HRANA U ZDRAVLJU I BOLESTI
FOOD IN HEALTH AND DISEASE

HRANA U ZDRAVLJU I BOLESTI
FOOD IN HEALTH AND DISEASE

Journal FOOD IN HEALTH AND DISEASE is indexed in:
CAB Abstracts database; Portal of Croatian Scientific Journals (HRČAK)

SADRŽAJ/CONTENT

PROCJENA RIZIKA ZA OBOLIJEVANJE OD KARCINOMA DEBELOG CRIJEVA STUDENTSKE POPULACIJE

Ines Banjari*, Tihana Ostrognjaj

Zavod za ispitivanje hrane i prehrane, Prehrambeno-tehnološki fakultet Osijek,
Sveučilište Josipa Jurja Strossmayera u Osijeku
F. Kuhača 20, HR-31000 Osijek, Hrvatska

Sažetak

Uvod: Karcinom debelog crijeva (CRC) jedan je od vodećih zdravstvenih problema u svijetu, a u Hrvatskoj je drugi uzročnik smrti. CRC je treći po incidenciji i mortalitetu u oba spola, uz značajne razlike po županijama. Etiologija uključuje interakciju nasljeđa, prehrane i životnih navika. Utvrđeno je kako je 90% svih slučajeva CRC-a u direktnoj vezi s prehrambenim navikama. Studentska populacija zbog promjene životne sredine, povećanja samostalnosti i promjene životnih uloga, te povećanog broja obveza mijenja prehrambene navike, najčešće nepovoljno. Prehrambene navike usvojene u ovom periodu se prolongiraju u kasnijim razdobljima života, a incidencija i mortalitet CRC-a značajno rastu iznad 40 godina.

Cilj: Procijeniti rizične čimbenike za obolijevanje od CRC-a koji su povezani s prehrambenim i životnim navikama studentske populacije.

Materijali i metode: 446 studenata (23 godine; 37,2% muški, 62,8% žene) ispunilo je anonimnu anketu koja je razvijena za potrebe ovog istraživanja. Obzirom na odgovore rangirani su u kategoriju niskog, srednjeg ili visokog rizika za karcinom debelog crijeva.

Rezultati: Utvrđena je prisutnost velikog broja rizičnih čimbenika povezanih s CRC-om: visoka prevalencija povećane tjelesne mase (23,1%) i pretilosti (6,1%), pušenja (30,0%), niska razina fizičke aktivnosti (25,6% neaktivno, 30,3% sezonski aktivno), visok unos alkoholnih pića (20,6% tjedno, 1,4% dnevno), nizak unos ribe, voća i povrća i visok unos mesa i mesnih proizvoda, preferencija jako slane i začinjene (ljute) hrane, te pozitivna obiteljska anamneza (47,5% s predispozicijom, 12,1% s pozitivnom anamnezom na CRC). Utvrđene su značajne razlike po spolu (p<0,001) i obiteljskoj anamnezi.

Zaključak: Najznačajniji rizični čimbenici za CRC povezani s prehrambenim navikama su visok unos mesa i mesnih prerađevina, preferencija slane i začinjene hrane te nizak unos ribe, voća i povrća. Utvrđen je značajno viši rizik kod studenata, a osobito je zabrinjavajuće što je kod studenata s pozitivnom obiteljskom anamnezom utvrđen najveći rizik za CRC.

Ključne riječi: karcinom debelog crijeva, studenti, procjena rizika, prehrambene navike, životne navike

Uvod

Kolorektalni karcinom (CRC) treći je najčešće dijagnosticirani karcinom u svijetu i zauzima treće mjesto u mortalitetu od zloćudnih bolesti muškaraca i žena (Cooper, 2011). Veći dio otpada na karcinomi kolona, dok ostatak čini karcinom rektuma (Siegel i sur., 2013), što je i razlog zašto se obično smatraju sinonimima. Etiologija CRC-a je multifaktorijalna i uključuje složenu interakciju između nasljeđa i okolišnih čimbenika (Johnson i sur., 2013). Najveći broj karcinoma javlja se u sporadičnom obliku, dok na oko 15 % utječu nasljedni čimbenici (Debas, 2003). Važno je istaknuti kako je ovo jedini tip karcinoma za koji je utvrđena izuzetno visoka povezanost s prehranom od gotovo 90 % (Banjari i Fako, 2014).

U većini razvijenih zemalja (npr. Sjedinjene Američke Države) incidencija stagnira ili čak opada, što se može povezati s promjenama u načinu prehrane, stilu življenja, te široke primjene mjera za rano otkrivanje, te učinkovitije liječenje i rehabilitaciju (Strnad i Šogorić, 2010). S druge strane, zemlje u tranziciji poput Češke ili Slovačke pokazuju porast incidencije CRC-a (Center i sur., 2009). Prema zadnjim dostupnim podacima za Hrvatsku CRC drugi je najčešći oblik karcinoma u muškaraca (iza karcinoma pluća; 15 %) i u žena (iza karcinoma dojke; 13 %)

*Corresponding author: ines.banjari@ptfos.hr

(HZJZ, 2013). Važno je istaknuti kako mortalitet raste sporije nego incidencija CRC-a (Strnad i Šogorić, 2010).

Rizični čimbenici povezani s etiologijom CRC-a se mogu podijeliti u dvije skupine. Prvo su rizični čimbenici koji se mogu kontrolirati, a koji su povezani s prehrambenim i životnim navikama te nepromjenjivi čimbenici, odnosno dob i obiteljska anamneza (Banjari i Fako, 2014). Iako rizični čimbenici mogu utjecati na razvoj karcinoma, većina ih izravno ne uzrokuje karcinom. Važno je istaknuti kako je za CRC specifično upravo to što prehrambene i životne navike nadilaze genetsku predispoziciju osobe za razvoj karcinoma (Johnson i sur., 2013).

U visokorizične skupine ubrajaju se osobe s pozitivnom obiteljskom anamnezom (bliski srodnici oboljeli od CRC-a), oboljeli s ranije potvrđenim adenomatoznim polipima debelog crijeva, bolesnici s ulceroznim kolitisom i Crohnovom bolešću te prethodnom anamnezom drugih karcinoma (Zovak, 2007). Upalna bolest crijeva (IBD) pokazuje najveći rizik za CRC (Johnson i sur., 2013). Povećan rizik je utvrđen za dijabetes mellitus tipa 2, hiperinzulinemiju i visoke koncentracije inzulin-sličnog faktora rasta (IGF) (Banjari i Fako, 2014).

Rizik znatno raste nakon 40. godine života, a 90 % svih karcinoma otkrije se u osoba starijih od 50 godina. Procjenjuje da se 2 do 3 % CRC pojavljuje kod pacijenata mlađih od 40 godina, uz tendenciju rasta prevalencije (Mitchell, 2012).

Veliki broj istraživanja ukazao je kako je pretilost povezana s agresivnijim CRC-a (Gribovskaja-Rupp i sur., 2011, Campbell i sur., 2011). Osim toga, viši indeks tjelesne mase (BMI) u usporedbi s normalnim BMI je povezan s većom smrtnošću. Važno je napomenuti kako je utjecaj pretilosti veći za muškarce, no ulaskom u postmenopauzu omjer se mijenja prema ženama (Banjari i Fako, 2014) što je i razlog zašto se preventivno davanje hormonalne terapije smatra zaštitnim čimbenikom (Brkić i Grgić, 2006). Također je centralna distribucija masnog tkiva (visceralni adipozitet) povezana s povećanim rizikom, neovisno o BMI (Banjari i Fako, 2014; Perera i sur., 2012).

Fizička aktivnost u trajanju od 30 minuta/dan pokazuje 11 % smanjenje rizika za CRC i 12 % smanjenje rizika za rak kolona prema zadnjoj

meta-analizi (Perera i sur., 2012). Za rekreativnu fizičku aktivnost je utvrđeno smanjenje rizika, no bez statističke značajnosti, dok generalno gledano dnevna fizička aktivnost smanjuje rizik za CRC 3 %, a rak kolona za 8 % (Perera i sur., 2012).

Pušači imaju povećani rizik za CRC, posebice ukoliko je pušenje kombinirano s niskim unosom voća i povrća (Banjari i Fako, 2014). Osobe koje su prestale pušiti imaju manji rizik od trenutnih pušača, no za njih se rizik za CRC izjednačava s rizikom nepušača nakon najmanje 20 godina nepušenja. Konzumacija alkohola, posebice u muškaraca, također je povezana s povećanim rizikom za CRC (Perera i sur., 2012).

Prehrambene navike za koje je utvrđena pozitivna korelacija s povećanim rizikom za CRC su: visok unos mesnih prerađevina (kao što su slanina, kobasice, hrenovke) i crvenog mesa (govedina, svinjetina ili janjetina) te unos zasićenih masnoća (Banjari i Fako, 2014). S druge strane, smanjenje rizika za CRC je utvrđeno za visok unos voća i povrća te sukladno tome i visok unos prehrambenih vlakana, konzumaciju cjelovitih žitarica, unos omega-3 masnih kiselina te cijeli niz vitamina, minerala i fitonutrijenata (karotenoidi, vitamini C i E, folna kiselina, selen, izotiocijanati i polifenoli) (Banjari i Fako, 2014).

