje ZOS3167C, Gorenje d.d., Velenje, Slovenija);
- globokozamrzovalna omara (SANYO MDF-U3386S, SANYO Electronic Co., Ltd., Japonska).

Reagenti

Za pripravo raztopin in vzorcev smo uporabili reagente in standarde čistosti, primerne za delo s plinsko kromatografijo (GC) (Priloga H). Delovne raztopine standardov za identifikacijo s koncentracijami 2 mg/ml smo pripravljali tedensko, raztopine standarda, ki smo ga uporabljali kot interni standard za kvantifikacijo maščobnih kislin, pa dnevno.

Statistična analiza

Pridobljene podatke smo obdelali in statistično analizirali z uporabo računalniškega programa Excel Microsoft Office 2010 in računalniškega programa za statistično analizo SPSS 21.0 (Statistical Package for Social Sciences). Uporabili smo naslednje statistične metode: metodo opisne statistike (srednja vrednost, standardni odklon, interval zaupanja, frekvenčna porazdelitev), Spearman's rho korelacijski koeficient, test ANOVA.

2.2.4.3 Rezultati

Odstotek identificiranih površin MEMK v HM je znašal $96,94 \pm 0,49\%$. Skupaj smo identificirali 31 maščobnih kislin, od tega 13 NMK, 7 ENMK in 11 VNMK (Priloga I).

Vsebnost DHK v zrelem HM je bila (srednja vrednost \pm S.D.) $0,26 \pm 0,15$ ut. %. Največji vpliv na vsebnost DHK v HM (padajoči vrstni red) ima:

- uživanje rib/izdelkov/sadežev + prehranskih dopolnil MD (rho = 0,600);
- uživanje rib/izdelkov/sadežev MD (rho = 0,406) in
- uživanje rib/dopolnil/sadežev + prehranskih dopolnil MN (rho = 0,385) (Preglednica 3).

Preglednica 3: Vsebnost DHK v zrelem HM v povezavi z uživanjem rib/izdelkov/sadežev in prehranskih dopolnil z DHK.

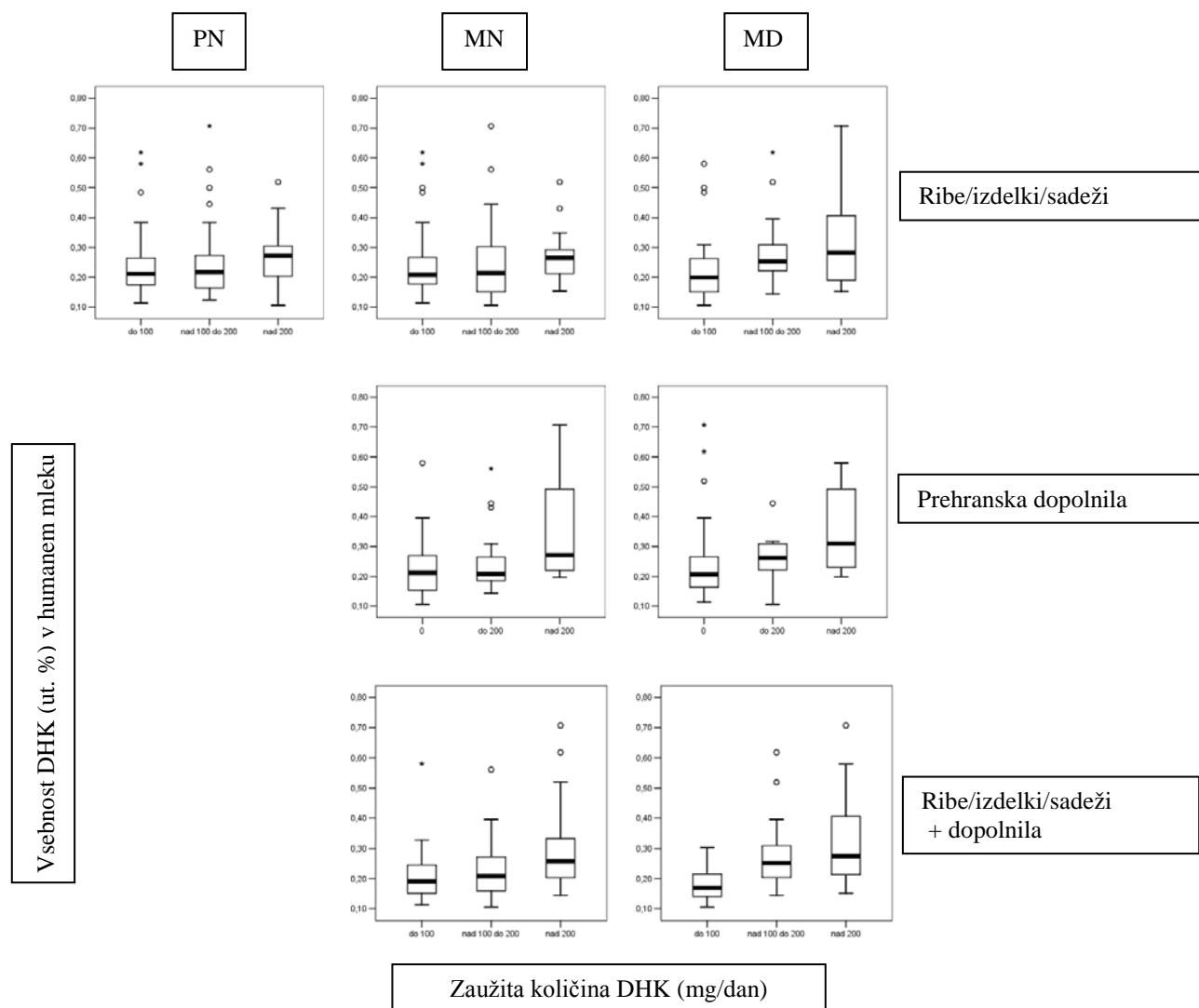
Table 3: DHK content in mature human milk in relation to fish, seafood and fish products and food supplements with DHK intake.

N = 101	Ribe/izdelki/sadeži		Prehranska dopolnila		Ribe/izdelki/sadeži + prehranska dopolnila	
	rho	P	rho	P	rho	P
PN	0,261	< 0,01				
MN	0,170	= 0,09	0,297	< 0,01	0,385	< 0,01
MD	0,406	< 0,01	0,377	< 0,01	0,600	< 0,01

Korelacija signifikanta pri stopnji 0,01 (2-tailed).

Ribe/izdelki/sadeži: rible, ribji izdelki in morski sadeži; Prehranska dopolnila z DHK PN – leto dni pred nosečnostjo; MN – med nosečnostjo; MD – med dojenjem (prvi mesec po porodu).

Vpliv na vsebnost DHK (ut. %) v HM je opazen, ko je vnos iz rib/izdelkov/sadežev nad 200 mg DHK/dan ($P < 0,05$), oziroma vpliv je še večji, če prištejemo še prispevek DHK iz prehranskih dopolnil ($P < 0,05$) (Slika 4).



Slika 4: Vsebnost DHK v HM (ut. %) v povezavi z uživanjem DHK iz rib/izdelkov/sadežev (ribe, ribji izdelki, morski sadeži) in prehranskih dopolnil z DHK (srednja vrednost \pm S.D. s prikazanimi osamelci) v času enega leta pred nosečnostjo (PN), med nosečnostjo (MN) in med prvim mesecem dojenja (MD) razdeljene v tri podskupine vnosa (≤ 100 , 100–200 in > 200 mg DHK/dan).

Figure 4: DHK content in mature human milk (wt. %) in relation to DHK intake from fish, fish products, seafoods and food supplements with DHK (median \pm S.D. with outliers) before pregnancy (PN), during pregnancy (MN) as well as during first month of lactation (MD) divided into three subgroups (≤ 100 , 100–200, and > 200 mg DHK/day).

3 RAZPRAVA

Predstavljena prehranska študija je prva tovrstna študija v Sloveniji (www.moje-mleko.si), v katero smo vključili nosečnice in doječe matere ter proučevali vpliv prehrane v krajšem (4PD 1 in 4PD 2) in daljšem časovnem obdobju (VPŽ1 in VPŽ2) na maščobno-kislinsko sestavo HM.

3.1 SOCIODEMOGRAFSKI, ZDRAVSTVENI IN ANTROPOMETRIČNI PODATKI

V kliničnem delu študije je sodelovalo 144 parov mati-otrok. Povprečna starost prostovoljk je bila 30,6 leta. Kot kažejo podatki Inštituta za varovanje zdravja Republike Slovenije (IVZ) iz leta 2010, je to za kar 0,4 leta višja povprečna starost nosečnic v Sloveniji (IVZ, 2012). To bi lahko pomenilo, da se trend naraščanja povprečne starosti nosečnic/mater še nadaljuje.

Podobno kot poroča IVZ za leto 2010, je tudi v našem primeru več kot polovica prostovoljk (60 %) otroke rodila prvič, in podobno kot leta 2010 dobra tretjina prostovoljk (40 %) drugič (IVZ, 2012). IVZ za leto 2010 po anamnestičnih podatkih poroča, da je v času nosečnosti kadilo 11,1 % nosečnic v Sloveniji. Kajenje povezujejo s stopnjo izobrazbe, in sicer največ kadilk naj bi bilo med manj izobraženimi nosečnicami (IVZ, 2012). V okviru naše študije ugotavljamo, da je kadilo 2 % prostovoljk (N = 3), ena je imela srednješolsko izobrazbo, preostali dve univerzitetno. Glede na izobrazbeno strukturo prostovoljk v primerjavi s podatki IVZ-ja za leto 2010 ugotavljamo, da naš vzorec ni reprezentativen, saj imamo v naši študiji 81 % prostovoljk z visoko ali višjo izobrazbo, medtem ko je bilo slovensko povprečje iz leta 2010 36,3 % (IVZ, 2012).

Glede na stan prostovoljk ugotavljamo, da jih 54 % živi v zunajzakonski skupnosti, 44 % je poročenih in 2 % je mater samohranilk. Po podatkih IVZ za vso Slovenijo je v zunajzakonski skupnosti v letu 2010 živilo 35,7 % parov, poročenih pa je bilo 39,5 % (IVZ, 2012).

Večina prostovoljk v naši študiji je eno leto po porodu dosegla svojo telesno maso pred zanositvijo, ne glede na začetno telesno maso (Slika 3). Poleg tega je naklon krivulj v vseh treh skupinah (ITM: < 18,5; od 18,5 do 25 in > 25) podoben.

V letu 2010 je bilo po podatkih IVZ v Sloveniji rojenih 48,4 % deklic in 51,6 % dečkov (IVZ, 2012). V naši študiji je bilo razmerje podobno, in sicer od 144 dojenčkov je bilo 46 % deklic in 54 % dečkov. Povprečna gestacijska starost za deklice je bila 39,2 tedna, za dečke pa 39,5 tedna. Dečki so se v povprečju rodili 3,5 dneva pozneje, prav tako so v povprečju težji od deklic (Slika 4).

Izklučno dojenih dojenčkov v prvem mesecu starosti je bilo 73 %, v prvih treh mesecih 68 %. Podatka o številu izključno dojenih dojenčkov v starosti šestih mesecev nimamo. Delno dojenih dojenčkov ob dopolnjenem 12. mesecu starosti je bilo 42 %. Trajanje dojenja ni bilo statistično značilno odvisno od starosti matere ($P = 0,66$), čeprav so starejše matere (nad 36 let) v naši študiji dojile svoje dojenčke dlje časa kot mlajše.

3.2 PREHRANSKI VNOS NA OSNOVI 4PD

Prehrano slovenskih nosečnic in doječih mater smo ugotavljali s 4PD. Ugotovili smo, da prehranski vnos ni optimalen za kar 25 % prostovoljk MN in 41 % prostovoljk MD. Vnos NMK in natrija (soli) je previsok, vnos LCP (predvsem DHK) in nekaterih vitaminov (folna kislina, vitamin D, vitamin E) ter mineralov (magnezij, železo, fluorid, jod) pa prenizek. Z neustreznim prehranskim vnosom imajo težave tako v deželah v razvijajočem se kot tudi razvitem svetu (Haileslassie in sod., 2013; Kim in sod., 2013; Ogechi, 2014; Vidal in sod., 2015). V zadnjem času največjo pozornost v času nosečnosti in dojenja posvečajo predvsem trem hranilom, in sicer vnosu DHK, železu in folni kislini.

3.2.1 Energijski vnos

Energijski vnos pri nosečnicah (2074 kcal/dan; razpon: 1121–3722 kcal/dan) in doječih materah (2126 kcal/dan; razpon: 1155–3918 kcal/dan) je bil glede na Referenčne vrednosti za vnos hranil (Referenčne vrednosti ..., 2004) (2555 in 2935 kcal/dan, za zmerno telesno aktivnost) prenizek in sicer 81 in 72 % (Preglednica 1). V literaturi obstaja nekaj študij iz drugih držav, ki so prišle do podobnih zaključkov glede energijskega vnosa nosečnih in doječih mater. V Seulu poročajo o energijskem vnosu med nosečnostjo 1872 in med dojenjem 1924 kcal/dan (Kim in sod., 2013). V Etiopiji so določili energijski vnos 2013 kcal/dan za doječe matere (Haileslassie in sod., 2013) in 2279 kcal/dan prav tako za doječe matere v Nigeriji (Ogechi, 2014). Neposredna primerjava teh podatkov ni mogoča, glede na to, da so bile uporabljene različne metode za določitev energijskega vnosa nosečnic in doječih mater. Vsekakor je pomemben vzrok, ki ga je treba upoštevati pri pravilni interpretaciji podatkov, tudi neustrezeno poročanje, navadno o prenizkih količinah in vrsti zaužite hrane, saj naj bi bilo, glede na podatke iz literature, kar 45 % nosečnic z nerealnim energijskim vnosom (McGowan in McAuliffe, 2012). Poleg tega smo v našem primeru za vse prostovoljke kot izhodiščno vrednost za energijski vnos vzeli vrednost energijskega vnosa pri zmerni telesni aktivnosti. Za natančnejšo oceno bi potrebovali podatek o telesni aktivnosti za vsako prostovoljko posebej. Da bi se izognili napačni interpretaciji podatkov in izgubi prostovoljk iz nabora zaradi neustreznega poročanja o bodisi prenizkem kot tudi previsokem energijskem vnosu, smo uporabili statistično utež, s katero smo glede na podan energijski vnos standardizirali vsa ostala proučevana hranila (Willett in Stampfer, 1986).