Studenti su podložni lošim prehrambenim navikama zbog promjene životne sredine, brojnih novih obveza te promjene životnih uloga i povećane odgovornosti. Povećana je tendencija preskakanja obroka (posebice doručka), smanjen je izbor hrane, češće konzumiraju nezdravu hranu za međuobrok („snack") te konzumiraju obroke s neadekvatnim sastavom nutrijenata. Sve ove navike negativno utječu na njihovu mentalnu aktivnost, ali i cjelokupno zdravstveno stanje. Uz loše prehrambene navike javljaju se i loše životne navike, kao što su smanjenje tjelesne aktivnosti, konzumacija alkohola te pušenje (Banjari i sur., 2011).

Iz svega navedenog proizlazi osnova za navedeno istraživanje čiji su ciljevi procijeniti rizične čimbenike za obolijevanje od CRC koji su prisutni među studentima, a u vezi su s karakteristikama prehrane i životnim navikama. Osim toga, cilj je utvrditi u kojoj je mjeri obiteljska anamneza studenata pozitivna na CRC.

Materijali i metode

Hipoteze istraživanja

Prema postavljenim ciljevima istraživanja postavljene su sljedeće radne hipoteze:

1. Stanje uhranjenosti studentske populacije ukazuje na značajnu prevalenciju povećane tjelesne mase i pretilosti.
2. Životne navike studenata su nepovoljne, prvenstveno niska razina fizičke aktivnosti i visoka prevalencija pušenja.
3. Pozitivnost obiteljske anamneze na CRC je u značajnoj mjeri prisutna u promatranoj populaciji studenata.
4. Najznačajniji rizični čimbenici povezani s prehrambenim navikama su visok unos mesa, jako začinjene hrane, nizak unos ribe te svježeg voća i povrća.

Metode i metodologija istraživanja

Za potrebe istraživanja razvijen je online anketni upitnik koji je kreiran uporabom internetskog javno-dostupnog programa za kreiranje anketnih upitnika, odnosno Google drive program. Primjena ovog programa je omogućila potpunu anonimnost ispitanika. Poveznica na online anketu je bila dostupna na stranicama knjižnica fakulteta Sveučilišta u Osijeku, te angažmanom studentskih organizacija (npr. studntski zbor). Vrijeme potrebno za ispunjavanje ankete je iznosilo 10 minuta, a nakon ispunjavanja ankete odgovori su se automatski spremali u centralnu bazu u formi MS Office Excel dokumenta. Ukupno je prikupljeno 476 anketa, od koji je 30 anketa (6,3 % anketa je izostavljeno iz obrade) izostavljeno zbog nepotpunih podataka, te je konačan broj anketa uzetih u daljnju obradu iznosio 446. Anketu je ispunilo 166 studenata i 280 studentica, prosječne starosti 23 godine (19 do 34 godine) (Tablica 1). Najveći je dio ispitanika bio iz Osječko-baranjske županije (41,5 %), zatim sjeverne Hrvatske, odnosno grada Zagreba i Varaždinske i Međimurske županije (31,2 %), Istarske županije (15,1 %), te drugih dijelova Hrvatske (12,2 %). Upitnik je u sebi sadržavao dio o općim karakteristikama ispitanika (dob, spol, fakultet studiranja te socio-ekonomske karakteristike), dio o općim prehrambenim (npr. broj obroka, učestalost konzumiranja svih skupina namirnica) i životnim navikama (npr. pušenje, fizička aktivnost, konzumiranje dodataka prehrani) te dio koji je konstruiran prema literaturno dostupnim podacima o rizičnim čimbenicima za razvoj CRC-a, a koji su povezani s prehrambenim i životnim navikama (npr. konzumacija začinjene hrane, suho-mesnatih proizvoda, alkohola).
Antropometrijski podaci o tjelesnoj masi i visini su prikupljeni metodom samobilježenja, iz kojih se dalje izračunavao BMI te je učinjena kategorizacija prema svjetskim preporukama (WHO, 2006).

Tablica 1. Opće karakteristike ispitivane studentske populacije (N = 446)
Table 1. General characteristics of the participating student population (N = 446)

		n	%
Spol	muški	166	37,2
	ženski	280	62,8
Životna sredina	selo	343	76,9
	grad	103	23,1
Od kako studirate živite	s roditeljima	127	28,7
	podstanar sam	239	53,9
	u studentskom domu	77	17,4
Novčani iznos kojim raspolažete cijeli mjesec	do 1000 kn	157	35,2
	1000-2000 kn	192	43,3
	> 2000 kn	96	21,5

Za kreirani je anketni upitnik razvijen sustav bodovanja. Sustav bodovanja je baziran na ranije elaboriranim rizičnim čimbenicima za koje je istraživanjima utvrđena povezanost s povećanim rizikom za obolijevanje od CRC-a. Princip dodjeljivanja bodova je bio takav da je odgovor za

koji je istraživanjima utvrđena visoka povezanost s obolijevanjem od CRC-a dodijeljen maksimalni broj bodova (5 bodova, maksimalni broj bodova 114). Na osnovu ostvarenih bodova ispitanici su svrstani skupinu s niskim, srednjim ili visokim rizikom za obolijevanje od CRC-a.

Obrada podataka

Grafička obrada podataka je napravljena pomoću MS Office Excel alata (inačica 2007, Microsoft Corp., USA), dok je za statističku obradu podataka korišten programski sustav Statistica (inačica 12.0, StatSoft Inc., USA), uz odabranu razinu značajnosti od p = 0,05.
Nakon ispitivanja normalnosti razdiobe numeričkih podataka primjenom Kolmogorov-Smirnov testa, za daljnju statističku analizu korišteni su neparametrijski testovi. Za usporedbu kategoričkih podataka unutar i među skupinama korišten je Fisherov egzaktni test. Razlike između dvije zavisne skupine numeričkih podataka su testirane Wilcoxonovim testom. Razlike između tri zavisne skupine testirane su neparametrijskim Friedmanovim testom. Razlike između dvije nezavisne skupine testirane su neparametrijskim Mann-Whitney U testom, a razlike između više nezavisnih skupina neparametrijskim Kruskal-Wallis testom. Za izračun korelacija numeričkih podataka korišten je Spearmanov test korelacije. Svi prikupljeni kategorički podaci predstavljeni su apsolutnim i relativnim frekvencijama, dok su numerički podaci opisani aritmetičkom sredinom i standardnom devijacijom, a u slučaju raspodjela koje nisu slijedile normalnu razdiobu medijanom i interkvartilnim rasponom.

Rezultati i rasprava

Kategorizacijom studenata prema izračunatom BMI je utvrđeno kako je većina studenata (68,4 %) normalnog stanja uhranjenosti, no prevalencija povećane tjelesne mase (23,1 %) i pretilosti (6,1 %) je značajna. Dobiveni podaci ukazuju na nepovoljan pozitivan trend koji se očituje u povećanoj prevalenciji povećane tjelesne mase i pretilosti u studentskoj populaciji, a prema ranije provedenim istraživanjima na istoj populaciji (Žiža, 2012; Banjari i sur., 2011). Osim toga, utvrđena je statistički značajna razlika po spolu (p<0,001); studenti imaju viši BMI od studentica (Tablica 2). Povećana tjelesna masa i pretilost su značajan rizični čimbenik za CRC (Banjari i Fako, 2014), a utvrđena prevalencija ukazuje kako je ovo značajan rizični čimbenik u ispitivanoj populaciji studenata. Jednako tako, vidljivo je kako je rizik veći za studente (viši prosječni BMI). Obzirom na kategoriju BMI-a, 23,1 % studenata je imalo povećanu tjelesnu masu (BMI 25,0 – 29,9 kg/m2), a 2,2 % studenata je bilo pretilo (BMI ≥30,0 kg/m2). Dobiveni rezultati potvrđuju prvu hipotezu.

Tablica 2. Stanje uhranjenosti ispitivane populacije studenata (N = 446) po spolu, a prema izračunatom indeksu tjelesne mase (BMI)
Table 2. State of nourishment of the participating student population (N = 446) by gender, and according to the calculated body mass index (BMI)

		n	Srednja vrijednost ± SD	Raspon (min – max)	p
Spol	muški	166	24,5 ± 2,8	16,4 – 31,1	<0,001*
	ženski	280	21,9 ± 2,9	15,6 – 32,9	

Mann-Whitney U test, * označava statističku značajnost kod p=0,05

Povećanjem svijesti o brizi za vlastito zdravlje povećava se i kvaliteta prehrambenih i životnih navika, a čije je poboljšanje u direktnoj vezi sa smanjenjem rizika za CRC (Banjari i Fako, 2014; Banjari, 2014). Čak se 67,5 % studenata izjavilo kako brine o vlastitom zdravlju (Tablica 3). S obzirom na pušenje, rezultati ukazuju kako je došlo do pozitivnih promjena. Obzirom na istraživanje koje je provela Žiža (2012) primjetan je pad broja pušača (24,0 % vs 26,0 %) i povećani broj bivših pušača (14,8 % vs 7,9 %) (Tablica 3). Razlog ovakvih pozitivnih promjena može bit rigoroznije mjere koje se provode na razini države, od propagandnih (intenzivna medijska promidžba negativnih strana duhanskog dima na zdravlje kroz sve medije) do financijskih (više cijene duhan-

skih proizvoda). Dakle, obzirom da je pušenje u pozitivnoj korelaciji s CRC-om (Ćurković i sur., 2010.; Banjari i Fako, 2014) ovakva se promjena može ocijeniti nadasve pozitivnom.