3.2.2 Vnos makrohranil in DHK

Razmerje med vnosom glavnih makrohranil (ogljikovi hidrati, mašcobe, beljakovine) je ustrezno.

Ogljikovi hidrati prispevajo 52 % energijskega vnosa pri nosečih in 51 % pri doječih materah. V času nosečnosti so skupni sladkorji predstavljeni 23 %, v času dojenja pa 19 % skupnega energijskega vnosa (Fidler Mis in sod., 2014).

Vnos prehranskih vlaknin je bil tako MN (25 g/dan) kot tudi MD (22 g/dan) nižji od priporočil (30 g/dan; SACN, 2015), kar je po vsej verjetnosti povezano s splošnim prepričanjem slovenskih doječih mater, da sveža zelenjava in sadje povzročata trebušne krče (kolike) dojenčkom, za kar pa ne obstaja znanstvenih osnov (Critch, 2011). Vnos prehranskih vlaknin je pri naših prostovoljkah MN in MD precej večji kot na Kitajskem (14,1 g prehranskih vlaknin/dan MN (Wang in sod., 2000) in 10,3 g prehranskih vlaknin/dan MD (Chen in sod., 2012)), v Seulu (21,4 g prehranskih vlaknin/dan MN in 22 g prehranskih vlaknin/dan MD (Kim in sod., 2013)) in v ZDA (17,8 g prehranskih vlaknin/dan MN (Tovar in sod., 2009)).

Delež energije iz maščob je ustrezен (nosečnice in doječe matere v primerjavi s priporočili) (33 in 34 % namesto 30-35 % (WHO/FAO, 2003; Referenčne vrednosti ..., 2004; Koletzko in sod., 2007; Koletzko in sod., 2008)), neustrezná je sestava maščob, saj zaužijejo previsok delež NMK (11,5 in 12,1 % namesto < 10 % (WHO/FAO, 2003; Referenčne vrednosti ..., 2004), a prenizek delež ENMK (8,6 in 8,1 % namesto ≥ 10 % (Referenčne vrednosti ..., 2004)) in VNMK (4,8 in 4,5 % namesto od 6 do 10 % (WHO/FAO, 2003) oziroma od 7 do 10 % (Referenčne vrednosti ..., 2004)).

Deleži energije iz maščob za nosečnice so različni ter zelo odvisni od prehranskih navad in okolja. Na Kitajskem (Wang in sod., 2000) in v ZDA (Tovar in sod., 2009) so deleži energije iz maščob pri nosečnicah naslednji (NMK, ENMK, VNMK): 13,7; 8,6; 10,2 % ter 11,2; 10,5 in 8,9 %.

Vnos holesterola (srednja vrednost ± S.D.) pri nosečnicah znaša 274 ± 366 mg/dan, pri doječih materah pa 295 ± 318 mg/dan. V povprečju niso presegle maksimalnega dopustnega vnosa (300 mg/dan) (WHO/FAO, 2003; Referenčne vrednosti ..., 2004).

Beljakovine prispevajo povprečno 15 % energijskega vnosa v obeh skupinah, kar je v skladu s priporočili (Referenčne vrednosti ..., 2004).

Alkohol prispeva povprečno 0,2 % energijskega vnosa pri nosečnicah in 0,3 % pri doječih materah, kar je previsoko, saj mednarodna priporočila med nosečnostjo in dojenjem odsvetujejo uživanje alkohola (IARD, 2015).

Vnos tekočine iz hrane in pijače je bil ustrezен tako MN kot MD. Nosečnice so v povprečju zaužile 3233 ± 1085 ml tekočine/dan od priporočenih 2700 ml/dan. MD so zaužile 3349 ± 1503 ml tekočine/dan od priporočenih 3100 ml/dan (Referenčne vrednosti ..., 2004). Vnos tekočine s pijačo pri naših prostovoljkah je podrobnejše raziskala in opisala Hribar s sod. (2015). Ugotovili so, da MN popijejo 406 ml in MD 403 ml pijač s sladkorjem/dan (definicija: Preglednica 1, c opomba) ter 1640 ml MN oziroma 1942 ml vode in mineralne vode/dan MD. Tako MN kot tudi MD je energijski vnos s tekočinami znašal 6,2 %, kar je za 24 % višje od skupne količine dovoljenega prostega sladkorja tako v tekoči kot tudi trdni oblici ($\leq 5\%$ celodnevnega energijskeva vnosa) (WHO/FAO, 2003; WHO, 2015).

DHK

Vnos DHK s prehrano znaša (srednja vrednost \pm S.D.) 92 ± 152 mg/dan MN in 108 ± 199 mg/dan MD, namesto priporočenih ≥ 200 mg DHK/dan (Koletzko in sod., 2007) (Preglednica 1). Skladno s priporočili ima zadosten prehranski vnos le 12 % nosečnic in 16 % doječih mater.

3.2.3 Vnos mikrohranil

Nosečnice in doječe matere s prehrano v povprečju niso dosegle priporočenih dnevnih potreb (Referenčne vrednosti ..., 2004) (% doseženega priporočenega dnevnega vnosa za noseče in doječe matere) za vnos: folne kisline (64 in 57 %), vitamina D (10 in 13 %) (German Nutrition Society, 2012), vitamina E (98 in 60 %), magnezija (nezadosten vnos samo MD, in sicer 88 %), železa (48 in 76 %), fluorida (10 in 6 %) ter joda (51 in 47 %). Vnos natrija je bil MN in MD višji od zgornje dopustne vrednosti (34 in 40 % nad priporočili (WHO/FAO, 2003)) (Preglednica 2), kar kaže na previsok vnos soli s prehrano.

Folna kislina (B_9)

Vnos folne kisline s prehrano MN znaša 386 ± 118 $\mu\text{g}/\text{dan}$ in MD 339 ± 99 $\mu\text{g}/\text{dan}$, kar je bistveno prenizek vnos od priporočenih 600 $\mu\text{g}/\text{dan}$ (Referenčne vrednosti ..., 2004) (Preglednica 2). S pomočjo anketnega vprašalnika smo pridobili podatek o uživanju folne kisline kot prehranskega dopolnila (Prilogi C in D). Ugotovili smo, da folno kislino v obliki prehranskih dopolnil MN uživa 39 % prostovoljk (Benedik in sod., 2013). V študiji, opravljeni leta 2010 v Sloveniji, v kateri je sodelovalo 259 prostovoljk, so ugotovili, da jih le 19 % uživa zadostne količine folne kisline (Antolič in sod., 2010). MN in MD so potrebe po folni kislini povečane, predvsem zaradi normalnih fizioloških sprememb organizma pri materi in optimalnega razvoja ter rasti plodu oziroma novorojenčka (Lamers, 2011). Zato folno kislino svetujejo uživati v obliki prehranskih dopolnil vsaj 4 mesece pred začetkom nosečnosti in z uživanjem nadaljevati med 1. tretjino nosečnosti (Referenčne vrednosti ..., 2004). V prejšnji pilotni študiji ($N = 69$) smo ugotovili, da uporaba prehranskih dopolnil med nosečnostjo in dojenjem sicer izboljša prehranski vnos hranil, a je 46,4 % preiskovank uvrščenih v skupino z revnim prehranskim statusom (zadovoljiv vnos dveh ali manj preiskovanih hranil). Poleg tega nobena od preiskovank ni doseglila priporočenega vnosa šestih ali več preiskovanih hranil (Puš in sod., 2013).

Vitamin D

Zaužile so premalo vitamina D ($2,0 \mu\text{g}/\text{dan}$ MN in $2,5 \mu\text{g}/\text{dan}$ MD, namesto $20,0 \mu\text{g}/\text{dan}$ (German Nutrition Society, 2012)). Pomanjkanje vitamina D v času nosečnosti in dojenja se povezuje z neželenimi zdravstvenimi posledicami pri nosečnici in doječi materi ter plodu oziroma otroku, predvsem pojavu zgodnje osteoporoze pri odraslih in rahitisa pri otrocih (Mulligan in sod., 2010; Dawodu in Wagner, 2012; Wagner in sod., 2012).

Železo

Vnos železa s prehrano MN znaša $14,5 \pm 7,4$ mg/dan od priporočenih 30 mg železa/dan MN, vnos železa s prehrano MD pa znaša $15,2 \pm 7$ mg/dan od priporočenih 20 mg železa/dan MD (Preglednica 2) (Referenčne vrednosti ..., 2004). Približno 75 % od vseh diagnosticiranih slabokrvnosti (anemij) MN je zaradi pomanjkanja železa (Goonewardene in sod., 2012). Prevalenca anemij zaradi pomanjkanja železa med nosečnicami v Sloveniji na podlagi reprezentativnih podatkov je bila 18,9 % v letu 2005 (McLean in sod., 2009) in 25,2 % v letu 2011 (Stevens in sod., 2013).

Fluorid

Vnos fluorida s prehrano pri MN je znašal 0,3 mg/dan in 0,2 mg/dan MD, kar je prenizko glede na priporočenih 3,1 mg/dan (Referenčne vrednosti ..., 2004)). V literaturi poročajo o podobni vsebnosti fluorida v prehrani nosečnic (0,35 mg/dan) (Maheshwari in sod., 1983). Fluorid je hranilo, ki ima pomembno vlogo pri preprečevanju nastajanja zognega kariesa (Maheshwari in sod., 1983).

Natrij

Prostovoljke so v povprečju zaužile preveč natrija. MN so zaužile 2684 ± 897 mg/dan in MD 2801 ± 972 mg/dan od priporočenih < 2000 mg natrija/dan. Študija iz leta 2009, ki je bila opravljena v Sloveniji in je temeljila na podlagi nakupov hrane gospodinjstev, je pokazala vnos 1860 mg natrija/dan, kar je sicer v skladu s priporočili, vendar je potrebno upoštevati, da so v študiji uporabili, manj natančno metodo za izračun skupnega natrija v prehrani (Hlastan Ribič in sod., 2014). Visok vnos natrija kaže na visok vnos soli v prehrani pri slovenskih nosečnicah in doječih materah, kar je povezano z visokim krvnim tlakom, ki ima neugoden vpliv na plod in otroka, tudi pozneje v življenju. Dokazano je, da imajo otroci mater, ki so s prehrano zaužile velike količine soli, povečano tveganje za težave z razvojem in funkcijo ledvic in uravnavanjem krvnega tlaka (Coimbra in sod., 2012).

Pre- in postnatalna prehranska podpora v Sloveniji

V Sloveniji obstajajo tako imenovane šole za starše (financira jih država), ki sicer niso obvezne, so pa priporočljive za vse nosečnice v zadnji tretjini nosečnosti in njihove partnerje. V okviru omenjene šole je tudi tematski sklop namenjen prehrani med nosečnostjo in dojenjem, ki ga je treba posodobiti in nadgraditi. Poleg šole za starše imamo v Sloveniji urejeno in dobro organizirano patronažno službo, ki nudi strokovno in praktično pomoč, tudi prehransko, na domu v času nosečnosti in prvo leto po porodu (Navodila ..., 1998). Kljub razmeroma dobri organiziranosti in dostopnosti služb za pre- in postnatalno podporo rezultati naše študije kažejo na neučinkovito implementacijo najnovejših prehranskih smernic v prakso, kar govori o tem, da bo treba spremeniti strategijo nacionalne prehranske politike v Sloveniji, v okvir katere sodi prehransko svetovanje nosečnicam in doječim materam, kar sta predlagali že Evropska komisija in Mednarodno društvo za proučevanje maščobnih kislin in maščob (Koletzko in sod., 2007; Koletzko in sod., 2008).

3.3 PREHRANSKE NAVADE

Po naših informacijah je to prva tovrstna študija, kjer ugotavljamo povezavo med prehranskimi navadami vnosa rib/izdelkov/sadežev in prehranskih dopolnil v treh obdobjih: PN (v tem obdobju nismo beležili vnosa DHK s prehranskimi dopolnili), MN in MD z vsebnostjo DHK v HM (Preglednica 3).

Korelacija med prehranskim vnosom DHK MN in MD ter vsebnostjo DHK v HM je v literaturi že opisana (Makrides in sod., 1996; Fidler in sod., 2000a; Dunstan in sod., 2007; Koletzko in sod., 2014), ni pa opisane korelacije med prehranskim vnosom DHK PN, MN in MD tako, kot smo to izvedli v naši študiji (Preglednica 3).

Ženske v rodnem obdobju naj bi zaužile vsaj 200 mg DHK/dan, kar dosežejo z eno do dvema porcijama morskih rib na teden, vključno z eno porcijo mastnih rib (Koletzko in sod., 2007; Koletzko in sod., 2008). V primerih, ko ženska ne uživa rib, naj bi zadosten vnos DHK zagotovila s prehranskimi dopolnili (ribje olje, druga prehranska dopolnila z DHK) (De Giuseppe in sod., 2014). V kliničnih študijah preizkušena zgornja še sprejemljiva količina prehranskega vnosa DHK je 1000 mg/dan (Koletzko in sod., 2007). Prehranska dopolnila z rastlinskim oljem niso dobri vir DHK, ampak ALK, ki pa ima nizek faktor pretvorbe v DHK, < 9 % pri ženskah, pri moških je faktor pretvorbe še bistveno nižji, < 1 % (DeFilippis in Sperling, 2006; Williams in Burdge, 2006; Abedi in Mohammad Ali, 2014). V naši študiji smo proučevali navade uživanja rib/izdelkov/sadežev ter prehranskih dopolnil. Znano je, da imajo lahko osebe s kroničnimi obolenji, kot so na primer alergije (Johansson in sod., 2011), ali osebe z različnimi genetskimi spremembami (polimorfizmi) v genih FADS1 in/ali FADS2, ki so povezane z n-6 in n-3 maščobnimi kislinami (Xie in Innis, 2008) nižjo vsebnost DHK v mleku kljub zadostnemu prehranskemu vnosu. Iz naše študije nismo izključili nobene prostovoljke, saj niso poročale o hujših kroničnih obolenjih, polimorfizmov pa nismo določali.