Tablica 3. Osobni stav o brizi za vlastito zdravlje i životne navike ispitivane populacije studenata (N = 446)
Table 3. Personal attitudes towards own health and lifestyle habits of the participating student population (N = 446)

		n	%	p
Smatrate li da se brinete za svoje zdravlje?	ne	129	28,9	<0,001*
	nije me briga	8	1,8	
	da	301	67,5	
Uzimanje dodataka prehrani	nikada	231	51,8	0,432
	svakodnevno	38	8,5	
	povremeno	172	38,6	
Fizička aktivnost	totalno sam neaktivan/a	114	25,6	<0,001*
	rekreiram se svaki dan bar 30 minuta kroz cijelu godinu	143	32,1	
	rekreiram se 2-3 puta u tjednu kada je lijepo vrijeme (sezonski)	135	30,3	
	bavim se sportom aktivno	45	10,1	
Pušenje	nikada	270	60,5	<0,001*
	prestao/la	66	14,8	
	pušim	107	24,0	

Fischerov egzaktni test, *označava statističku značajnost kod p=0,05

Iako se uzimanje dodataka prehrani smatra jednim od pokazatelja brige za vlastito zdravlje (Pollak, 2008), opravdanost uzimanja istih u smislu prevencije karcinoma je i više nego upitno (Banjari, 2014). Dobiven su podaci u skladu s ranijim rezultatima (Žiža, 2012; Banjari i sur., 2011) i u slučaju uzimanja to je najčešće u vrijeme prehlada. Statistički značajna razlika je utvrđena za razinu fizičke aktivnosti ispitivane populacije studenata (p<0,001). Neaktivno je 25,6 % studenata, dok se sportom aktivno bavi 10,0 % ispitivane populacije. Unatoč povremenoj fizičkoj aktivnosti, važno je istaknuti kako je 30,3 % studenata aktivno sezonski, a njih 32,1 % je aktivno cijele godine (Tablica 3). Dobiveni rezultati ukazuju na pozitivne promjene u vidu povećane fizičke aktivnosti među studentima, u odnosu na ranije dostupne podatke (Žiža, 2012). Ipak je razina redovite fizičke aktivnosti niska, što je u pozitivnoj vezi s rizikom za CRC (Perera i sur., 2012; Banjari i Fako, 2014). Osim što povećana razina fizičke aktivnosti pozitivno utječe na smanjenje rizika za CRC (Cummings i Bingham, 1998; Banjari i Fako, 2014; Kushi i sur., 2006), ima i brojne druge povoljne učinke na organizam. Već drugi dan nakon

smanjenja tjelesne aktivnosti, mentalna aktivnost se smanjuje do 50 %, koncentracija opada, raste napetost i drugo (Kisenauskaitė i Paškevičienė, 2011). Najveći dio studenata alkohol konzumira na mjesečnoj bazi (63,2 %), a zatim na tjednoj bazi (20,6 %), dok ih 1,4 % konzumira na dnevnoj bazi (rezultati nisu prikazani). Istraživanjem na Sveučilištu u Rijeci utvrđeno je kako čak 97 % studenata (oba spola) konzumira alkohol povremeno, muškarci češće od žena (Giacometti i Buretić-Tomljanović, 2013.). Rezultati ukazuju na pozitivne promjene u smislu životnih navika, djelomično potvrđujući drugu hipotezu. Ipak, potrebno je uložiti još aktivnosti, ponajprije edukativnih kako bi promjene bile učinkovitije i brže. Obiteljska anamneza ispitivane populacije studenata ukazuje na značajan broj zdravstvenih problema u 87 % studenata (Sl. 1). U ovisnosti o vrsti zdravstvenog problema, najveći dio otpada na bolesti i stanja koja nisu uključena u etiologiju CRC-a (79,4 %), dok je 12,1 % studenata imalo pozitivnu obiteljsku anamnezu na CRC. Istraživanja su pokazala kako je genetska predispozicija važan čimbenik rizika, no ipak ne ključni (Johnson i sur., 2013.). Ipak, ne treba zanemariti

visoku pojavnost bolesti koje su istraživanjima povezane s povećanim rizikom za CRC (47,5 %), poput dijabetesa tipa 2 ili nekih drugih tipova gastrointestinalnih karcinoma (Banjari i Fako, 2014). Važno je istaknuti kako je prevalencija CRC-a u najbližih rođaka ispitivane populacije studenata u skladu sa statističkim podacima dostupnima za Hrvatsku (12,1 % naprema 13 do 15 %) (HZJZ, 2013).
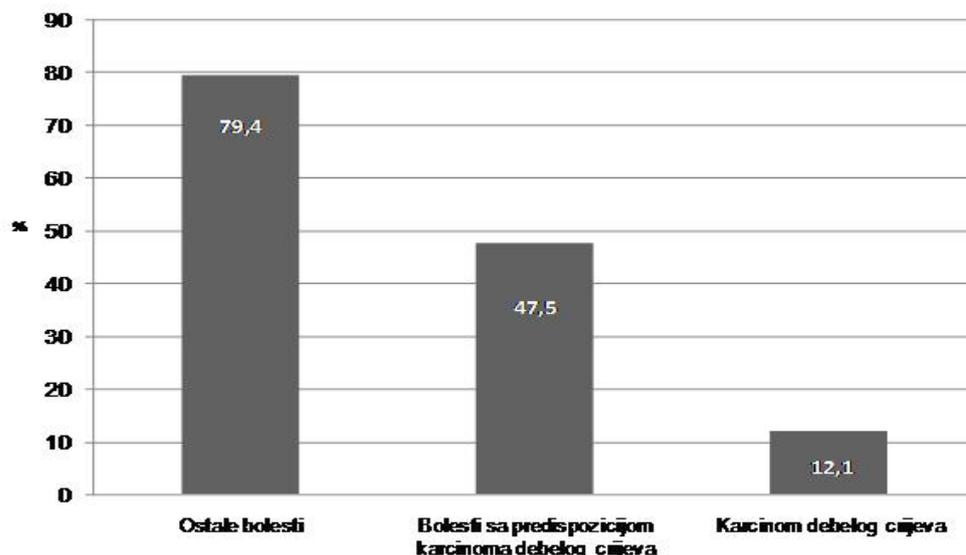
Slika 1. Genetska predispozicija za kolorektalni karcinom ispitivane populacije studenata (N = 446) prema obiteljskoj anamnezi
Fig. 1. Genetic predisposition for colorectal carcinoma of the participating student population (N = 446) according to family anamnesis
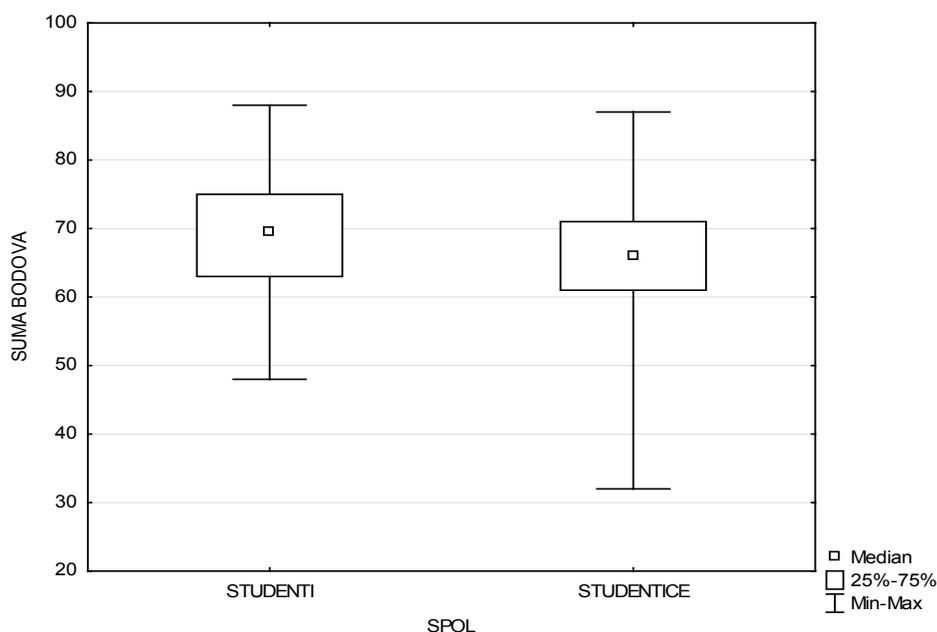
Slika 2. Procjena rizika za obolijevanje od karcinoma debelog crijeva prema sumi bodova ostvarenih anketom o prehrambenim i životnim navikama, po spolu za ispitivanu populaciju studenata (N = 446) (napomena autora: viši broj bodova je u vezi s većim rizikom za CRC)
Fig. 2. Risk assessment for colorectal cancer according to the sum points gained through questionnaire on dietary and lifestyle habits, and depending on gender for the participating population of students (N = 446) (authors' note: the higher the sum points the higher the risk for CRC)

Općenito gledano, prehrambene navike koje predstavljaju odliku tzv. zapadnjačkog načina prehrane su u direktnoj vezi s povećanim rizikom od CRC-a (Banjari i Fako, 2014; Banjari, 2014; Kushi i sur., 2006). Načn prehrane koji se dovodi u vezu s CRC-om karakteriziraju visok unos zasićenih masnoća, crvenog mesa i mesnih prerađevina, te jednostavnih ugljikohidrata, kao i učestalom konzumacijom brze hrane i jako začinjene hrane (Banjari i Fako, 2014; Kushi i sur., 2006; Banjari, 2014). S druge strane, veći broj obroka tijekom dana, visok unos svježeg voća i povrća te cjelovitih žitarica, visok unos prehrambenih vlakana, visok unos ribe i omega-3 masnih kiselina, uz dovoljan unos vode su brojnim istraživanjima potvrđeni kao zaštitni čimbenici u smislu rizika za CRC (Banjari i

Fako, 2014; Perera i sur., 2012; Johnson i sur., 2013). Anketa koja je razvijena za potrebe ovog istraživanja je uključivala pitanja o svim ovim čimbenicima povezanim s prehrambenim i životnim navikama. Sustav bodovanja koji je razvijen korelira s rizikom za CRC; što je viši broj bodova to su prehrambene i životne navike lošije te je rizik za CRC veći. Utvrđena je statistički značajna razlika za sumu bodova između studenata i studentica (p<0,001; Sl. 2) gdje su studenti (69 bodova, raspon 48 – 88) ostvarili značajno više bodova od studentica (66 bodova, raspon 32 – 87). Ukoliko uzmemo u obzir kako su studenti imali i statistički značajno viši BMI od studentica (Tablica 2), rizik za CRC je dodatno povećan.
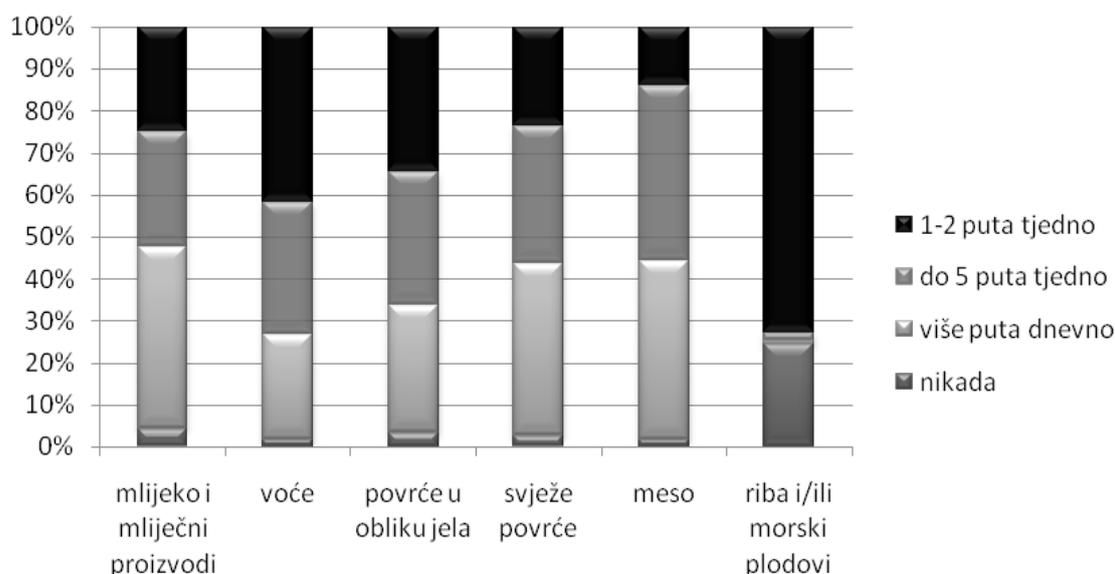
Slika 3. Učestalost konzumacije pojedinih skupina namirnica u ispitivanoj populaciji studenata (N = 446)
Fig. 3. Consumption of specific food groups in the participating student population (N = 446)

Studenti najčešće konzumiraju (Sl. 3) mlijeko i mliječne proizvode (43,5 % više puta dnevno i 24,9 % do 5 puta tjedno) te meso (42,4 % više puta dnevno te 41,3 % do 5 puta tjedno). Voće se u manjoj mjeri od povrća konzumira na dnevnoj bazi, bez obzira radi li se o svježem ili povrću u obliku jela (24,9 % naprema 40,6 % svježe povrće, 26,2 % povrće u obliku jela). S druge strane, voće se u manjoj mjeri od povrća ne konzumira nikada (1,79 % naprema 2,7 % svježe povrće, 3,6 % povrće u obliku jela). Rezultati su u skladu s ranijim podacima o nedovoljnom unosu voća i

povrća među studentskom populacijom (Žiža, 2012; Banjari i sur., 2011; Ćurin i sur., 2006), čime je upitan unos vitamina i minerala (Ćurin i sur., 2006), ali i prehrambenih vlakana (Banjari i Fako, 2014). Riba i morski plodovi najčešće se konzumiraju 1-2 puta tjedno (72,2 %), dok ih nikada ne konzumira 24,4 % studenata. Nizak unos ribe je povezan s niskim unosom omega-3 masnih kiselina koje imaju pozitivan učinak na prevenciju CRC-a (Banjari i Fako, 2014).
U ispitivanoj je populaciji utvrđena statistički značajna korelacija po svim promatranim

aspektima začinjenosti hrane (Tablica 4). Najveći dio studenata preferira slanu i dobro začinjenu hranu koja se najčešće konzumira nekoliko puta mjesečno. Ovi su rezultati zabrinjavajući i svakako bi trebalo poraditi na podizanju svijesti o štetnim učincima ovakve hrane po zdravlje. Djelomično se rezultati mogu objasniti činjenicom da najveći dio studenata koji su sudjelovati u istraživanju dolazi iz regije Slavonije i Baranje koja je poznata po tradicionalnim jako začinjenim jelima (slana, s puno paprike i ljuta jela). Dobiveni rezultati o prehrambenim navikama potvrđuju četvrtu hipotezu istraživanja.

Tablica 4. Prehrambene navike i osobni stavovi vezani uz konzumaciju soli i drugih začina ispitivane populacije studenata (N = 446)

Table 4. Dietary habits and personal attitudes on consumption of salt and other spices of the participating student population (N = 446)

		n	%	p
Dodajete li sol svo- jim obrocima?	nikada	119	26,7	*0,001**
	samo kada jelo nije dovoljno slano	279	62,6	
	gotovo uvijek i prije nego probam jelo	45	10,1	
Volite li jesti ljutu hranu?	ne, ni najmanje	59	13,2	*0,022**
	vrlo slabo ljutu	93	20,9	
	umjereno ljutu	218	48,9	
	jako ljutu	60	13,5	
	ekstremno ljutu	13	2,9	
Ako jedete ljutu hranu, makar i malo, koliko često jedete takvu hranu?	svaki dan	2	0,5	*0,006**
	nekoliko puta tjedno	81	18,2	
	nekoliko puta mjesečno	173	38,8	
	samo za posebne prilike	162	36,3	
Ako možete birati, birate jelo:	sa paprikom	310	69,5	*0,008**
	bez paprike	128	28,7	
Kada sagledate svoju prehranu, smatrate da je vaša hrana:	potpuno nezačinjena i blaga	18	4,0	*0,001**
	sva su jela umjereno začinjena	201	45,1	
	poneko jelo je jače začinjeno, no generalno je umjer- eno začinjena	198	44,4	
	sva hrana je sa puno začina	27	6,1	

Fischerov egzaktni test, *označava statističku značajnost kod p=0,05

Zaključci

Stanje uhranjenosti, životne i prehrambene navike studenata odražavaju brojne negativne karakteristike koje se dovode u direktnu vezu s povećanim rizikom za CRC. Studentice imaju jače izraženu svijest o utjecaju stanja uhranjenosti, životnih i prehrambenih navika na zdravlje, što se očituje kroz lošije navike među studentima i nepovoljnije stanje uhranjenosti.

Literatura

Banjari I (2014) Ditch and Switch. How much supplements do we actually need? Medicinski pregled 67(7-8). In press

Banjari I, Fako J (2014) The importance of an up-to-date evidence based diet planning for colorectal cancer patients. Archive of Oncology. In press

Banjari I, Kenjerić D, Mandić ML, Nedeljko M (2011) Is fad diet a quick fix? An observational study in a Croatian student group. Period biol 13:377-381

Brkić T, Grgić M (2006) Kolorektalni karcinom. Medicus 15:89–97

Campbell PT, Newton CC, Dehal AN, Jacobs EJ, Patel AV, Gapstur SM (2011) Impact of body mass index on survival after colorectal cancer diagnosis: The cancer prevention study-II, nutrition cohort. J Clin Oncol 30:42-52

Center MM, Jemal A, Ward E (2009) International trends in colorectal cancer incidence rates. Cancer epidem biomar 18:1688-1694

Cooper M (2011) Is colorectal cancer preventable? NZMJ 124:96-99

Cummings JH, Bingham SA (1998) Diet and the prevention of cancer. Brit Med J 317:1636–1640

Ćurin K, Knezović Z, Marušić J (2006) Kakvoća prehrane u studentskom centru u Splitu. Med. Jad. 36: 93-100

Ćurković S, Caput-Jogunica R, Gričar I, Zvonarek N (2010) Istraživanje o pušačkim navikama studenata Sveučilišta u Zagrebu. U Zbornik radova 19. ljetne škole kineziologa RH, str. 98-103, Hrvatski kineziološki savez, Zagreb

Debas HT (2003) Gastrointestinal Surgery: Pathophysiology and Management. Springer, USA

Giacometti J, Buretić-Tomljanović A (2013) Prevalencija dislipidemije u studentskoj populaciji Sveučilišta u Rijeci. Medicina fluminensis 49:184-192

Gribovskaja-Rupp I, Kosinski L, Ludwig KA (2011) Obesity and Colorectal Cancer. Clin Colon Rectal Surg 24:229-43

Hrvatski zavod za javno zdravstvo, Registar za rak (2013) Incidencija raka u Hrvatskoj 2011., HZJZ, Bilten br. 36, Zagreb

Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. (2013) Meta-analyses of colorectal cancer risk factors. Cancer Causes Control 24:1207-1222

Kisenauskaitė R, Paškevičienė D (2011) The research of physical condition, physical activity and nutrition of teacher education students. Papers on Anthropology 20:185-198