Iz VPŽ1 in VPŽ2, s katerima smo ugotavljali prehranske navade vnosa rib/izdelkov/sadežev in prehranskih dopolnil, smo določili povprečen dnevni vnos DHK (mg DHK/dan) (Benedik in sod., 2014). Ugotovili smo, da je prehranski vnos DHK iz rib/izdelkov/sadežev in prehranskih dopolnil MN in MD nižji od priporočenih vsaj 200 mg DHK/dan (Koletzko in sod., 2007; Koletzko in sod., 2008), razen MN, ko je prispevek DHK iz prehranskih dopolnil visok ($80,8 \pm 5,8$ mg DHK/dan). Prehranska dopolnila z DHK izboljšajo celoten vnos DHK, kar so potrdili tudi v nekaterih drugih študijah (Bergmann in sod., 2008; Delgado-Noguera in sod., 2010; Jia in sod., 2015). Povprečen dnevni celokupni vnos DHK (mg/dan), določen na podlagi VPŽ, je višji od celokupnega dnevnega vnosa, pridobljenega iz 4PD (92 ± 152 mg DHK/dan MN in 108 ± 199 mg DHK/dan MD), kar so ugotovili tudi v nekaterih drugih študijah (McNaughton in sod., 2007; Ingram in sod., 2012). Razlika med dobljenima vrednostma vnosa DHK s pomočjo VPŽ in 4PD je verjetno zaradi različnega obdobja spremeljanja prehrane (280 dni proti 4 dni MN ter 28 dni proti 4 dni MD). Poleg tega se lahko zgodi, da prostovoljka sicer uživa ribe/izdelke/sadeže, vendar jih pred in med štiridnevnim vodenjem 4PD ni zaužila. Določena napaka je bila narejena tudi zaradi nepopolnih podatkov o vsebnosti DHK za posamezne vrste rib v OPKP, tako smo vzeli povprečno vrednost vsebnosti DHK najbliže sorodnih rib. Seveda je treba poudariti še pomanjkljivost same VPŽ metode, saj gre za

pridobitev ocene uživanja rib, ki pa je zelo subjektivna. Prostovoljke, vključene v študijo, so verjetno precenile pogostost uživanja rib. Poleg tega je treba tudi upoštevati, da si verjetno niso zapomnile, koliko rib ter katero vrsto so zaužile. Iz tega razloga je treba poudariti, da z našim vprašalnikom (VPŽ) pridobimo samo približno informacijo o pogostosti uživanja rib in velikostih porcije, ne pa natančne vrednosti. Nasprotno, podatek o uživanju prehranskih dopolnil je bilo lažje pridobiti in je tudi bolj natančen, saj so bili poznani vrsta, količina in doziranje za vsako dopolnilo posebej, poleg tega pa se je prehransko dopolnilo uživalo redno, načeloma daljše časovno obdobje.

Analiza HM je pokazala, da je vsebnost DHK v zrelem HM (srednja vrednost \pm S.D.) $0,26 \pm 0,15$ ut. % (razpon: 0,09–1,34 ut. %), kar je nekoliko nižje kot v podatkih iz literature za Evropo, ZDA, Azijo in Afriko, kjer navajajo kot srednjo vrednost 0,32 ut. % (razpon: 0,06–1,4 ut. %) (Sanders, 1999; Brenna in sod., 2007).

V povprečju je zaužilo ≥ 1 porcijo rib/izdelkov/sadežev/teden (1 porcija definirana kot minimalen vnos rib/izdelkov/sadežev/teden tekom vseh treh obdobij in v povprečju znaša; PN = 203 g/teden, MN = 175 g/teden, MD = 182 g/teden) 68,9 % prostovoljk PN, 57,1 % MN in 62,3 % MD. Prehranska dopolnila je uživalo 35,9 % MN in 21,4 % MD. Miklavčič in sod. (2011) so opravili študijo na slovenskih nosečnicah, vendar na drugi skupini prostovoljk (N = 585; 513 iz ljubljanske regije z okolico ter 72 iz Izole, Kopra, Pirana, Idrije, in Kočevja), in ugotovili, da zaužijejo 25 g sladkovodnih in morskih rib ter ribjih izdelkov/dan. Po študiji Ministrstva za kmetijstvo gozdarstvo in prehrano Republik Slovenije iz leta 2008 na letni ravni odrasel slovenec v povprečju zaužije 9,1 kg sladkovodnih in morskih rib, kar je za 2,6 kg več kot leta 2004 (MKGPRS, 2008).

Na vsebnost DHK v HM ima največji vpliv skupen prehranski vnos DHK iz rib/izdelkov/sadežev in prehranskih dopolnil MD ($\rho = 0,600$; $P < 0,01$), sledi obdobje MN ($\rho = 0,385$; $P < 0,01$) ter obdobje PN (v tem obdobju podatka o prehranskih dopolnilih nismo beležili) ($\rho = 0,261$; $P < 0,01$) (Preglednica 3).

Vpliv prehranskih dopolnil z DHK na vsebnost DHK v HM je večji, če zaužijejo vsaj 200 mg DHK/dan (Slika 4). Rezultati so primerljivi s podatki iz literature, kjer so ugotavljali, kako prehranska dopolnila z DHK vplivajo na vsebnost DHK v HM (Boris in sod., 2004; Dunstan in sod., 2007; Bergmann in sod., 2008; Haugen in sod., 2008; Carlson, 2009; Imhoff-Kunsch in sod., 2011; Sherry in sod., 2015). Študija, ki so jo opravili Imhoff-Kunsch in sod. (2011) je pokazala, da dodatek 400 mg DHK/dan od sredine nosečnosti vse do prvega meseca po porodu vpliva na višjo vsebnost DHK v HM kot v kontrolni skupini ($0,20 \pm 0,06$ namesto $0,17 \pm 0,07$ ($P < 0,01$)). Do podobnih sklepov so prišli tudi v dansi študiji, kjer so prav tako prostovoljkam dodajali ribje olje od zadnje tretjine nosečnosti do meseca dni po porodu ter pri tem ugotovili, da se vsebnost DHK v HM zviša (1,4 ut. %), in sicer bolj, kot če bi jim dodajali ribje olje le MN (0,6 ut. %) (Boris in sod., 2004). Do podobnih sklepov so prišli tudi Dunstan in sod. (2007), ko so prostovoljkam dodajal ribje olje (2,2 g DHK/dan) od zadnje tretjine nosečnosti do 6. tedna po porodu ter ugotovili, da je ut. % DHK višji kot v kontrolni skupini ($0,42 \pm 0,2$ namesto $0,25 \pm 0,1$ ut. %) ($P < 0,001$).

V naši študiji je bila korelacija med vsebnostjo DHK v HM in uživanjem rib/izdelkov/sadežev PN nizka ($\rho = 0,261$; $P < 0,01$), kar je verjetno posledica siceršnjega nižjega uživanja rib med Slovenci (MKGPRS, 2008). Domnevamo, da se je posledično sorazmerno manj DHK vgradilo v maščobno tkivo ženske. Razpolovni čas DHK v maščobnem tkivu ženske je ocenjen na dve leti (Katan in sod., 1997), vendar je delež vgrajene DHK zelo nizek (0,1 % ali nižji) (Hodson in sod., 2008).

V naši študiji prehranska dopolnila z DHK uživa 35,9 % MN in 21,4 % MD, medtem ko na Norveškem 58,6 % nosečnic (Haugen in sod., 2008), na podeželju v Nemčiji 27,8 % v času nosečnosti in 16,8 % med dojenjem (Libuda in sod., 2014), v Muenchnu pa 41,8 % nosečnic (Becker in sod., 2011). Uporaba prehranskih dopolnil v naši študiji ni v korelacijski s starostjo ali izobrazbo prostovoljk, kar pa ne velja za prej omenjeni norveško in nemško študijo (Haugen in sod., 2008; Libuda in sod., 2014). Predvidevamo, da imamo premajhen vzorec za razlikovanje statističnih razlik med uporabo prehranskih dopolnil in izobrazbo, saj ima večina prostovoljk (81 %), vključenih v našo študijo, visokošolsko izobrazbo. Za primerjavo v norveški študiji je bilo vključenih 19,8 % prostovoljk z visokošolsko izobrazbo (Libuda in sod., 2014).

Prav tako ni bilo statistično značilne razlike med vnosom DHK iz rib/izdelkov/sadežev in geografskimi regijami, verjetno zato, ker je večina prostovoljk iz LJ. V študiji, kjer so raziskovali vnos rib v povezavi z vsebnostjo živega srebra v laseh otroka in matere, opravljeni v Sloveniji, so ugotovili nižji prehranski vnos rib na podeželju, kar je nekako pričakovano (Becker in sod., 2014; Castaño in sod., 2015).

Prednosti in slabosti študije

Ena od prednosti naše študije je, da smo vanjo vključili razmeroma veliko število prostovoljk in da smo imeli nizek osip (12 %). Poleg tega smo spremljali prehranski vnos MN in MD pri istih prostovoljkah. Vseeno je pri tem potrebna previdnost pri interpretaciji naših rezultatov na širšo populacijo, saj naš vzorec prostovoljk ni reprezentativen. Prostovoljke so v glavnem prihajale iz LJ in so bile više izobražene kot je izobrazbena struktura v Sloveniji (podiplomska: 17 %; visoka in višješolska: 64 %; srednješolska in poklicna: 19 %, osnovnošolska ali manj: 0 %). Izobrazbena struktura med rodnimi ženskami v Sloveniji je naslednja: podiplomska: 2,4 %; višješolska: 35,7 %; srednješolska: 52,9 %, osnovnošolska ali manj: 9 % (SURS, 2015). Stopnja izobrazbe in z njo povezani prihodki vplivajo na boljše prehranske navade posameznikov, kar je tudi opisano v literaturi (Rifas-Shiman in sod., 2009; McGowan in McAuliffe, 2012; Brunst in sod., 2014). Zato na podlagi omenjenih podatkov domnevamo, da je prehrana nosečnic in doječih mater v Sloveniji še slabša kot kažejo podatki v naši študiji.

Natančnost pri vodenju prehranskega dnevnika se lahko med prostovoljkami zelo razlikuje glede na različne dejavnike, kot sta izobrazba in socioekonomski status, ter nekatere druge lastnosti (nosečnost, materinstvo). Poleg tega lahko prostovoljka vodenje prehranskega dnevnika nekoliko prilagodi ali med vodenjem poenostavi svoje prehranske navade z namenom, da bo vodenje prehrane lažje.

3.4 SKLEPNE UGOTOVITVE

V doktorski disertaciji smo postavili več raziskovalnih hipotez z namenom, da ugotovimo, kakšno je stanje prehrane pri nosečnicah in doječih materah v Sloveniji.

Hipotezo 1, da je štiridnevni tehtan elektronski prehranski dnevnik (e-4PD) enakovreden ali celo boljši od papirnega štiridnevnega tehtanega prehranskega dnevnika (p-4PD), smo potrdili. Še več, naredili smo tudi primerjavo med e-4PD, ki temelji na Odprtih platformih za klinično prehrano (OPKP) ter med računalniškim programom (s-4PD) (Prodi 5.9 Exper Plus, Nutri-Science, Stuttgart, Germany, 2011) in ugotovili, da so rezultati ovrednotenja prehranskih dnevnikov s spletno aplikacijo in računalniškim programom v visoki korelacijski za energijsko vrednost in vsebnost makrohranil. Izkazalo se je, da bi lahko spletno aplikacijo OPKP rutinsko uporabljali v klinični praksi in za potrebe različnih populacijskih študij, saj bi pacienti/prostovoljci sami vodili svoj prehranski dnevnik, dietetik pa bi lahko na koncu samo še preveril vnos. S tem bi precej zmanjšali čas, ki ga sicer dietetik potrebuje za analizo prehranskega dnevnika. Poleg tega smo natančnost podajanja rezultatov pri OPKP tudi fizično testirali v laboratoriju, ko smo analizirali sestavo posameznih obrokov ter določili vsebnost energije, makro- in mikrohranil, na koncu pa še primerjali z izpisom iz OPKP.

Hipotezo 2, da prehrana slovenskih nosečnic in doječih mater odstopa od prehranskih priporočil, smo tudi potrdili. Namreč dokazali smo, da zaužijejo ustrezeno količino maščob, le razmerje med NMK, ENMK in VNMK ni ustrezeno. Zaužijejo preveč NMK in premalo VNMK, zlasti DHK. Zaužijejo tudi preveč soli in skupnih sladkorjev. Zaužijejo premalo folne kisline, vitaminov D in E, železa, fluorida ter joda. Rezultati naše študije niso reprezentativni, saj smo v njej imeli visok odstotek visoko izobraženih prostovoljk (79 %). Glede na to, da je izobrazba in z njo povezan prihodek pomemben dejavnik pri poseganju po bolj zdravih živilih, je verjetno splošna situacija v Sloveniji še precej slabša.

Hipotezo 3, da prehranske navade, zlasti uživanje rib/izdelkov/sadežev in prehranskih dopolnil v PN in MN, vplivajo na vsebnost LCP, zlasti DHK v HM, smo delno potrdili. Ugotovili smo namreč, da predvsem prehrana MD pomembno vpliva na vsebnost DHK v HM med tem ko prehrana PN nima velikega vpliva. Prehranska dopolnila, ki vsebujejo DHK, pomembno prispevajo k višji vsebnosti DHK v HM.

Z našo študijo smo dobili vpogled v prehranski vnos DHK s hrano in prehranskimi dopolnili ter prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil v povezavi z vsebnostjo DHK v HM. Prvič smo dobili tudi podrobnejši vpogled o uživanju prehranskih dopolnil med slovenskimi nosečnicami in doječimi materami. Dobljeni rezultati so osvetlili problematiko prehrane pri slovenskih nosečnicah in doječih materah ter so tako dobro izhodišče za ustvarjanje nove nacionalne prehranske politike za noseče in doječe matere. Poleg tega opravljeno delo predstavlja tudi dodatno referenco in izhodišče za nadaljnje raziskovanje na tem področju.

3.4.1 Kaj je novega za stroko?

Naša študija ima pomemben prispevek tudi za razvoj stroke na področju prehrane, predvsem na področju vodenja 4PD, saj smo ugotovili, da se p-4PD lahko nadomesti z e-4PD oziroma lahko prostovoljka samostojno vodi svoj e-4PD v aplikaciji OPKP ob predpostavki, da je računalniško dovolj usposobljena. Zadnje predstavlja velik časovni prihranek za dietetika, saj mu ni več treba fizično vnašati prehranskega dnevnika v OPKP ali Prodi ali kateri drug program, temveč ga lahko enostavno takoj pregleda in začne z ovrednotenjem v spletnem okolju OPKP. Ugotovili smo tudi, da je e-4DP večino primerljiv s s-4PD.