Kushi LH, Byers T, Doyle C, Bandera EV, McCullough M, Gansler T, Andrews KS, Thun MJ (2006) American Cancer Society guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin 62:254-281

Mitchell P (2012) Colorectal cancer in the young. Future Medicine Ltd 1:355–358

Perera PS, Thompson RL, Wiseman MJ (2012) Recent evidence for colorectal cancer prevention through healthy food, nutrition, and physical activity: implications for recommendations. Curr Nutr Rep 1:44–54

Pollak L (2008) Dodaci prehrani i hrana za posebne prehrambene potrebe. Medicus 17:47-55

Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63:11-30

Strnad M, Šogorić S (2010) Rano otkrivanje raka u Hrvatskoj. Acta Medica Croatica 64:461-468

Zovak M (2007) Korelacija ekstranodalnih depozita i klinickopatoloških faktora u bolesnika operiranih zbog mucinoznog kolorektalnog karcinoma: Disertacija. Medicinski fakultet, Zagreb

Žiža N (2012) Prehrambene i životne navike studenata Sveučilišta u Osijeku: Diplomski rad, Prehrambeno–tehnološki fakultet, Osijek

ESTIMATION OF COLORECTAL CANCER RISK FACTORS RELATED TO NUTRITIONAL AND LIFESTYLE HABITS IN STUDENTS

Ines Banjari, Tihana Ostrognjaj

Department of Food and Nutrition Research, Faculty of Food Technology Osijek, University of Osijek, F. Kuhača 20, HR-31000 Osijek, Croatia

Summary

Introduction: Colorectal carcinoma (CRC) is one of the leading public health concerns around the globe and the second death cause in Croatia. By incidence and mortality CRC is on the third place in both genders, with significant differences through regions. Etiology incudes interaction between inheritance, nutrition and lifestyle habits. Around 90% of all CRC cases are in direct correlation with the diet. Student population because of change in place of living, higher independence and change in life roles, and increased number of obligations change nutritional habits, usually unfavourably. Nutritional

habits established in this period are prolonged further through life, and the incidence and mortality due to CRC are showing significant increase from 40 years on.

Aim: To estimate the risk factors for CRC related to dietary and lifestyle habits in student population.

Materials and methods: 446 students (23 years; 37.2% male, 62.8% female) completed an anonymous questionnaire developed specifically for this research. Ranking into low, medium or high risk group was made according to their answers.

Results: Number of CRC risk factors are highly abundant: high prevalence of overweight (23.1%) and obesity (6.1%), smoking (30.0%), low level of physical activity (25.6% inactive, 30.3% seasonally active), high consumption of alcohol (20.6% weekly, 1.4% daily), low consumption of fish, fruits and vegetables and high consumption of meat and meat products, preference of food high in salt and spices, and positive family anamnesis (47.5% with predisposition, 12.1% with positive anamnesis). Significant difference was found for gender ($p < 0,001$) and family anamnesis.

Conclusion: The most important CRC risk factors related to nutritional habits are high consumption of meat and meat products, preference of salty and spicy foods, as well as low intake of fish, fruits and vegetables. Significantly higher risk was determined among male students, and of particular concern is that the highest risk of CRC was found among students with positive family anamnesis.

Key words: colorectal carcinoma, students, risk estimation, nutritional habits, lifestyle habits

POKAZATELJI UHRANJENOSTI I NUTRITIVNA POTPORA ADOLESCENTICA OBOLJELIH OD ANOREKSIJE NERVOZE

Vedrana Škoro Petranović[1], Orjena Žaja[2], Ines Banjari[3]

[1]Klinički bolnički centar Sestre milosrdnice, Služba prehrane, Vinogradska 29, HR-10000 Zagreb, Hrvatska, vedrana.skoro@kbcsm.hr
[2]Klinički bolnički centar Sestre milosrdnice, Klinika za pedijatriju, Vinogradska 29, HR-10000 Zagreb, Hrvatska
[3]Prehrambeno-tehnološki fakultet Osijek, Zavod za ispitivanje hrane i prehrane, F. Kuhača 20, HR-31000 Osijek, Hrvatska

Sažetak

Uvod: Anoreksija nervoza (AN) predstavlja trajan poremećaj odnosa prema hrani i izgledu vlastitog tijela koji dovodi do znatnog oštećenja tjelesnog zdravlja i psiho-emocionalnog funkcioniranja. Pridružene metaboličke komplikacije zbog fizioloških osobitosti razdoblja rasta i spolnog razvoja mogu biti potencijalno ireverzibilne, izvjesno i fatalne ukoliko su kasno prepoznate.

Cilj: Istražiti vrijednosti antropometrijskih parametara, elektrolitska odstupanja te ispitati trajanje i broj hospitalizacija ovisno o primjeni, trajanju i kalorijskoj vrijednosti nutritivne potpore kod adolescentica oboljelih od AN.

Materijali i metode: Retrospektivna studija je provedena na 171 hospitaliziranoj pacijentici Klinike za pedijatriju KBC-a Sestre milostrdnice u Zagrebu, prosječne dobi 14,8 ± 2,5 godina,uvidom u medicinsku dokumentaciju i antropometrijska mjerenja.

Rezultati: Neki od parametara poput tjelesne mase, postotka idealne tjelesne mase i indeksa tjelesne mase bili su značajno niži od očekivanih s obzirom na dob, a kod elektrolita nisu pronađena veća odstupanja. Ispitanice koje su dnevno primale 1068,7 ± 425,5 kcal nutritivne potpore provele su duže vrijeme u bolnici, ali su bile manji broj puta hospitalizirane.

Zaključak: Pravilnim i odgovarajućim dijagnostičkim pristupom, ovaj poremećaj prehrane moguće je otkriti u ranijoj fazi kada funkcija intervencije i multidisciplinarne stručne pomoći (liječnik specijalist, psihijatar, psiholog, klinički nutricionist) može spriječiti daljnje razvijanje bolesti i popratne zdravstvene komplikacije.

Ključne riječi: Anoreksija nervoza, adolescentice, antropometrija, elektroliti, nutritivna potpora, multidisciplinarna pomoć

Uvod

Poremećaji prehrane su izrazito opasni poremećaji ponašanja, a nastaju kao rezultat međusobnog djelovanja niza čimbenika poput poremećaja ličnosti, emocionalnih poremećaja, pritiska obitelji i okoline, ali i opsjednutosti mislima o mršavošću (Vidović, 1998).Posljedice koje su uzrokovane poremaćajima prehrane su brojne i pogađaju sve tjelesne sustave (Sharpe i Potts, 2006). Anoreksija nervoza (AN) jeporemećaj prehrane karakeriziranodbijanjem hrane, željom za mršavošću, strahom od debljanja, odražavanjem tjelesne mase ispod minimalne normalne granice te amenorejom u žena (Folnegović-Šmalc, 2010).Kod AN razlikujemo restriktivni tip i bulimični tip AN (ADA, 2006). Zbog prisutnosti bolesti kroz duže vremensko razdoblje ne može se sa sigurnošću reći koliki je točan postotak oboljelih osoba od AN prije nego li je sama bolest klinički ustanovljena, a u većoj je mjeri zastupljena u razvijenim zemljama. U95% slučajeva zahvaća žene i to većinom adolescentice (70%) (ADA, 2006) koje već u toj osjetljivoj dobiisprobavaju brojne dijete kako bi kontrolirale tjelesnu masu (Banjari i sur., 2011). U muškoj populaciji, najviše su zahvaćeni muškarci homoseksualne orijentacije (ADA, 2006). Stopa morbiditeta i mortaliteta je među najvišim uspoređujući s drugim psihičkim bolestima.

Provođenje raznovrsnih dijeta unatoč izrazitoj mršavosti, konzumiranje niskokaloričnih namirnica, opsjednutost brojanjem kalorija, mjerenje i vaganje porcija hrane, čitanje raznovrsnih knjiga o dijetama, proučavanje deklaracija na prehrambenim proizvodima,laganje o unosu hrane, te skrivanje i bacanje hrane česti su simptomi oboljelih (Folnegović-Šmalc, 2010).Većina pripada srednjim ili gornjim društvenim slojevima,

*Corresponding author: vedrana.skoro@kbcsm.hr

inteligentni su, kompulzivni i sitničavi te imaju vrlo visoko mjerilo uspjeha (Ambrosi-Randić, 2003).Nadutost, abdominalne tegobe i opstipacija, bradikardija, niski krvni tlak, endokrine promjene, hipotermija, depresija, lanugo dlačice i edemi stanja su koja se često javljaju u pacijenata oboljelih od AN. Menstruacije, u jako pothranjenih bolesnica, obično prestaju. Dehidracija, metabolička alkaloza i niski serumski kalij mogu se pogoršati povraćanjem i korištenjem laksativa ili diuretika (Halmi i Falk, 2006). Dolazi i do smanjenja koštanog i mišićnog tkiva, osteoporoze i osteopenije, proteinske i energetske malnutricije, anemije, ispadanja kose, hipoglikemije, hiperkolesterolemije, slabe koncentracije, umora i smanjenog rasta (ADA, 2006). Karakterističan je gubitak 15% i više tjelesne mase kod mlade osobe koja se boji debljine, amenoreja u trajanju najkraće 3 mjeseca, poricanje bolesti, vrlo niska količina tjelesne masti i iskrivljena slika vlastitog tijela (Sharpe i Potts, 2006). Blagi oblici bolesti su često neotkriveni i rijetko dovode do smrti, no u teških oblika, bez liječenja, smrtnost je blizu 10%. Polovica bolesnika, uz liječenje, vraća većinu izgubljene mase te se endokrini poremećaji i druge komplikacije povlače, u 25% slučajeva dolazi do recidiva, a u preostalih 25% ishod je loš te ostaju trajne tjelesne i psihičke komplikacije (Saccomani i sur., 1998).