Na podlagi 4PD smo pridobili natančne podatke o prehrani prostovoljk MN in MD. Skupaj s 4PD in vprašalnikom VPŽ, v okviru katerega smo pridobili celovit vpogled v prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil že PN, MN in MD, smo določili vnos DHK (mg/dan) in povezali z maščobno-kislinsko sestavo HM.

4 SKLEPI

Na podlagi rezultatov doktorske disertacije lahko podamo naslednje sklepe:

- Kemijska analiza obroka je pokazala, da so tako energija kot druga makro- in mikrohranila v visoki korelaciji s podatki, ki jih poda OPKP.
- p-4PD se lahko nadomesti z e-4PD oziroma lahko prostovoljka samostojno vodi svoj e-4PD v aplikaciji OPKP ob predpostavki, da je računalniško dovolj usposobljena.
- Analize prehranskih dnevnikov z aplikacijo OPKP (e-4PD) ali računalniškim programom Prodi (s-4PD) so večinoma primerljive.
- Prehrana slovenskih nosečnic in doječih mater ni optimalna, zaužijejo preveč NMK, in soli, premalo pa DHK, vitaminov (B₉, D in E), železa, fluorida ter joda.
- Vnos DHK s hrano pri slovenskih nosečnicah in doječih materah ni zadosten. Ob upoštevanju vnosa DHK s prehranskimi dopolnilni je prehranski vnos z DHK zadosten samo MN.
- Prehranska dopolnila z DHK, predvsem MD, zelo prispevajo k vsebnosti DHK v HM.
- Vsebnost DHK v HM slovenskih doječih mater je primerljiva z vsebnostjo DHK v HM drugih študij, ki jo navajajo v literaturi (Brenna in sod., 2007).

5 POVZETEK (SUMMARY)

5.1 POVZETEK

Doktorska disertacija je del večje prospективne opazovalne klinične raziskave (Vloga materinega mleka v razvoju črevesne mikrobiote dojenčka, krajše Moje-mleko: www.moje-mleko.si), ki vključuje slovenske nosečnice in doječe matere ter njihove novorojenčke, usmerjene v ugotavljanje povezav med prehrano matere, vsebnostjo dolgoverižnih večkrat nenasicienih maščobnih kislin (LCP) in sestavo mikrobiote v humanem mleku (HM), otrokovo črevesno mikrobioto in zdravjem. Zdrava prehrana eno leto pred nosečnostjo (PN), med nosečnostjo (MN) in mesec dni po porodu (MD) pomembno vpliva na zdravje dojenčka in otroka vse v odraslo dobo. Nacionalnih podatkov o prehranskem vnosu v srednji Evropi, še posebej v Sloveniji, primanjkuje.

V študijo so bile vključene prostovoljke iz treh slovenskih regij: Primorske (Nova Gorica/Izola (NG/IZ)); Osrednjeslovenske (Ljubljana in okolica (LJ)) ter Štajerske (Maribor in okolica (MB)). Od decembra 2010 do oktobra 2012 je bilo v študiju vključenih 294 nosečnic. 174 prostovoljk, od tega 149 iz LJ z okolico, je sodelovalo tudi v kliničnem delu študije. Povprečna starost prostovoljk, ki so sodelovale v kliničnem delu študije, je bila $30,6 \pm 4,3$ leta. Prostovoljke so bile visoko izobražene, 79 % jih je imelo vsaj visokošolsko izobrazbo.

V okviru kliničnega dela študije smo s pomočjo anketnih vprašalnikov pridobili sociodemografske in antropometrične podatke o materah. Prav tako smo pridobili podatke o prehranskem vnosu s pomočjo 4-dnevnega tehtanega prehranskega dnevnika (4PD) za obdobji MN in MD ter podatke o prehranskih navadah s pomočjo vprašalnika o uživanju rib, ribjih izdelkov in morskih sadežev (rib/izdelkov/sadežev) ter prehranskih dopolnil (VPŽ) za vsa tri obdobja (PN, MN in MD). Opravljene so bile tudi antropometrične meritve ter odvzeti vzorci zrelega HM za določitev maščobno-kislinske sestave. Prostovoljke so vodile 4PD v papirni oblikih (p-4PD) oziroma, če so želele in jim je čas dopuščal, so lahko vzporedno vodile tudi spletno obliko 4PD (e-4PD), ki je prvi slovenski spletni prehranski dnevnik, ki smo ga želeli v naši študiji preizkusiti in evalvirati.

V prvem koraku smo preverili metodo ocene hranil z uporabo slovenske spletne aplikacije (OPKP; Odprta platforma za klinično prehrano, OPKP, <http://opkp.si>, Ljubljana, Slovenija). Ker je najbolj zaželena potrditev neposredna primerjava izračunanih vrednosti z vrednostmi, dobljenimi s kemijsko analizo živil, smo opravili kemijsko analizo in izračun za vzorčen niz dnevnih obrokov ($N = 20$) ter rezultate primerjali med seboj. Naslednja stopnja je bila oceniti uporabnost aplikacije OPKP pri uporabnikih, prostovoljkah. V povprečnih vsebnostih energije, celotne prehranske vlaknine, vode, makrohranil in izbranih mineralov ter elementov v sledovih Ca, Fe, Mg, Zn, Na, P, Cu ter I ni bilo statistično značilnih razlik. Opazili smo statistično pomembne razlike med povprečno izračunano in analitično vrednostjo selena ($P = 0,004$). Prostovoljke so za izpolnjevanje p-4PD porabile enako časa kot za izpolnjevanje e-4PD. Večina prostovoljk (75 %) je raje izpolnjevala e-4PD. Sklenili smo, da sta p-4PD in e-4PD primerljiva med seboj, da je e-4PD priročnejši

za uporabnika (dietetika ali prostovoljko) ter ima pomembne logistične in stroškovne prednosti.

V drugem koraku smo preverili, ali se lahko p-4PD nadomesti z uporabo e-4PD. Primerjali smo ujemanje različnih živil ($N = 1103$) iz p-4PD in e-4PD za energijo ter 48 hranilnih snovi, ki so jih vodile nosečnice prostovoljke iste štiri dni ($N = 16$). Raziskovalni dietetik je p-4PD vnesel v e-4PD. Ugotovili smo visoko korelacijo med p-4PD in e-4PD. Za 45 hranil ni bilo statistično pomembnih razlik, bile pa so za proste sladkorje ($P < 0,001$), alfa-linolensko ($P = 0,041$), folno ($P = 0,036$) in pantotensko kislino ($P = 0,023$). Sklenili smo, da sta p-4PD in e-4PD primerljiva med seboj.

V tretjem koraku smo izvedli primerjavo med e-4PD in s-4PD. p-4PD, ki so ga vodile nosečnice prostovoljke ($N = 63$), je izkušeni klinični dietetik vnesel v e-4PD in s-4PD. Rezultate za izračunani energijski vnos ter vnos 45 makro- in mikrohranil s pomočjo e-4PD in s-4PD smo statistično primerjali. Ugotovili smo, da ima visoko korelacijo ($> 0,800$) med metodama 12 prehranskih parametrov (energija, ogljikovi hidrati, skupne maščobe, beljakovine, voda, kalij, kalcij, fosfor, skupna prehranska vlaknina, vitamin C, folna kislina in stearinska kislina) zmerno (od 0,600 do 0,799) za 18 hranil, šibko (od 0,400 do 0,599) za 11 hranil, medtem ko za 5 hranil ni bilo korelacije (arahidonska kislina, niacin, alfa-linolenska kislina, fluor, skupni sladkorji). Sklenili smo, da so rezultati ovrednotenja prehranskih dnevnikov z e-4PD in s-4PD v visoki korelaciji za energijsko vrednost in vsebnost makrohranil. Na podlagi rezultatov teh treh korakov smo se odločili za uporabo aplikacije OPKP pri nadaljnji analizi in vrednotenju p-4PD.

Trenutno prehrano prostovoljk ($N = 174$) smo spremljali z metodo 4PD dvakrat, in sicer MN in MD.

Vnos energije in hranil smo primerjali z Referenčnimi vrednostmi za vnos hranil (Referenčne vrednosti ..., 2004; German Nutrition Society, 2012), WHO/FAO priporočili (WHO/FAO, 2003; WHO, 2015) ter priporočilom Evropske komisije in Mednarodnega društva za proučevanje maščobnih kislin in maščob (Koletzko in sod., 2007; Koletzko in sod., 2008). Energijski vnos pri nosečnicah (2074 kcal/dan) in doječih materah (2126 kcal/dan) je bil prenizek glede na Referenčne vrednosti za vnos hranil (Referenčne vrednosti ..., 2004). Ženske zaužijejo prevelik delež NMK (energijski vnos (%)): 11,5 % MN in 12,1 % MD namesto $< 10\%$ (WHO/FAO, 2003; Referenčne vrednosti ..., 2004), vendar prenizek delež ENMK (8,6 in 8,1 % namesto $\geq 10\%$ (Referenčne vrednosti ..., 2004)) in VNMK (4,8 in 4,5 % namesto od 6 do 10 % (WHO/FAO, 2003) ozziroma od 7 do 10 % (Referenčne vrednosti ..., 2004)). Vnos dokozaheksajenojske kislune (DHK) je prav tako prenizek (srednja vrednost \pm S.D.): (MN: 92 ± 152 , MD: 108 ± 199 namesto ≥ 200 mg/dan) in dosega le 46 % priporočene vrednosti (Koletzko in sod., 2007; Koletzko in sod., 2008) MN in za 54 % MD. Prav tako so zaužile velike količine prostih sladkorjev (119 ± 24 g/dan ($23 \pm 6\%$ energijskega vnosa) MN in 104 ± 28 g/dan ($19 \pm 5\%$ energijskega vnosa) MD), natrija (MN: 34 %, MD: 40 % nad priporočili (WHO/FAO, 2003)). Med mikrohranili prihaja do prenizkega vnosa folne kislune ($\mu\text{g}/\text{dan}$) (MN: 386 ± 118 , MD: 339 ± 99 namesto 600 $\mu\text{g}/\text{dan}$), vitamina D ($\mu\text{g}/\text{dan}$) (MN: 2 ± 2 , MD: $2,5 \pm 2,5$ namesto 20 $\mu\text{g}/\text{dan}$), vitamina E (mg/dan) (MN: $12,8 \pm 5,3$, MD: $10,2 \pm 4,3$ namesto 13 mg/dan MN in 17 mg/dan MD), železa (MN: $14,5 \pm 7,4$, MD: $15,2 \pm 7$ namesto 30 mg/dan

MN in 20 mg/dan MD), fluorida (MN: $0,3 \pm 0,3$, MD: $0,2 \pm 0,1$ namesto 3,1 mg/dan) ter joda (MN: $101,1 \pm 44,1$; MD: $93,3 \pm 49,1$ namesto od 200 do 230 μg /dan MN in od 200 do 260 μg /dan MD). Ugotovili smo, da prehrana slovenskih nosečnic in doječih mater ni optimalna, zaužijejo NMK, skupnega sladkorja in soli, premalo pa LCP (zlasti DHK), folne kisline, vitaminov D in E, železa, fluorida ter joda.

Kot smo že omenili, smo z VPŽ proučevali prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil v vseh treh obdobjih (PN, MN, MD). V HM smo s pomočjo plinske kromatografije s plamenskim ionizacijskim detektorjem (GC-FID) identificirali skupaj 31 maščobnih kislin, vključno z DHK. Skupaj je bila v študijo vključena 101 prostovoljka.

Določili smo korelacijo med vnosom DHK, izračunanim na podlagi prehranskih navad uživanja rib/izdelkov/sadežev, za vsa tri opazovana obdobja: PN, MN in MD. V povprečju je zaužilo ≥ 1 porcijo rib/izdelkov/sadežev/eden (25 g/dab) 68,9 % prostovoljk PN, 57,1 % MN in 62,3 % MD. Prehranska dopolnila je uživalo 35,9 % MN in 21,4 % (za obdobje PN nismo spraševali po uporabi prehranskih dopolnil).

Vnos DHK (mg DHK/dan; povprečje \pm S.E.) iz rib/izdelkov/sadežev in prehranskih dopolnil za vsa tri proučevana obdobja; PN ($194 \pm 11,2$ mg DHK/dan iz rib/izdelkov/sadežev), MN (246 ± 8 mg DHK/dan, od tega $165,4 \pm 10,0$ mg DHK/dan iz rib/izdelkov/sadežev in $80,8 \pm 5,8$ mg DHK/dan iz prehranskih dopolnil) ter MD (191 ± 7 mg DHK/dan, od tega $149,5 \pm 9,4$ mg DHK/dan iz rib/izdelkov/sadežev ter $42,2 \pm 3,8$ mg DHK/dan iz prehranskih dopolnil). Vnos DHK iz rib/izdelkov/sadežev in prehranskih dopolnil ni bil v korelaciji niti z izobrazbo, geografsko regijo niti s starostjo prostovoljke (velja za vsa tri opazovana obdobja: PN, MN, MD). Vsebnost DHK v zrelem HM je bila (srednja vrednost \pm S.D.) $0,26 \pm 0,15$ ut. % ter je odvisna predvsem od prehranskih navad uživanja rib/izdelkov/sadežev in prehranskih dopolnil MD ($\rho = 0,600$, $P < 0,01$).

Povprečen vnos DHK iz prehranskih navad uživanja rib/izdelkov/sadežev je v vseh treh obdobjih nižji od priporočenega (vsaj 200 mg DHK/dan; (Koletzko in sod., 2007; Koletzko in sod., 2008)). Vnos DHK se je zelo izboljšal zaradi uživanja prehranskih dopolnil z DHK. Ob sočasnem upoštevanju prehranskega vnosa DHK s prehranskimi navadami uživanja rib/izdelkov/sadežev in prehranskih dopolnil prostovoljke MN dosežejo priporočeni dnevni vnos DHK (246 mg/dan). Ženske v rodni dobi naj se spodbuja na nacionalni ravni, da bodo imele zadosten prehranski vnos DHK (vsaj 200 mg DHK/dan), kar dosežejo z eno do dvema porcijama morskih rib na teden, vključno z eno porcijo mastnih rib (Koletzko in sod., 2007; Koletzko in sod., 2008).