Oboljeli od AN često odbijaju ili odustaju od liječenja (Pike, 1998). Svaki program liječenja AN trebao bi obuhvaćati vođenje prehrane, redovito medicinsko praćenje te psihoterapiju. Vrlo je važan stadij bolesti jer o stadiju u kojem se bolest prepozna i spremnosti na suradnju sa stručnim osobama, ovisit će način liječenja (Kos, 2010).Najbolje rezultate daje liječenje koje uz povratak tjelesne mase osigurava i normalizaciju prehrambenih navika. Iako većina pacijenata verbalizira svoju želju za oporavkom, teško ih je uvjeriti na liječenje koje će ih povratiti na medicinski prihvatljivu tjelesnu masu. Prognoza za adolescente je bolja nego za odrasle; oporavak se bilježi kod otprilike 70% pacijenata, hospitalizirani pacijenti koji dovrše liječenje imaju uspješniji ishod od nehospitaliziranih, a obiteljska terapija se pokazala najučinkovitijom (Guarda, 2008). Što je klinička slika ozbiljnija na početku liječenja i što je hospitalizacija kraća, rezultati liječenja će biti lošiji te će vjerojatnost recidiva biti veća (Saccomani i sur., 1998). Ukoliko postoji sumnja na AN, uzima se anam-

neza, provodi se tjelesni pregled, laboratorijske pretrage i kožni testovi alergije. Antropometrijska mjerenja govore o prehrambenim navikama kroz dulje vremensko razdoblje i ukazuju na eventualnu malnutriciju i pothranjenost(Lee i Nieman, 2010) te ako je tjelesna masa manja od 80% one koja se predviđa s obzirom na visinu treba posumnjati na pothranjenost (Kos, 2010); indeks tjelesne mase (ITM) koji korigira masu tijela s visinom, a dobivena brojka uspoređuje se sa standardom te ukazuje na normalnu tjelesnu masu, pretilost ilistanje pothranjenosti (kod djece i adolescenata promatraju se percentili na osnovu grafikona rasta specifičnih za dob i spol);izračunavanje idealne tjelesne mase pojedinca (IBW, engl. *ideal body weight*) uzevši u obzir visinu, dob i spol osobe, a podrazumijeva optimalne udjele pojedinih komponenti tijela (masnog tkiva, koštane mase, mišićne mase i vode);kožni nabor stražnje strane nadlaktice iznad tricepsa mišića, TSF, (engl. *triceps skinfold thickness*), debljinom kožnog nabora se procjenjuje postotak masnog tkiva u tijelu. Mjeri se i MAC (engl. *upper mid-arm circumference ili prosječni opseg srednjeg dijela nadlaktice*) i određuje AMC (engl. *arm muscle circumference ili prosječna vrijednost područja mišića srednjeg dijela nadlaktice*)(Lee i Nieman, 2010). Prosječni opseg srednjeg dijela nadlaktice u žena iznosi 28 ± 6 cm, a prosječne vrijednosti područja mišićja srednjeg dijela nadlaktice u žena iznose 30 ± 7 cm2. Ukoliko su izmjerene vrijednosti manje od 75% standarda (ovisno o dobi) smanjena je nemasna tjelesna masa (Jurčić i sur., 2008).

Kod oboljelih od AN biokemijski parametri se, u većini slučajeva, zadržavaju unutar granica normale, no u teškim slučajevima dolazi do razvoja poremećaja ravnoteže elektrolita i minerala (Nova i sur., 2004).Može doći do hiponatremijekoja je povezana s deficitom natrija koji se gubi zbog uporabe diuretika ili prilikom povraćanja (Caregaro i sur., 2005), a zbog istih razloga moguće je stanje hipokalijemije i hipokloremije (Field i sur., 2006).Zbog deficita kalcija osteoporoza se u oboljelih od AN razvija u 38 do 50% slučajeva i povezana je s neadekvatnom formacijom kostiju (Legroux-Gerot i sur., 2005), no i ako ne dođe do razvoja osteoporoze, AN uzrokuje ozbiljne promjene na kosturu adolescentica (Balenović i sur., 2008; Oswiecimska i sur., 2007). Deficit fosfora i magnezija je vrlo rijedak, ali uslijed tzv. *refeeding sindroma* može doći do

razvoja hipofosfatemije (Štraus i Dodig, 2009). Sama anamneza uključuje i pitanja o unosu hrane gdje nutricionist provodi upitnik o prehrani, zapisuje popis svih namirnica koje su se konzumirale u posljednja 24 sata ili upućuje na vođenje vlastitog dnevnika prehrane.Prehrambenu potporu, čiji je cilj povećanje nemasne tjelesne mase, trebaju mnogi pothranjeni pacijenti. U oboljelih od AN hranjenje na usta može biti otežano pa se koriste bihevioralne mjere, poput ohrabrivanja prilikom jela, nuđenja omiljenih jela, malih obroka ili točan raspored obroka. Kada su bihevioralne mjere neučinkovite prehrambena potpora se inducira oralno, enteralnim putem ili parenteralno (Kos, 2010). Prema smjernicama za liječenje AN, parenteralnu prehranu nije potrebno uvoditi, osim u situacijama kada je nemoguće uvesti nazogastričnu sondu. Uvođenjem parenteralne prehrane u takvih pacijenata povratit će se ravnoteža elektrolita, riješiti će se problem dehidracije te kardiovaskularni problemi nastali zbog pothranjenosti organizma (Mehler i Weiner, 2007). Kada tjelesna masa u kratkom roku padne ispod 75% idealne važno je pravovremeno intervenirati. Prehrambeno liječenje započinje uz primjenu 30 do 40 kcal/kg/dan, što tijekom hospitalizacije rezultira povećanjem mase do 1,5 kg/tjedno, a do 0,5 kg/tjedno tijekom ambulantnog liječenja. Elementarnim kalcijem u dozama od 1200 do 1500 mg/dan i vitaminom D u dozama od 600 do 800 IU/dan liječi se gubitak koštane mase. Antipsihotici mogu pomoći pri dobivanju na tjelesnoj masi i ublažiti strah od debljanja (Schebendach, 2008).

Materijali i metode

U istraživanje je bilo uključeno 171 djevojčica i djevojaka u dobi od 8 do 23 godine, koje su bile hospitalizirane na Odjelu za gastroenterologiju Klinike za pedijatriju KBC-a „Sestre milosrdnice" u Zagrebu s dijagnozom AN prema DSM-IV (eng. *Diagnostic and Statistical Manual of Mental Disordersili* Dijagnostički i statistički priručnik mentalnih poremećaja) kriterijima (APA, 1994) u razdoblju od 1999. do 2011. godine.Podaci su dobiveni tjelesnim pregledom i antropometrijskim mjerenjima tjelesne mase (TM) koja se mjeri medicinskom vagom s utezima (Seca, UK) i tjelesne visine (TV) koja se mjeri pomoću antropometra bez obuće.Idealna tjelesna masa (IBW) je određena iz NHANES

percentilnih krivulja za tjelesnu masu po dobi za djevojčice (NCHS, 2002), a udio idealne tjelesne mase se računa prema jednadžbi:

$$\% \, IBW = (TM \, (kg) \, / \, IBW \, (kg)) \, x \, 100$$

Mjerenja potkožnog masnog tkiva mjere se kaliperom na nadlaktici desne ruke s vanjske strane i nadlaktice desne ruke s unutarnje strane, kako bi se dobila vrijednost kožnog nabora iznad tricepsa (TSF), prosječan opseg srednjeg dijela nadlaktice (MAC) te se pomoću tih vrijednosti izračuna prosječna vrijednost područja mišića srednjeg dijela nadlaktice (AMC)prema jednadžbi:

$$AMC(mm) = MAC \, (cm) - [0.314 \, xTSF \, (mm)]$$

Pratila se godina pojave bolesti i trajanje bolesti u mjesecima, kao i tjelesna masa prije bolesti te se gubitak kilograma od početka bolesti (% gubitka) računa prema jednadžbi:

$$\% \, gubitka = [(TM \, prije \, anoreksije - TM) \, / \, TM \\ prije \, anoreksije] \, x \, 100$$

Odmah pri primitku u bolnicu vadila se krv ispitanicama kako bi se odredili elektroliti (Na, K, Cl, Ca, P, Mg) u laboratoriju Bolnice prema standardnim metodama (HRN EN ISO 15189, 2008).

Podaci o nutritivnoj potpori prikupljeni su iz medicinske dokumentacije ispitanica. U slučaju ispitanica koje su bile hospitalizirane više puta prikupljeni su samo podaci koji su se odnosili na prvi boravak u bolnici. Uz prisutnost ili odsutnost nutritivne potpore prikupljeni su i podaci o vrsti eneteralnog pripravka koji je korišten za ispitanice kod kojih je taj podatak bio dostupan, kalorijskoj vrijednosti primjenjenoj putem nutritivne potpore kroz 24 sata, vrijeme trajanja nutritivne potpore u danima te jesu li ispitanice uz enteralni pripravakunosile peroralno i uobičajenu bolničku hranu. Također je zabilježena duljina trajanja hospitalizacije; u slučaju kada je bilo više od jedne hospitalizacije uključen je samo podatak o trajanju prve hospitalizacije te ukupan broj hospitalizacija.