Z našo študijo smo dobili vpogled v prehranski vnos DHK s hrano in prehranskimi dopolnili ter prehranske navade uživanja rib/izdelkov/sadežev in prehranskih dopolnil v povezavi z vsebnostjo DHK v HM. Dobljeni rezultati so osvetlili problematiko prehrane pri slovenskih nosečnicah in doječih materah ter so lahko dobro izhodišče za izboljšanje prehrane na nacionalni ravni.

5.2 SUMMARY

This PhD thesis is a part of a wider prospective observational clinical study on Slovenian pregnant and lactating women and their infants, focused on the establishment of linkage among the mother's diet, human milk (HM) long-chain polyunsaturated fatty acids (LCP) and microbiota composition, the child's gut microbiota and health (The role of human milk in the development of a breast fed child's intestinal microbiota, in short the "My-Milk" study available at: www.moje-mleko.si/en). Proper nutrition one year before pregnancy (PN), during pregnancy (MN) and during the first month after birth (MD) plays an important role in maternal health and fetal growth and development. A nationally representative dietary intake in Central Europe, especially in Slovenia, is lacking.

In the "My-Milk" study, volunteers ($N = 294$) from three Slovenian regions: NG/IZ – Nova Gorica/Izola (coastal/Mediterranean region); LJ – Ljubljana (central region); MB – Maribor (western edge of the Podravje region, close to Austria) were recruited into the "My-Milk" study from December 2010 to October 2012, of which 174 participated in the clinical part of the study. The average age of the volunteers included in the clinical part of the study was 30.6 ± 4.3 years. The volunteers were highly educated; 79 % had at least a university degree and they came mainly from the capital of Slovenia (LJ; $N = 149$).

In the clinical part of the study, socio-demographic and anthropometric data on mothers were collected by questionnaires, dietary intake by 4-day weighted dietary record (4PD) for MN and MD was assessed and dietary habits of fish, fish products and seafood (fish/products/seafood) by adopted food frequency questionnaire (VPŽ), and dietary supplements consumption was assessed for all three periods: PN, MN and MD. Anthropometrical measurements were also performed and mature human milk samples were collected for fatty acids analysis. The volunteers reported the dietary intake on paper-based 4PD (p-4PD) and, if they had time and were willing, also on web-based 4PD (e-4PD). e-4PD is the first Slovenian web-based dietary record, which we wanted to test and evaluate.

We first validated the nutrient estimation method applied using the Slovenian Open Platform for Clinical Nutrition (OPKP; www.opkp.si/en). Because the most desirable validation is direct comparison of the calculated values with the values obtained from a chemical analysis of the same food, we performed a chemical analysis and a calculation for a representative set of daily meals ($N=20$) and compared the data. The secondary aim was to evaluate the usability of OPEN for dietary assessment by the volunteers. We found no statistically significant differences in the mean contents of energy, total dietary fiber, water, macronutrients and selected essential minerals: Ca, Fe, Mg, Zn, Na, P, Cu and I. There was a difference in the mean calculated and analytical values of selenium ($P=0.004$). Volunteers found p-4PD equally time-consuming as e-4PD. The majority of the volunteers (75%) preferred e-4PD. We conclude that e-4PD is a useful and cost effective tool for both dieticians and volunteers.

Secondly, we compared p-4PD to e-4PD. We compared the matching of different food items ($N=1.103$) from p-4PD and e-4PD for energy and 48 nutrients among 16 pregnant volunteers, with 4PDs for the same individuals, matched for the same four days. p-4PD

were coded into e-4PD independently by the same research dietitian. We noted a high correlation between p-4PD and e-4PD. There were statistically insignificant differences for 45 nutrients, but not for free sugars ($P<0.001$), alpha-linolenic acid ($P=0.041$), folate ($P=0.036$) and pantothenic acid ($P=0.023$). We conclude that p-4PD and e-4PD are comparable methods.

Thirdly, we compared e-4PD with s-4PD. s-4PD is widely used in Slovenian clinical practice. A volunteer group of pregnant women ($N=63$) completed p-4PDs. These records were entered by an experienced research dietitian into e-4PD and s-4PD. The results for calculated energy intake, as well as 45 macro- and micronutrient intakes, were statistically compared. Twelve nutritional parameters (energy, carbohydrates, fat, protein, water, potassium, calcium, phosphorus, dietary fiber, vitamin C, folic acid and stearic acid) were in high correlation (>0.800), 18 in moderate (0.600-0.799) and 11 in weak correlation (0.400-0.599), while 5 (arachidonic acid, niacin, alpha-linolenic acid, flour, total sugars) did not show any statistical correlation. We conclude that e-4PD and s-4PD are in high correlation for energy and macronutrient content.

Based on these results, we decided to use OPKP for further analysis of 4PD, into which all p-4PD were entered and evaluated.

The diet of the volunteers ($N=174$) was twice assessed by 4PD: MN and MD. Dietary intake was calculated and compared with the Central European (D-German, A-Austrian, CH-Swiss, D-A-CH) recommendations (Reference values ..., 2004; German Nutrition Society, 2012), World Health Organization/Food and Agriculture Organization recommendations (WHO/FAO, 2003; WHO, 2015) as well as with the European Commission with the International Society for the Study of Fatty Acids and Lipids (Koletzko et al, 2007; Koletzko et al, 2008). We found that energy intake was below D-A-CH recommendations (Reference values ..., 2004) (MN: 2.074 ± 377 kcal/day, MD: 2.126 ± 501 kcal/day). The women consumed excessive amounts of saturated fatty acids (NMK) (energy intake (%)): 11.5 % MN and 12.1 % MD vs. recommended <10 %, and an insufficient amount of monounsaturated fatty acids (ENMK) (8.6 % MN and 8.1 % MD vs. ≥ 10 % (Reference values..., 2004)) and polyunsaturated fatty acids (VNMK) (4.8 % MN and 4.5 % MD vs. 6-10 % (WHO/FAO., 2003) or 7-10 % (Reference values ..., 2004)). Docosahexaenoic acid (DHK) intake (median \pm S.D.) was also insufficient: (MN: 92 ± 152 , MD: 108 ± 199 vs. recommended ≥ 200 mg/day) (Koletzko et al., 2007; Koletzko et al, 2008). They also consumed excessive amounts of total sugars (119 ± 24 g/day MN and 104 ± 28 g/day MD) and sodium (MN: 34 %, MD: 40 % of WHO/FAO upper limit) (WHO/FAO, 2003; WHO, 2015). Among micronutrients, compared to D-A-CH recommendations (Reference values ..., 2004; German Nutrition Society, 2012), insufficient intakes of folate (MN: 386 ± 118 , MD: 339 ± 99 vs. recommended 600 $\mu\text{g}/\text{day}$), vitamin D (MN: 2.0 ± 2.0 , MD: 2.5 ± 2.5 vs. 20 $\mu\text{g}/\text{day}$), vitamin E (MN: 12.8 ± 5.3 , MD: 10.2 ± 4.3 vs. 13 mg/day MN and 17 mg/day MD), iron (MN: 14.5 ± 7.4 , MD: 15.2 ± 7.0 vs. 30 mg/day MN and 20 mg/day MD), fluoride (MN: 0.3 ± 0.3 , MD: 0.2 ± 0.1 vs. 3.1 mg/day) and iodine (MN: 101.1 ± 44.1 , MD: 93.3 ± 49.1 vs. 200-230 $\mu\text{g}/\text{day}$ MN and 200-260 $\mu\text{g}/\text{day}$ MD) were reported. We conclude that the diet of Slovenian pregnant and lactating women is not optimal. It is characterized by excessive intake of NMK, total

sugars and salt and deficient intake of LCP (especially DHK), folic acid, vitamin D, vitamin E, iron, fluoride and iodine.

As mentioned, an adapted VPŽ method was used to determine DHK intake from habitual fish/products/seafood as well as supplements intake, assessed through three periods: PN, MN and MD. Fatty acid composition (of 31 fatty acids) of HM at one month *post-partum*, especially the content of DHK, was assessed by gas chromatography with a flame ionization detector (GC-FID). One hundred and one volunteers were included in this part of the study. A correlation of dietary DHK and its content in HM was determined in all three periods (PN, MN, MD). The consumption of ≥ 1 fish/products/seafood meal (25 g/day) was reported by 68.9 % of volunteers PN, 57.1 % MN and 62.3 % MD. The intake of supplements was reported by 35.9 % MN and 21.4 % MD (we did not ask the volunteers to report the use of supplements for the period PN). Taking fish/products/seafood and supplementation into account, the DHK intake was (mean \pm S.E.) 194.9 ± 11.2 mg/day PN (only fish/products/seafood), 246.2 ± 11.5 mg /day (165.4 ± 10.0 mg DHK/day from fish/products/seafood and 80.8 ± 5.8 mg DHK/day from supplements) MN and 191.7 ± 9.8 mg /day (149.5 ± 9.4 mg DHK/day from fish/products/seafood and 42.2 ± 3.8 mg DHK/day from supplements) MD. DHK intake from fish/products/seafood PN, MN and MD was not correlated with education, geographical region or age; the same was noted for supplements. The (median \pm S.D.) DHK content in HM was 0.26 ± 0.15 wt. %, respectively. DHK in HM was in positive, dose-response correlation with maternal fish/products/seafood and supplements consumption MD ($\rho=0.600$, $p<0.01$). We conclude that in all three periods, women on average failed to reach the recommended DHK intake with the reported habitual fish/products/seafood intake. The recommended DHK intake (above 200 mg DHK/day) was reached only MN on account of fish/products/seafood and supplements intake together (246 mg DHK/day). Encouragement on a national level to increase the dietary intake of fish to one to two portions of sea fish per week, including oily fish, during all three periods (PN, MN and MD), would be of benefit.

Our study gives an insight into the dietary intake of DHK from food and supplements, as well as from dietary habits of fish/products/seafood and supplements consumption in relation to its DHK content in HM. The results shed light on the inadequate nutrition of Slovenian pregnant and lactating women and this is a starting point for targeted actions to improve nutrition on a national level.

6 VIRI

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Velika zahvala gre tudi **sodelavcem Kliničnega oddelka za gastroenterologijo, hepatologijo in nutricionistiko** in sicer predstojniku oddelka prof. dr. Roku Orlu, glavni medicinski sestri oddelka Eriki Šmid, zdravnikom specialistom prim. Marjeti Sedmak, doc. dr. Matjažu Homanu, asist. mag. Darji Urlep Žuzej, asist. mag. Jerneju Breclju in asist. Tini Kamhi Trop ter diplomiranim medicinskim sestrar Andreji Lajhar, Klavdiji Medja, Maji Primec, Nini Slemenšek, Aniti Smajlović in zdravstvenim tehnikom Simoni Dobravec, Urošu Gorjupu, Dariu Havoju, Nini Ivančič, Nataši Kaisersberger, Bojani Kalan, Mojci Kranjc, Denisu Lukanciču, Mateji Mohar, Jeleni Petrošanec, Nataši Podlogar, Renati Salamon, Ireni Šivic, Vesni Sprajcar, Dragici Šramel Stefanović, Metki Zajc in Štefki Žvokelj. Zahvalil bi se rad tudi psihologinji Valentini Kralj Stefanova, vzgojiteljici Sandri Ban, tajnici Marjetki Košir in administratorki Darji Strnad.

Velika zahvala gre tudi **sodelavcem Kliničnega oddelka za neonatologijo** in sicer predstojnici oddelka prof. dr. Darji Paro Panjan, glavni medicinski sestri oddelka Ani Galič, zdravnikom specialistom asist. dr. Borutu Brataniču, doc. dr. Petji Fister, Aneti Soltirovska Šalamon, Jani Krivec in Gregorju Nosanu ter diplomiranim medicinskim sestrar Janji Gržinić, Albini Gubanc, Nives Jesenko, Arneli Karabegović, Martini Lekan, Marijani Pranjić, Regini Šstrukelj in zdravstvenim tehnikom Nini Ambrož, Adrijani Borovac, Zdenki Cigoj, Almi Hodžić, Ramzi Kurtović, Marjeti Klemenc, Rebeki Kubik, Sanji Milovanović, Nini Palčič, Aniti RadićSimoni Švigelj, Anji Tancek, Fehriji Žilić, Urški Mošnik, Edini Sitnić in Barbari Pintar Jazbec.

Zahvala gre tudi **sodelavcem iz Službe za dietoterapijo in bolniško prehrano** in sicer vodji službe Andreji Širca Čampa ter kliničnima dietetikoma Heleni Kobe in Tomažu Poredošu. Zahvala gre tudi vodji kuhinje Ljubici Barič ter dietnim kuharjem Dragici Češnovar, Majdi Anžlovar, Dominiki Jovanovič, Borisu Cimpricu, Lidiji Đorđević, Nadi Mudrič in Zlati Šokec ter kuharjem Žigi Dobrotinšku, Gordani Ofak, Anici Urban, Refiki Durakovič, Miri Subotić, Ivanki Dakič, Semiri Rošić ter kuharjem mlečne kuhinje Mariji Novak, Tijani Vrabičić, Darji Planinšek in Nadi Trebec ter kuharskim pomočnikom Slobodanki Milošević, Jadranki Erjavec, Luku Rozmanu, Mariji Klišanin, Borisu Žontarju, Semiri Ponjević, Milanki Lazič, Đurđici Mesarič, Suzani Jesenček, Zorici Kuzmič in Halidi Bukvar. Zahvala tudi kuhinjskemu ekonomistu Tomotu Bejuku in kuhinjskemu skladniščniku Stanetu Kuzmiču.

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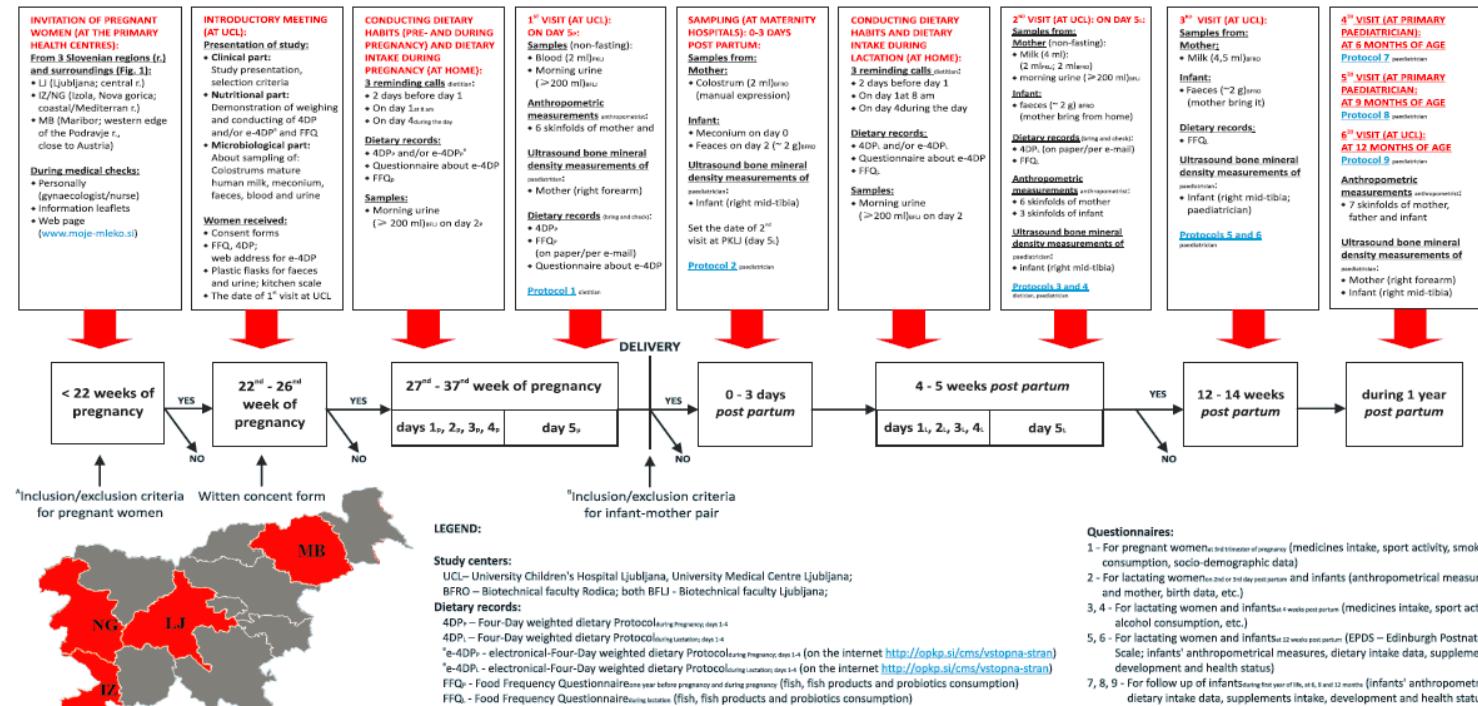
Iz srca se zahvaljujem tudi vsem drugim, s katerimi sem sodeloval v času svojega raziskovalnega dela na Pediatrični kliniki UKC Ljubljana in Biotehniški fakulteti, Oddelku za živilstvo in Oddelku za zootehniko.

NAJLEPŠA HVALA VSEM!

PRILOGE

PRILOGA A: Časovni potek študije Moje-mleko (Bogovič Matijašić in sod., 2014)

ANNEX A: Time frame of »My-Milk« study (Bogovič Matijašić et al., 2014)



^aInclusion criteria:

Otherwise healthy pregnant women, plan to fully breastfeed their baby for at least 6 weeks, willingness to write a 4DP twice: during pregnancy and again at 4 weeks post partum (one day should be Saturday or Sunday).

^aExclusion criteria:

Autoimmune chronic disease, acute and chronic infections, increased risk of premature delivery.

^bInclusion criteria:

Full-term, otherwise healthy, fully or partially breast-fed infants (≥ 37 gest. weeks, with normal gestation mass)

^bExclusion criteria:

Known metabolic or gastrointestinal disorders of infant, acute infectious disease, sickness of mother or infant during the study.



PRILOGA B: 4-dnevni tehtani prehranski dnevnik
ANNEX B: 4-day weighted dietary record



Pediatrična klinika
Dietna posvetovalnica
doc. dr. Nataša Fidler Mis, univ. dipl. inž.
Helena Kobe, univ. dipl. inž.
Bohoriceva 20
1525 Ljubljana

PREHRANSKI DNEVNIK ZA NOSEČNICE / DOJEČE MATERE

Št. preiskovanje:	Kraj in datum:
--------------------------	-----------------------

Prosiva, da prehranski dnevnik vodite **štiri dni**. Prehranujte se enako kot običajno. Vpišite datum in dan v tednu. Za en dan lahko uporabite več kot eno stran. Pišite čim bolj čitljivo. Najbolj natančno lahko vodite dnevnik, če se prehranujete doma. Natančneje kot boste vodili prehranski dnevnik, bolj natančno bova lahko ocenili vaš vnos energije in hranil.

1. stolpec Ura: Vsakokrat, ko kaj pojeste ali popijete, vpišite v 1. stolpec čas (npr. 8:00). Po možnosti zapisujte sproti. Prehranski dnevnik imejte vedno pri sebi.

2. ter 3. stolpec: Količina ter Živila/jedi, pijače: Vpisujte tehtane oz. merjene količine:

- živil: **g** = gram; **dag** = dekagram; **ČŽ** = čajna žlička; **JŽ** = jedilna žlica,
- pijač: **ml** = mililiter; **dl** = deciliter.

Potrebujete gospodinjsko tehtnico in posodo z oznakami volumna. V primeru, da živila niste uspeli stehtati, lahko izjemoma napišete velikost porcije (npr. 2 kosa kruha, 1 kos torte z 1 žlico smetane).

Natančno beležite vse kar zaužijete in popijete (hrano, pijačo in prehranske dodatke):

- dodatek soli, kisa, začimb, dišavnic (npr. 0,5 g morske soli; 1 list sveže bazilike);
- živila s katerimi obogatite jedi (npr. 1 ČŽ sladkorja, 1 JŽ mleka v prahu, 10 g naribanega parmezana, 1 JŽ kisle sметane);
- ali ste živila **tehtali surova** ali **kuhana** (npr. 100 g kuhanih testenin) ter **način priprave živila** (npr. losos na žaru, pečen krompir);
- **sestavo sendviča** ali **recept jedi** (npr. pravilno: 60 g bela žemlja, 10 g gauda sir, 3 g maslo, 10 g kuhan pršut, 7 g kisle kumarice; nepravilno: 90 g sendvič);
- **ime živila oz. napitka ter proizvajaleca** (npr. piščančja hrenovka, Perutnina Ptuj; jabolčni nektar, Fructal);
- **vrsto mesa** in ali ste ga tehtali skupaj s kostmi ali brez (npr. piščančje bedro tehtano brez kosti, pojedel brez kože); ime sadja in ali ste ga tehtali z lupino ali brez (npr. celo jabolko z olupkom)
- **količino in vrsto maščobe** (npr. 3 ml olivenega olja pri dušenju rezeka). Pri solati odštejte olje, ki ostane v posodi (npr. dodate 5 ml sojinega olja, v posodi ostane 2 ml; v dnevnik zapišite 3 ml sojinega olja). Napišite vsebnost maščobe v izdelkih (npr. navadni jogurt s 3,2 % mlečne maščobe; kranjska klobasa z 22 g maščob na 100 g izdelka);
- pri napitkih izmerite volumen soka in vode ločeno (npr. 1 dl 100 % jabolčnega soka in 2 dl vode).

Primer pravilno izpolnjenega prehranskega dnevnika (del dnevnika)

Ura	Količna	Živila / jedi , pijače
7:30	200 ml	Mleko s 3,2 % mlečne maščobe, Ljubljanske mlekarne
	100 g	Beli pšenični kruh (Žito Ljubljana)
	40 g	Sirni namaz s tuno s 25 g maščob/100 g izdelka (Ljubljanske mlekarne)
	70 g	Zelena paprika (sveža)
9:45	100 g	Actimel Jogurtov napitek z 1,6 % mlečne maščobe, okus: navadni (Danone)
	60 g	Graham žemljica (pekarna Grosuplje)
	120 g	Sveže jabolko
12:20	150 ml	Goveja juha z jušno zakuhom (60 g zakuh v liter juhe)
	50 g	Piščančje bedro (dušeno, tehtano brez kosti; zaužil brez kože)

Prehranski dnevnik

1. dan: _____

Prehranski dnevnik

2. dan: _____

Prehranski dnevnik

3. dan: _____

Prehranski dnevnik

4. dan: _____

PRILOGA C: Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil pred in med nosečnostjo (VPŽ1)

ANNEX C: Food frequency questionnaire for period one year before pregnancy and during pregnancy for fish, fish products and food supplements consumption (VPŽ1)



Št. preiskovanje: Kraj in datum: _____

Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil pred in med nosečnostjo

Zahvaljujemo se vam, da ste si vzeli čas za sodelovanje v anketi. Vprašalnik nam bo dal informacije o vaših prehranskih navadah zgoraj naštetih živil. Prosimo vas, da odgovarjate povsem iskreno.



velika srednja mala
porcija

Slika 1: Primeri velikosti porcije: velika (175 g), srednja (125 g), mala (100 g)
(Prirejeno po: http://oehha.ca.gov/fish/so_cal/index.html)

PRIMER IZPOLNJEVANJA ANKETE:

V vsako vrstico vnesite dva križca (X): 1. x za pogostost uživanja; 2. x za velikost porcije (desni trije stolpci).

	Katere vrste rib in morskih sadežev ste najpogosteje uživali v času pred nosečnostjo? (vse RAZEN konzerv in namazov)									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
MORSKE RIBE:												
Sardela	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>							

Iz odgovora je razvidno, da ste pred nosečnostjo uživali sardele 1 krat na mesec in sicer srednje veliko porcijo (125 g/teden).



Anketa

Splošna vprašanja

1. Kako bi opisali vašo prehrano:	pred nosečnostjo	med nosečnostjo
Uživam: meso in ribe	<input type="checkbox"/>	<input type="checkbox"/>
ribe, ne mesa	<input type="checkbox"/>	<input type="checkbox"/>
meso, ne rib	<input type="checkbox"/>	<input type="checkbox"/>
Sem laktovo-vegetarijanka (uživam tudi mleko, mlečne izdelke ter jajca).	<input type="checkbox"/>	<input type="checkbox"/>
Sem laktovo-vegetarijanka (uživam tudi mleko in mlečne izdelke).	<input type="checkbox"/>	<input type="checkbox"/>
Sem veganka.	<input type="checkbox"/>	<input type="checkbox"/>

V primeru, da NE uživate rib, ribjih izdelkov ter morskih sadežev nadaljujete prosim z vprašanjem št. 8 na strani 9.

2. Pri pripravi hrane (vnesite križec v vsako okno, ki odraža vaš način priprave hrane):

- a) skoraj vedno uporabljam **surovo maslo** za kuhanje in pečenje
- b) skoraj vedno uporabljam **svinjsko mast** za kuhanje in pečenje
- c) skoraj vedno uporabljam **rastlinsko olje** za kuhanje in pečenje, katero: _____
- d) skoraj vedno uporabljam **margarino** za kuhanje in pečenje, katero: _____
- e) drugo: _____
- f) NE uporabljam maščobe za kuhanje in pečenje.

3. Ali uživate različno količino rib in ribjih izdelkov glede na letni čas? NE DA,

Če DA, v katerem letnem času pojestе največ: RIB pomlad poleti jeseni pozimi
RIBJIH IZDELKOV pomlad poleti jeseni pozimi



Vprašanja (4 - 5) za obdobje enega leta PRED NOSEČNOSTJO

	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
4.1 MORSKE RIBE:												
Brancin	<input type="checkbox"/>											
Ciplji	<input type="checkbox"/>											
Girice	<input type="checkbox"/>											
Losos	<input type="checkbox"/>											
Morski list	<input type="checkbox"/>											
Morski pes	<input type="checkbox"/>											
Orada	<input type="checkbox"/>											
Ostič	<input type="checkbox"/>											
Polenovka	<input type="checkbox"/>											
Sardela	<input type="checkbox"/>											
Sardina	<input type="checkbox"/>											
Skuša	<input type="checkbox"/>											
Stanik	<input type="checkbox"/>											
Škarpena	<input type="checkbox"/>											
Tuna	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											



	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
4.2 SLADKOVODNE RIBE:												
Postrv	<input type="checkbox"/>											
Krap	<input type="checkbox"/>											
Lipan	<input type="checkbox"/>											
Ščuka	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											
4.3 MORSKI SADEŽI:												
Školjke	<input type="checkbox"/>											
Kozice	<input type="checkbox"/>											
Jastog	<input type="checkbox"/>											
Ostrige	<input type="checkbox"/>											
Rakovice	<input type="checkbox"/>											
Lignji	<input type="checkbox"/>											
Sipe	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											
4.4 OSTALO:												
Kaviar	<input type="checkbox"/>											
Surimi	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											



5. Kako pogosto in kako pripravljene ribe, ribje izdelke in morske sadeže ste uživali v času enega leta pred nosečnostjo?										Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
5.1 MORSKE RIBE:												
suрова	<input type="checkbox"/>											
prekajena	<input type="checkbox"/>											
pečena ali dušena	<input type="checkbox"/>											
ocvrta	<input type="checkbox"/>											
5.2 SLADKOVODNE RIBE:												
suрова	<input type="checkbox"/>											
prekajena	<input type="checkbox"/>											
pečena ali dušena	<input type="checkbox"/>											
ocvrta	<input type="checkbox"/>											
5.3 MORSKI SADEŽI:												
suровi	<input type="checkbox"/>											
pečeni ali dušeni	<input type="checkbox"/>											
ocvrti	<input type="checkbox"/>											
5.4 RIBJI IZDELKI:												
ribje konzerve, katere:	<input type="checkbox"/>	grami/obrok: _____										
ribji namazi, kateri:	<input type="checkbox"/>	grami/obrok: _____										
drugo (napišite ime živila):	<input type="checkbox"/>	grami/obrok: _____										



Vprašanja (6 - 8) za obdobje NOSEČNOSTI!