Podaci su obrađeni primjenom programa MS Office Excel, (2010 Microsoft, USA) uz primjenu parametrijskog t-testa za razinu značajnosti od p=0,05. Svi su rezultati izraženi kaosrednja vrijednost uz prikaz standardne devijacije (± SD) te

minimum i maksimum.

Rezultati i rasprava

Raspon starosne dobi u ispitivanom uzorku je bio od 8 do 23 godine, prosječno 14,8 ± 2,5godina; najzastupljenije su 14-godišnjakinje (23,97%), a što je u skladu s očekivanjima jer prema istraživanjima AN pogađa adolescentice i to najčešće 14-godišnjakinje (Halmi i sur., 1979). Indeks tjelesne mase ispitanica u uzorku iznosi prosječno 15,77 ± 1,99 kg/m2, a prosječan pos-

totak idealne tjelesne mase iznosi 77,57 ± 9,83 % (Tablica 1) što odgovara stanju teške pothranjenosti (Kos, 2010). Tjelesna masa ispitanica kreće od 22 do 65,7 kg, prosječno 41,87 ± 7,21 kg.Dok se tjelesna masa ispitanica prije pojave bolesti kretala od 25 do 96 kg, prosječno je iznosila 54,13 ± 10,56 kg, uz prosječan gubitak 21,69 ± 8,58 % tjelesne mase s najvećim gubitkom kilograma od 47,85%. Prosječnotrajanje bolesti je 12,7 ± 14,2mjeseci, a 108 mjeseci (9 godina) najduže je trajanje bolesti (Tablica 1).

Tablica 1. Prikaz prosjeka, minimuma i maksimuma za: dob ispitanica, tjelesnu masu (TM), tjelesnu visinu (TV), idealnu tjelesnu masu (IBW), postotak idealne tjelesne mase (% IBW), indeks tjelesne mase (BMI), tjelesnu masu prije anoreksije (TM prije AN), postotak gubitka kilograma, dobi ispitanica kod pojave bolesti i trajanje bolesti, debljinu kožnog nabora tricepsa (TSF), opseg srednjeg dijela nadlaktice (MAC), područje mišića srednjeg dijela nadlaktice (AMC)(N = 171).
Table 1. Preview the average, minimum and maximum for: age, body weight, body height, ideal body weight, percentage of ideal body weight, body mass index, body weight before anorexia, weight loss percentage, the age of onset and disease duration, triceps skinfold thickness, upper mid-arm circumference, arm muscle circumference(N = 171).

Parametri	Prosjek	Minimum	Maksimum
Dob ispitanica (god)	14,8 ± 2,5	8	23
TM (kg)	41,87 ± 7,21	22	65,7
TV (m)	1,63 ± 0,08	1,3	1,87
IBW (kg)	54,13 ± 7,29	27,5	69,6
% IBW	77,57 ± 9,83	52,54	106,87
ITM (kg/m2)	15,77 ± 1,99	11,4	22,1
TM prije AN (kg)	54,12 ± 10,56	25	96
% gubitka	21,69 ± 8,58	2,6	47,85
Dob ispitanica kod pojave bolesti (god.)	14,4 ± 2,6	8	23
Trajanje bolesti (mj.)	12,7 ± 14,2	2	108
TSF (mm)	5,50 ± 3,31	0,8	29
MAC (mm)	19,83 ± 2,41	12	26,2
AMC (mm)	17, 96 ±2,37	8,89	24,3

Pokazalo se da je prva hospitalizacija trajala prosječno 34,9 ± 16,6 dana (Tablica 2), 70 (40,94%) ispitanica bilo je hospitalizirano samo jednom, dok su se 101 (59,06%) ponovno vraćale u bolnicu (Sl. 1). Pacijentice su hospitalizirane prosječno 2,0± 1,3 puta (Tablica 2).

Tablica 2. Prikaz prosjeka, minimuma i maksimuma za trajanje prve hospitalizacije u danima i ukupni broj hospitalizacija(N = 171).
Table 2. Preview the average, minimum and maximum for the duration of the first hospitalization in days and the total number of hospitalizations (N = 171).

	Trajanje prve hospitalizacije (dani)	Ukupni broj hospitalizacija (n)
Prosjek	34,9 ± 16,6	2,0 ± 1,3
Minimum	4	1
Maksimum	93	10

Primarna amenoreja nastupila je kod 35 ispitanica, odnosno 20,47%, a sekundarna kod 136 ispitanica, odnosno 79,53% (Tablica 3) što je i očekivano prilikom dijagnosticirane AN.

125 ispitanica (72,67%) je primalo nutritivnu potporu (Sl. 2)te su prosječno dnevno putem nutritivne potpore unosile 1068,69 ± 425,5 kcal.

Tablica 3. Prikaz udjela primarne i sekundarne amenoreje u ispitanica (N = 171).

Table 3. Preview share of primary and secondary amenorrhea in respondents (N = 171).

Amenoreja	Broj ispitanica (n)	Udio ispitanica (%)
Primarna	35	20,47
Sekundarna	136	79,53

Nutritivna potpora je trajala prosječno 28,5 ± 15,7 dana (Tablica 4). Od 1999. do 2004. godine nutritivna potpora se davala u 50% slučajeva, no od 2004. godine postaje uobičajena praksa pri

liječenju AN i primjenjuje se u većini slučajeva, 84,75%, kako je i propisano u smjernicama za liječenje AN (ADA, 2006).

Tablica 4. Prikaz prosječne kalorijske vrijednosti koja se daje tijekom 24 sata ispitanicama koje primaju nutritivnu potporu i prosječni broj dana trajanja nutritivne potpore.

Table 4. Preview of average calorific value that is given for 24 hours respondents who receive nutritional support, and the average number of days of nutritional support.

	Kalorijska vrijednost/24 h (kcal)	Trajanje nutritivne potpore (dani)
Prosjek	1068,7 ± 425,5	28,5 ± 15,7
Minimum	330	2
Maksimum	2640	90

Uobičajenu bolničku hranu *per os* uzimalo je 111 ispitanica(64,9%), 54 ispitanica (31,6%) je minimalno uzimalo bolničku hranu, dok su 6 ispitanica (3,5%)u potpunosti odbijale obroke (Tablica 5). Iz tih podataka može se zaključiti

kako je 64,9% ispitanica željelo i bilo spremno na oporavak, a ostalih 35,1% odbija liječenje i povratak na normalnu tjelesnu masu ih plaši, što je u skladu s drugim istraživanjima (Walsh i sur., 2001).

Tablica 5. Prikaz ispitanica prema uzimanju regularne bolničke hrane (N = 171).

Table 5. Preview of respondents according to intake of regular hospital food (N = 171).

Per os bolnička hrana	Broj ispitanica (n)	Udio (%)
Uzima	111	64,9
Minimalno uzima	54	31,6
Ne uzima	6	3,5
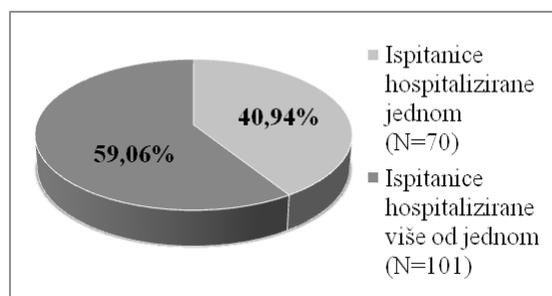
Slika 1. Prikaz udjela ispitanica hospitaliziranih jednom i ispitanica hospitaliziranih više od jednom (N = 171).
Fig. 1. Preview share of respondents hospitalized once and respondents are hospitalized more than once(N = 171).
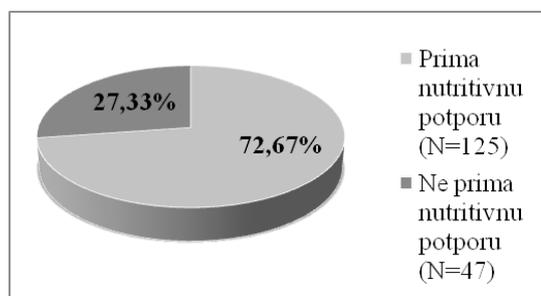
Slika 2. Prikaz udjela ispitanica koje primaju nutritivnu potporu (N = 171).
Fig.2. Preview share of respondents who receive nutritional support (N = 171).

Provjerom hipoteze da su ispitanice koje su primale nutritivnu potporu hospitalizirane manje puta od ispitanica koje nisu primalne nutritivnu potporu nije utvrđena statistički značajna razlika

(p=0,243), odnosno nema značajne razlike u broju hospitalizacija unutar dvije grupe ispitanica (Tablica 6).

Tablica 6. Utjecaj prisutnosti, odnosno odsutnosti nutritivne potpore na broj hospitalizacija i trajanje hospitalizacije u danima te postotak idealne tjelesne mase (% IBW) s obzirom na prisutnost, odnosno odsutnost nutritivne potpore (N = 53).
Table 6. The impact of the presence or absence of nutritional support on the number of hospitalizations and length of hospitalization in days and percentage of ideal body weight (IBW%) due to the presence or absence of nutritional support (N = 53).