6. Katerе vrste rib in morskih sadežev najpogosteje uživate tekom nosečnosti? (vse RAZEN konzerv in namazov)										Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
6.1 MORSKE RIBE:												
Brancin	<input type="checkbox"/>											
Cipliji	<input type="checkbox"/>											
Girice	<input type="checkbox"/>											
Losos	<input type="checkbox"/>											
Morski list	<input type="checkbox"/>											
Morski pes	<input type="checkbox"/>											
Orada	<input type="checkbox"/>											
Oslič	<input type="checkbox"/>											
Polenovka	<input type="checkbox"/>											
Sardela	<input type="checkbox"/>											
Sardina	<input type="checkbox"/>											
Skuša	<input type="checkbox"/>											
Slanik	<input type="checkbox"/>											
Škarpena	<input type="checkbox"/>											
Tuna	<input type="checkbox"/>											
Drugo (dopisite):	<input type="checkbox"/>											



	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
6.2 SLADKOVODNE RIBE:												
Postrv	<input type="checkbox"/>											
Krap	<input type="checkbox"/>											
Lipan	<input type="checkbox"/>											
Ščuka	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											
6.3 MORSKI SADEŽI:												
Školjke	<input type="checkbox"/>											
Kozice	<input type="checkbox"/>											
Jastog	<input type="checkbox"/>											
Ostrige	<input type="checkbox"/>											
Rakovice	<input type="checkbox"/>											
Lignji	<input type="checkbox"/>											
Sipe	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											
6.4 OSTALO:												
Kaviar	<input type="checkbox"/>											
Surimi	<input type="checkbox"/>											
Drugo (dopišite):	<input type="checkbox"/>											



	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
7.1 MORSKE RIBE:												
surova	<input type="checkbox"/>											
prekajena	<input type="checkbox"/>											
pečena ali dušena	<input type="checkbox"/>											
ocvrti	<input type="checkbox"/>											
7.2 SLADKOVODNE RIBE:												
surova	<input type="checkbox"/>											
prekajena	<input type="checkbox"/>											
pečena ali dušena	<input type="checkbox"/>											
ocvrti	<input type="checkbox"/>											
7.3 MORSKI SADEŽI:												
surovi	<input type="checkbox"/>											
pečeni ali dušeni	<input type="checkbox"/>											
ocvrti	<input type="checkbox"/>											
7.4 RIBJI IZDELKI:												
ribje konzerve, katere:	<input type="checkbox"/>	grami/obrok:										
ribji namazi, kateri:	<input type="checkbox"/>	grami/obrok:										
drugo (napišite ime živila):	<input type="checkbox"/>	grami/obrok:										



8. Ali uživate ribje olje tekom nosečnosti?

NE DA (katera, koliko časa, kako pogosto, zakaj): _____

8.1 Koliko:

- čajna žlička (5 g)
- jedilna žlica (10 g)
- ____ ml zaužitega ribjega olja
- kapsule (koliko: _____)

9. Ali uživate omega-3 maščobne kisline tekom nosečnosti v obliki prehranskih dopolnil?

NE DA (katera, koliko časa, kako pogosto, zakaj): _____

9.1 Koliko:

- čajna žlička (5 g)
- jedilna žlica (10 g)
- ____ ml zaužitega omega-3 olja
- kapsule (koliko: _____)

10. Ali uživate vitaminsko-mineralne dodatke tekom nosečnosti kot npr. Elevit, folna kislina, Femibion, šumeče tablete ipd.?

NE DA (katera, koliko časa, kako pogosto, koliko, zakaj): _____

Zahvaljujemo se vam za izpolnitve vprašalnika!

PRILOGA D: Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil tekom dojenja (VPŽ2)

ANNEX D: Food frequency questionnaire for period during lactation for fish, fish products and food supplements consumption (VPŽ2)



Št. preiskovanke: Kraj in datum: _____

Vprašalnik o pogostosti uživanja rib, ribjih izdelkov in morskih sadežev ter ostalih prehranskih dopolnil tekom dojenja

Zahvaljujemo se vam, da ste si vzeli čas za sodelovanje v anketi. Vprašalnik nam bo dal informacije o vaših prehranskih navadah zgoraj naštetih živil. Prosimo vas, da odgovarjate povsem iskreno.



velika srednja mala
porcija

Slika 1: Primeri velikosti porcije: velika (175 g), srednja (125 g), mala (100 g)
(Prirejeno po: http://oehha.ca.gov/fish/so_cal/index.html)

PRIMER IZPOLNJEVANJA ANKETE:

V vsako vrstico vnesite dva križca (X): 1. x za pogostost uživanja; 2. x za velikost porcije (desni trije stolpci).

Katere vrste rib in morske sadeže uživate tekom dojenja? (vse RAZEN konzerv in namazov)

	Pogostost uživanja								Velikost porcije			
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
MORSKE RIBE:												
Sardela	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>						

Iz odgovora je razvidno, da tekom dojenja uživate sardele 1 krat na mesec in sicer srednje veliko porcijo (125 g/teden).



Anketa

Splošna vprašanja

1. Kako bi opisali vašo prehrano:	tekom dojenja
Uživam: meso in ribe	<input type="checkbox"/>
ribe, ne mesa	<input type="checkbox"/>
meso, ne ribe	<input type="checkbox"/>
Sem laktovo-vegetarijanka (uživam tudi mleko, mlečne izdelke ter jajca).	<input type="checkbox"/>
Sem laktovo-vegetarijanka (uživam tudi mleko in mlečne izdelke).	<input type="checkbox"/>
Sam veganka.	<input type="checkbox"/>

V primeru, da NE uživate rib, ribjih izdelkov ter morskih sadežev nadaljujete prosim z vprašanjem št. 5 na strani 6.

2. Pri pripravi hrane (vnesite križec v vsako okno, ki odraža vaš način priprave hrane):

- a) skoraj vedno uporabljam surovo maslo za kuhanje in pečenje
- b) skoraj vedno uporabljam svinjsko mast za kuhanje in pečenje
- c) skoraj vedno uporabljam rastlinsko olje za kuhanje in pečenje, katero: _____
- d) skoraj vedno uporabljam margarino za kuhanje in pečenje, katero: _____
- e) drugo: _____
- f) NE uporabljam maščobe za kuhanje in pečenje.



Vprašanja (3 - 4) za obdobje DOJENJA

	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
3.1 MORSKE RIBE:												
Brancin	<input type="checkbox"/>											
Ciplji	<input type="checkbox"/>											
Girice	<input type="checkbox"/>											
Losos	<input type="checkbox"/>											
Morski list	<input type="checkbox"/>											
Morski pes	<input type="checkbox"/>											
Orada	<input type="checkbox"/>											
Oslič	<input type="checkbox"/>											
Polenovka	<input type="checkbox"/>											
Sardela	<input type="checkbox"/>											
Sardina	<input type="checkbox"/>											
Skuša	<input type="checkbox"/>											
Slanik	<input type="checkbox"/>											
Škarpena	<input type="checkbox"/>											
Tuna	<input type="checkbox"/>											
Drugo (dopisite):	<input type="checkbox"/>											



	Pogostost uživanja									Velikost porcije		
	nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
3.2 SLADKOVODNE RIBE:												
Postrv	<input type="checkbox"/>											
Krap	<input type="checkbox"/>											
Lipan	<input type="checkbox"/>											
Ščuka	<input type="checkbox"/>											
Drugo (dopisite):	<input type="checkbox"/>											
3.3 MORSKI SADEŽI:												
Školjke	<input type="checkbox"/>											
Kozice	<input type="checkbox"/>											
Jastog	<input type="checkbox"/>											
Ostrige	<input type="checkbox"/>											
Rakovice	<input type="checkbox"/>											
Lignji	<input type="checkbox"/>											
Sipe	<input type="checkbox"/>											
Drugo (dopisite):	<input type="checkbox"/>											
3.4 OSTALO:												
Kaviar	<input type="checkbox"/>											
Surimi	<input type="checkbox"/>											
Drugo (dopisite):	<input type="checkbox"/>											



4. Kako pogosto in kako pripravljene ribe, ribje izdelke in morske sadeže uživate tekom dojenja?		Pogostost uživanja								Velikost porcije			
		nikoli	1 x letno	2 x letno	1 x mesečno	2-3 x mesečno	1 x tedensko	2 x tedensko	3 x tedensko	≥4 x tedensko	velika	srednja	mala
4.1 MORSKE RIBE:													
surova		<input type="checkbox"/>											
prekajena		<input type="checkbox"/>											
pečena ali dušena		<input type="checkbox"/>											
ocvrta		<input type="checkbox"/>											
4.2 SLADKOVODNE RIBE:													
surova		<input type="checkbox"/>											
prekajena		<input type="checkbox"/>											
pečena ali dušena		<input type="checkbox"/>											
ocvrta		<input type="checkbox"/>											
4.3 MORSKI SADEŽI:													
surovi		<input type="checkbox"/>											
pečeni ali dušeni		<input type="checkbox"/>											
ocvrti		<input type="checkbox"/>											
4.4 RIBJI IZDELKI:											grami/obrok:		
ribje konzerve, katere:		<input type="checkbox"/>	grami/obrok:										
ribiji namazi, kateri:		<input type="checkbox"/>	grami/obrok:										
drugo (napišite ime živila):		<input type="checkbox"/>	grami/obrok:										

web: www.moje-mleko.si

(5/6)

e-mail:info@moje-mleko.si



5. Ali uživate ribje olje tekom dojenja?

NE DA (katera, koliko časa, kako pogosto, zakaj): _____

5.1 Koliko:

- čajna žlička (5 g)
- jedilna žličica (10 g)
- ____ ml zaužitega ribjega olja
- kapsule (koliko: _____)

6. Ali uživate omega-3 maščobne kislino tekom dojenja v obliki prehranskih dopolnil?

NE DA (katera, koliko časa, kako pogosto, zakaj): _____

6.1 Koliko:

- čajna žlička (5 g)
- jedilna žličica (10 g)
- ____ ml zaužitega ribjega olja
- kapsule (koliko: _____)

7. Ali uživate vitaminsko-mineralne dodatke tekom dojenja kot npr. Elevit, folna kislina, Femibion, šumeče tablete ipd.?

NE DA (katera, koliko časa, kako pogosto, koliko, zakaj): _____

Zahvaljujemo se vam za izpolnitve vprašalnika!

web: www.moje-mleko.si

(6/6)

e-mail:info@moje-mleko.si

PRILOGA E: Vprašalnik za pridobitev sociodemografskih in zdravstvenih podatkov prostovoljke v času nosečnosti

ANNEX E: Questionnaire to obtain socio-demographic and health data from the volunteers during pregnancy



Protokol za nosečnice 3. trimester

Protokol 1

VLOGA HUMANEGA MLEKA V RAZVOJU ČREVESNE MIKROBIOTE DOJENČKA

Št. preiskovanje: Kraj in datum: _____

Narodnost: _____ Kraj rojstva: _____ Kraj bivanja: _____

Okolje bivanja (obkrožite): mestno podeželsko

Ali ste že bili kdaj noseči (vključuje nosečnosti, ki so se normalno končale kot tudi nosečnosti, ki so se končale s splavom)?

NE DA, če da, katera nosečnost je to za vas: _____, koliko otrok imate: _____

Imate sestre ali brate: NE DA, če da, koliko: _____

Živite:

- | | |
|-------------------------------|----------------------|
| a) sami (z otroki) | d) drugimi sorodniki |
| b) s partnerjem (in z otroki) | d) s prijatelji |
| c) s starši | f) drugo: _____ |

Zakonski stan:

- | | |
|--------------------------|-----------------|
| a) poročena | d) vdova |
| b) izvezakonska skupnost | e) samohranilka |
| c) ločena | |

Izobrazba:

	osnovna šola	poklicna šola	srednja šola	višja šola	visoka šola	magisterija ali doktorat
Vaša	<input type="checkbox"/>					
Vašega partnerja	<input type="checkbox"/>					

Ali ste se že oz. ali se nameravate udeležiti šole za starše?

DA NE SEM SE JE ŽE UDELEŽILA PRI 1 OTROKU

Trenutni status zaposlitve:

	Vaš	Vašega partnerja
Zaposleni za polni delovni čas	<input type="checkbox"/>	<input type="checkbox"/>
Zaposleni za polovični delovni čas	<input type="checkbox"/>	<input type="checkbox"/>
Zaposleni na kmetiji	<input type="checkbox"/>	<input type="checkbox"/>
Samozaposlen (s.p.)	<input type="checkbox"/>	<input type="checkbox"/>
Brezposeln	<input type="checkbox"/>	<input type="checkbox"/>
Student/dijak	<input type="checkbox"/>	<input type="checkbox"/>
Rehabilitiran	<input type="checkbox"/>	<input type="checkbox"/>

Je vaše delovno mesto kakorkoli ogroženo zaradi nosečnosti? NE DA MOGOČE

Ali ste izpostavljeni pasivnemu kajenju:

doma oz. v prostem času: NE DA, če da, koliko ur na dan: _____
na delovnem mestu: NE DA; če da, koliko ur na dan: _____

Ali ste kadili preden ste postali noseči? NE DA, če da, koliko na dan:

a) 1-4 b) 5-14 c) 15-24 d) 25-34 e) 35-44 f) 45+

Ali trenutno kadite cigarete? NE DA, če da, koliko na dan:

a) 1-4 b) 5-14 c) 15-24 d) 25-34 e) 35-44 f) 45+

Ali vaš partner kadi? NE DA

Kako pogosto ste uživali kakrnekoli alkoholne pijače zadnje 3 meseca preden ste izvedeli, da ste noseči in kako pogosto jih uživate sedaj med nosečnostjo?

	Pred nosečnostjo	Med nosečnostjo
6-7 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>
4-5 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>
2-3 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>
1 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>
1-3 krat na mesec	<input type="checkbox"/>	<input type="checkbox"/>
Manj kot 1 krat na mesec	<input type="checkbox"/>	<input type="checkbox"/>
Nikoli	<input type="checkbox"/>	<input type="checkbox"/>

Prosim, označite, ali držijo sledeče trditve:

	Drži	Delno drži	Ne drži
Nerada telovadim, zato se tudi izogibam tovrstnim aktivnostim.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Telovadim z namenom, da izgubim telesno težo.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Telovadim, če uživam preveč hrane.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Ali se profesionalno ukvarjate s športom? NE DA, če da, s katerim: _____

Koliko stopnic dnevno prehodite v času nosečnosti?

a) dve stopnici ali manj b) 3-4 c) 5-9 d) 10-14 e) 15 ali več

Kakšna je vaša trenutna povprečna hitrost hoje?

a) ne morem hoditi b) počasna c) normalna d) hitra e) zelo hitra

Kako pogosto so fizične aktivnosti? (Označite prosim za zadnje 3 mesece preden ste izvedeli, da ste noseči ter za obdobje nosečnosti.)

	Zadnje 3 mesece pred nosečnostjo					V času nosečnosti				
	nikoli	1-3 x na mesec	1 x na teden	2 x na teden	≥ 3 x na teden	nikoli	1-3 x na mesec	1 x na teden	2 x na teden	≥ 3 x na teden
Hoja	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hitra hoja	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tek, orientacijski tek	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Kolesarjenje	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fitnes, dvigovanje uteži	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gimnastika/aerobika	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Ples (vse vrste standardnih plesov)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Smučanje	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sportne aktivnosti z žogo	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Plavanje	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Joga	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Plezanje	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Planinarjenje	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Telovadba za nosečnice	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pilates za nosečnice	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Drugo (kaj?):	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Kdaj ste v času nosečnosti fizično aktivnejši (obkrožite):

med tednom

med vikendom

med tednom in vikendom



Kako pogoste so fizične aktivnosti (v prostem času ali v službi) ob katerih se zadihate in/ali pričnete potiti?

	Zadnje 3 mesece pred nosečnostjo		V času nosečnosti	
	V prostem času	V službi	V prostem času	V službi
Nikoli	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Manj kot 1 enkrat na teden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3-4 krat na teden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5 ali večkrat na teden	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Zdravstveno stanje (diabetes, težave s ščitnico, ipd.): _____

Zdravila na recept (naštejte jih): _____

PRILOGA F: Vprašalnik za pridobitev sociodemografskih in zdravstvenih podatkov prostovoljke med dojenjem

ANNEX F: Questionnaire to obtain socio-demographic and health data from the volunteers during lactation



Protokol za doječe matere 4 tedne po porodu

Protokol 3

VLOGA HUMANEGA MLEKA V RAZVOJU ČREVESNE MIKROBIOTE DOJENČKA

Št. preiskovanie:

Kraj in datum: _____

Živite:

Zakonski stan:

- a) poročena d) vdova
 - b) izvenzakonska skupnost e) samohranilka
 - c) ločena

Ali ste izpostavljeni pasivnemu kajenju:

doma oz. v prostem času: NE DA, če da, koliko ur na dan: _____

Ali je vaš otrok izpostavljen pasivnemu kajenju? NE DA; če da, koliko ur/dan:

Ali trenutno kadite cigarete? NE DA, če da, koliko na dan:

- a) 1-4 b) 5-14 c) 15-24 d) 25-34 e) 35-44 f) 45+

Ali vaš partner kadi? NE DA

Kako pogosto uživate kakršnekoli alkoholne pijače od poroda naprej?

Kako pogosto izvrate kaki slike kot atertonite pija	Po porodu
6-7 krat na teden	<input type="checkbox"/>
4-5 krat na teden	<input type="checkbox"/>
2-3 krat na teden	<input type="checkbox"/>
1 krat na teden	<input type="checkbox"/>
1-3 krat na mesec	<input type="checkbox"/>
Manj kot 1 krat na mesec	<input type="checkbox"/>
Nikoli	<input type="checkbox"/>

Prosim, označite, ali držijo sledeće trditve:

	Drži	Delno drži	Ne drži
Nerada telovadim, zato se tudi izogibam tovrstnim aktivnostim.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Telovadim z namenom, da izgubim telesno težo.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Telovadim, če uživam preveč hrane.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Koliko stopnic dnevno prehodite v času po porodu?

- a) dve stopnici ali manj b) 3-4 c) 5-9 d) 10-14 e) 15 ali več

Kakšna je vaša trenutna povprečna hitrost hoje?

- a) ne morem hoditi b) počasna c) normalna d) hitra e) zelo hitra

Kako pogosto so fizične aktivnosti? (Označite prosim za zadnji mesec po porodu)

	Po porodu				
	nikoli	1-3 x na mesec	1 x na teden	2 x na teden	≥ 3 x na teden
Hoja	<input type="checkbox"/>				
Hitra hoja	<input type="checkbox"/>				
Tek, orientacijski tek	<input type="checkbox"/>				
Kolesarjenje	<input type="checkbox"/>				
Fitnes, dvigovanje uteži	<input type="checkbox"/>				
Gimnastika/aerobika	<input type="checkbox"/>				
Ples (vse vrste standardnih plesov)	<input type="checkbox"/>				
Smučanje	<input type="checkbox"/>				
Športne aktivnosti z žogo	<input type="checkbox"/>				
Plavanje	<input type="checkbox"/>				
Joga	<input type="checkbox"/>				
Plezanje	<input type="checkbox"/>				
Planinarjenje	<input type="checkbox"/>				
Telovadba	<input type="checkbox"/>				
Pilates	<input type="checkbox"/>				
Drugo (kaj?): _____	<input type="checkbox"/>				

Kdaj ste v času po porodu fizično aktivnejši (obkrožite):

med tednom

med vikendom

med tednom in vikendom

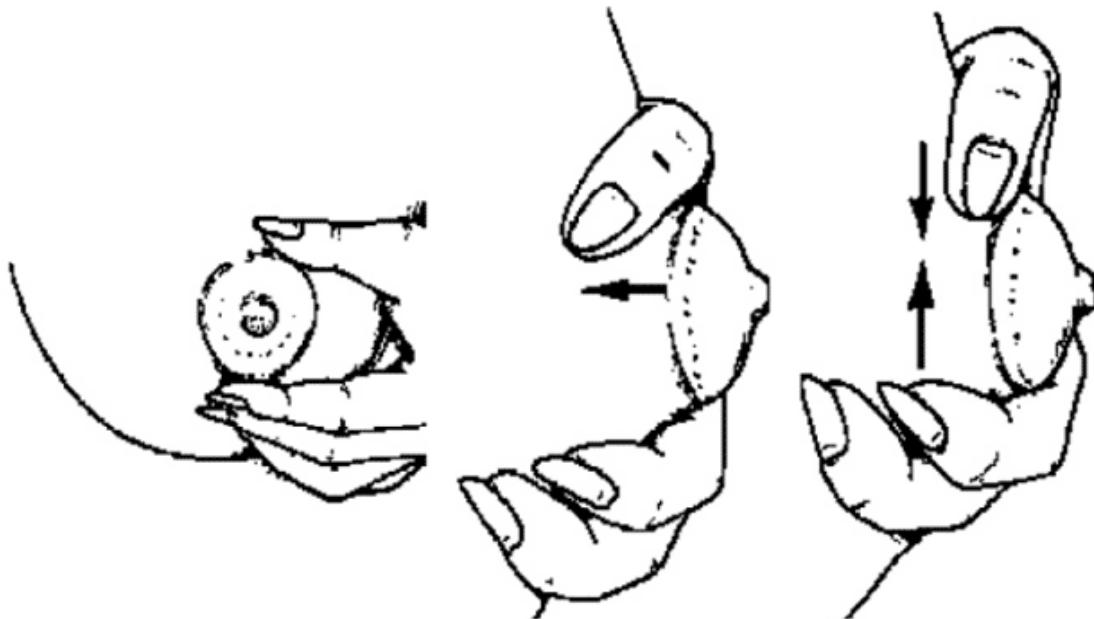
Kako pogoste so fizične aktivnosti ob katerih se zadihate in/ali pričnete potiti?

	Po porodu
Nikoli	<input type="checkbox"/>
Manj kot 1 enkrat na teden	<input type="checkbox"/>
1 krat na teden	<input type="checkbox"/>
2 krat na teden	<input type="checkbox"/>
3-4 krat na teden	<input type="checkbox"/>
5 ali večkrat na teden	<input type="checkbox"/>

Zdravstveno stanje (diabetes, težave s ščitnico, ipd.): _____

Zdravila na recept (naštejte jih): _____

PRILOGA G: Ročno izbrizgavanje humanega mleka (Davis, 2015)
ANNEX G: Manual expression of human milk (Davis, 2015)



PRILOGA H: Reagenti in standardi, ki smo jih uporabili v študiji
ANNEX H: Reagents and standards used in the study

Ime reagenta slovensko	angleško	Proizvajalec in kataloška številka reagenta
destilirana voda	destilated water	
etanol	ethanol 96 vol. %t	Sigma-Aldrich 24106
n-heksan	n-hexan	Sigma-Aldrich 32293
natrijev hidroksid	sodium hydroxide	Sigma-Aldrich 30620
metanol	methanol	Fluka 34860
diklorometan	dichloromethane	Sigma-Aldrich 32222
14 % borov trifluorid v metanolu	14 % boron trifluoride-methanol solution	Sigma-Aldrich SU-33021
Ime standardov		
mešanica MEMK	Supelco 37 component FAME Mix	Sigma-Aldrich CRM47885
metilni ester heneikozanojske kisline (IS)	methyl heneicosanoate	Sigma-Aldrich H3265
metilni ester tridekanojske kisline	methyl tridecanoate	Fluka 91558
metilni ester linolenske kisline (cis/trans)	linoleic methyl ester(cis/trans)	Supelco 47791
metil nonadekanoat	methyl nonadecanoate	Fluka 74208
PUFA no. 3	PUFA no. 3	Supelco 47085-U
metilni ester cis-5,8,11-eikozatrienojske kisline	cis-5,8,11-eicosatrienoic acid methyl ester	Sigma-Aldrich E6013

PRILOGA I: Seznam identificiranih maščobnih kislin v vzorcih humanega mleka (HM) v ut. %
ANNEX I: List of identified fatty acids in human milk samples (HM) in wt. %

Ime maščobne kisline ^a	Oznaka	Srednja vrednost ± S.D.
dekanojska	C10:0	1,16 ± 0,28
undekanojska	C11:0	0,01 ± 0,00
dodekanojska	C12:0	4,47 ± 1,49
tridekanojska	C13:0	0,03 ± 0,01
tetradekanojska	C14:0	5,46 ± 1,41
cis-9-tetradecenojska	C14:1 n-5	0,26 ± 0,09
pentadekanojska	C15:0	0,36 ± 0,10
cis-10-pentadecenojska	C15:1 n-5	0,00 ± 0,00
heksadekanojska	C16:0	23,23 ± 2,34
cis-9-heksadecenojska	C16:1 n-7	2,79 ± 0,62
heptadekanojska	C17:0	0,30 ± 0,06
oktadekanojska	C18:0	6,30 ± 1,04
Σ oktadecenojskih kislin ^b	Σ C18:1	38,24 ± 3,71
trans-9,12-oktadekadienojska	C18:2 n-6tt	0,06 ± 0,02
cis-9,12-oktadekadienojska	C18:2 n-6cc	13,97 ± 3,40
eikozanojska	C20:0	0,17 ± 0,03
cis-6,9,12-oktadekatrienojska	C18:3 n-6	0,14 ± 0,05
cis-9,12,15-oktadekatrienojska	C18:3 n-3	0,86 ± 0,49
cis-11-eikozenojska	C20:1 n-9	0,37 ± 0,06
cis-11,14-eikozadienojska	C20:2 n-6	0,29 ± 0,06
dokozanojska	C22:0	0,05 ± 0,01
cis-8,11,14-eikozatrienojska	C20:3 n-6	0,40 ± 0,09
cis-11,14,17-eikozatrienojska	C20:3 n-3	0,03 ± 0,01
cis-5,8,11,14-eikozatetraenojska	C20:4 n-6	0,52 ± 0,10
cis-13-dokozenojska	C22:1 n-9	0,08 ± 0,02
trikozanojska	C23:0	0,00 ± 0,00
cis-13,16-dokozadienojska	C22:2 n-6	0,03 ± 0,01
cis-5,8,11,14,17-eikozapentaenojska	C20:5 n-3	0,07 ± 0,06
tetrakozanojska	C24:0	0,04 ± 0,01
cis-15-tetrakozenojska	C24:1 n-9	0,04 ± 0,01
cis-4,7,10,13,16,19-dokozaheksanojska	C22:6 n-3	0,26 ± 0,15
	NMK	41,58 ± 4,57
	ENMK	42,22 ± 3,81
	VNMK	16,21 ± 3,65
	VNMK/NMK	0,40 ± 0,12
	n-3	1,22 ± 0,59
	n-6	14,98 ± 3,49
	Σ(n-3) + (n-6)	16,21 ± 3,65
	n-6/n-3	13,78 ± 4,67
	Skupen % maščob	3,44 ± 1,04

^aVrstni red navedenih maščobnih kislin je enak elucijskemu vrstnemu redu maščobnih kislin pod opisanimi kromatografskimi pogoji (poglavlje 2.2.4.2).

^b \sum oktadecenojskih kislin = trans-9-oktadecenojska + cis-9-oktadecenojska + cis-11-oktadecenojska kislina.

NMK = \sum (C10:0; C11:0; C12:0; C13:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:0; C22:0; C23:0; C24:0);

ENMK = \sum (C14:1 n-5; C15:1 n-5; C16:1 n-7; \sum C18:1; C20:1 n-9; C22:1 n-9; C24:1 n-9);

VNMK = \sum (C18:2 n-6tt; C18:2n-6cc; C18:3 n-6; C18:3 n-3; C20:2 n-6; C20:3 n-6; C20:3 n-3; C20:4 n-6;

C22:2 n-6; C20:5 n-3; C22:6 n-3).

PRILOGA J: Soglasje Komisije Republike Slovenije za medicinsko etiko za izvajanje študije Moj-mleko

ANNEX J: Approval from the Medical Ethics Committee of the Republic of Slovenia for the »My-Milk« study



KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

Asist. dr. Borut Bratanič, dr. med.
KO za neonatologijo, Pediatrična klinika, Univerzitetni klinični center Ljubljana
Bohoričeva 20, 1525 Ljubljana, in

Prof. dr. Irena Rogelj, dipl. ing. živil. tehnik.
Katedra za mlekarstvo, Biotehniška fakulteta
Jamnikarjeva 101, 1000 Ljubljana

Štev.: 32/07/10
Datum: 11. 8. 2010

Spoštovana gospod dr. Bratanič in gospa prof. dr. Rogelj,

Komisiji za medicinsko etiko (KME) sta 9. 6. 2010 poslala prošnjo za oceno načrta raziskave z naslovom:

"Vloga humanega mleka v razvoju črevesne mikrobiote¹ dojenčka."

KME je na seji 13. 7. 2010 ocenila, da je raziskava etično sprejemljiva, in Vam s tem izdaja svoje soglasje.

S spoštovanjem in lepimi pozdravi,

V imenu Komisije za medicinsko etiko:
prof. dr. Jože Trontelj

¹ pravilno: črevesnih mikrobiotov ali črevesne mikroflore

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