Parametar	Ispitanice koje nisu primale nutritivnu potporu	Ispitanice koje su primale nutritivnu potporu	*p*
Broj hospitalizacija (n)	2,0 ± 1,2	2,7 ± 2,0	0,243
Trajanje hospitalizacije (dani)	32,8 ± 14,5	45,7 ± 13,5	0,003*
% IBW	78,1 ± 9,3	71,6 ± 9,6	0,029*

T-test, *označava statističku značajnost kod p< 0,05

S druge strane, statistički značajna razlika je utvrđena za prosječno trajanje hospitalizacije (p=0,003), čime je potvrđena hipoteza da je u ispitanica koje su primale nutritivnu potporu trajanje hospitalizacije dulje nego u ispitanica koje nisu primale nutritivnu potporu. Ispitanice koje su primale nutritivnu potporu prosječno su bile hospitalizirane 45,7 dana, dok je prosječna

hospitalizacija pacijenticama bez nutritivne potpore bila 13 dana kraća (Tablica 6).Navedene hipoteze odnose se na razdoblje od 1999. do 2004. godine, kada je nutritivnu potporu primalo otprilike polovica ispitanica, a za razdoblje od 2004. godine kada je većina pacijentica primalo nutritivnu potporu nije napravljena statistička obrada zbog raskola u broju podataka.

Tablica 7. Utjecaj postotka idealne tjelesne mase (% IBW) na trajanje hospitalizacije (N = 171).
Table 7. The impact of the percentage of ideal body weight (IBW%) on the duration of hospitalization (N = 171).

	% IBW niži od 77%	% IBW viši od 77%	*p*
Trajanje hospitalizacije (dani)	40,3 ± 16,2	30,2 ± 15,6	0,002*

T-test, *označava statističku značajnost kod p< 0,05

U razdoblju od 1999. do 2004. godine nutritivna potpora je pripisivana samo ispitanicama s nižim postotkom idealne tjelesne mase (40,3 dana naprema 30,2 dana, p=0,002) (Tablica 7), a u skladu je s rezultatima drugih istraživanja koja su utvrdila kako je liječenje dugotrajnije ukoliko je klinička slika AN teža(Saccomani i sur., 1998).

Od 2004. do 2011. godine 100 od ukupno 118 pacijentica primalo je nutiritivnu potporu i kod njih je trajanje hospitalizacije bilo dulje nego u ispitanica koje nisu primale nutritivnu potporu, odnosno 34,2 ± 16,1 dana naprema 21,7 ± 22,6 dana, a broj hospitalizacija bio je manji, 1,8 ± 1,1 puta naprema 2,0 ± 1,2 puta (Tablica 8).

Tablica 8. Prikaz prosječnog trajanja i broja hospitalizacija za razdoblje od 2004. do 2011. godine (N = 118).
Table 8.Showing the average duration and the number of hospitalizations for the period 2004 to 2011 (N = 118).

Parametar	Ispitanice koje primaju nutritivnu potporu	Ispitanice koje ne primaju nutritivnu potporu
Broj ispitanica (n)	100	18
Trajanje hospitalizacije (dani)	34,2 ± 16,1	21,7 ± 22,6
Broj hospitalizacija (n)	1,8 ± 1,1	2,0 ± 1,2

Ti podaci se slažu s očekivanjima da se u hospitaliziranih pacijenata oboljelih od AN koji su duže hospitalizirani i koji su više dobili na tjelesnoj masi, javlja manje relapsa (Schebendach, 2008).

Razlozi odstupanja od referentnog intervala kod elektrolita mogu biti uzrokovani povraćanjem ili proljevom uzrokovanim zlouporabom laksativa, što će smanjiti razine kalija, natrija i klorida ispod referentnih vrijednosti (Connan i sur., 2000; Imbierowicz i sur., 2004) pa možemo zaključiti da je kod ispitanica (Tablica 9) sa sniženom razinom elektrolita prisutno takvo ponašanje. Razine kalcija, fosfata i magnezija u krvi su u nekoliko slučajeva iznad referentnog intervala. Hipermagneziju može uzrokovati unos laksativa, hiperkalcemiju i hiperfosfatemiju dehidracija (Field i sur., 2006; Misra i sur., 2004) pa možemo zaključiti da je u nekih ispitanica prisutno korištenje laksativa dok su druge pri primitku u bolnicu bile dehidrirane.

Tablica 9. Prikaz prosječnih vrijednosti elektrolita i udjela ispitanica čije su vrijednosti navedenih parametara niže, odnosno više od referentnih intervala.
Table 9. Showing the average value of the electrolyte and the share of respondents whose values are lower or more than the reference interval.

Biokemijski parametri	Prosjek	Udio ispitanica s vrijednostima nižim od referentnog intervala (%)	Udio ispitanica s vrijednostima višim od referentnog intervala (%)
Kalij (mmol/L)	4,05 ± 0,45	5,85	0
Natrij (mmol/L)	139,99 ± 2,56	1,75	0
Kloridi (mmol/L)	102,8 ± 3,58	1,75	7,02
Kalcij (mmol/L)	2,52 ± 0,11	0	15,79
Fosfati (mmol/L)	1,27 ± 0,21	0,58	23,98
Magnezij (mmol/L)	0,95 ± 0,47	6,43	11,7

Zaključak

AN je bolest koja pogađa većinom adolescentice, a među psihičkim bolestima ima najvišu stopu mortaliteta. Stoga je od iznimne važnosti kontinuirano educirati adolescente glede prehrane, tjelesne kondicije i načina življenja. Prevalencija AN je svake godine veća, a etiologija bolesti je još uvijek nepoznata. Djelovanjem multidisciplinarnog tima, koji zajedničkim radom prate metabolički status pacijenta i provode medicinsku nutritivnu terapiju, omogućuje se brži i bolji oporavak.Kako bi se spriječio recidiv svim je bolesnicima potrebno dugotrajno multidisciplinarno liječenje uz primjenu nutritivne potpore.

Literatura

1. Ambrosi-Randić N(2003) Razvoj poremećaja hranjenja, 1 izd., Naklada Slap, Zagreb.
2. American Dietetic Association. (2006) Nutrition Diagnosis: A CriticalStep in the Nutrition Care Process Anorexia nervosa. American Dietetic Association; Chicago, IL.
3. Balenović A, Žaja Franulović O, Jurčić Z, Vrkić N, Punda M, Kusić Z (2008) Poremećaj gustoće kostiju u djece s poremećajem hranjenja. Paediatr Croat 52 (Suppl 1):126-129.
4. Banjari I, Kenjerić D, Mandić ML, Nedeljko M (2011) Is a fad diet a quick fix? An observational study in a Croatian student group. Periodicum biologorum 113:377-381.
5. Caregaro L, Di Pascoli L, Favaro A, Nardi M, Santonastaso P (2005) Sodium depletion and hemoconcentration: Overlooked complications in patients with anorexia nervosa? Nutrition 21:438-445.
6. Connan F, Lightman S, Treasure J (2000) Biochemical and endocrine complications. Eur Eat Disord Rev 8:144-157.
7. Diagnostic and statistical manual of mental disorders (1994) American Psychiatric Association, Washington.
8. Field MJ, Burnett L, Sullivan DR, Stewart P(2006) Clinical biochemistry and metabolism. U: Davidson's Principles & Practice of Medicine, (Boon NA., Colledge NR, Walker BR, Hunter JAA, ured.), Elsevier Limited, Edinburgh, str. 419-454.
9. Folnegović-Šmalc V. (2010) Psihijatrija. U: MSD priručnik dijagnostike i terapije, (Ivančević Ž, ured.), Placebo, Split, str. 1665-1744.
10. Guarda AS (2008) Treatment of anorexia nervosa: Insights and obstacles. Physiol Behav 94:113-120.
11. Halmi KA, Falk JR (2006) Common physiological changes in anorexia nervosa. Int J Eat Disord 1:16-27
12. HRN EN ISO 15189: 2008, Medicinska biokemija – ispitivanja u području kliničke kemije, laboratorijske

hematologije i koagulacije, laboratorijske imunologije, toksikologije i molekularne dijagnostike.

13. Imbierowicz K, Curkovic I, Braks K, Geiser F, Liedtke R, Jacoby GE (2004) Effect of weight-regulating practices on potassium level in patients with anorexia or bulimia nervosa. Eur Eat Disord Rev 12:300-306.

14. Jurčić Z, Žaja Franulović O, Juran A (2008) Klinička ocjena pothranjenosti u anoreksiji nervozi. Paediatr Croat 52 (Suppl 1):91-101

15. Kos M. (2010) Poremećaji prehrane. U: MSD priručnik dijagnostike i terapije, (Ivančević, Ž., ured.), Placebo, Split, str. 1-61.

16. Legroux-Gerot I, Vignau J, Collier F, Cortet B (2005) Bone loss associated with anorexia nervosa. Joint Bone Spine 72:489-495.

17. Lee RD, Nieman DC(2010) Nutritional Assessment, 5. izd., McGraw-Hill Companies, Inc., New York.

18. Mehler PS, Weiner KL (2007) Use of total parenteral nutrition in the refeeding of selected patients with severe anorexia nervosa. Int J Eat Disord40:285-287.

19. Misra M, Aggarwal A, Miller KK, Almazan C, Worley M, Soyka LA, Herzog DB, Klibanski A (2004) Effects of anorexia nervosa on clinical, hematologic, biochemical, and bone density parameters in community-dwelling adolescent girls. Pediatrics 114:1574-1583

20. Nova E, Lopez-Vidriero I, Varela P, Toro O, Casas J, Marcos A (2004) Indicators of nutritional status in restricting-type anorexia nervosa patients: a 1-year follow-up study. Clin Nutr 23:1353-1359.

21. Oswiecimska J, Ziora K, Pluskiewicz W, Geisler G, Broll-Waska K, Karasek D, Dyduch A (2007) Skeletal status and laboratory investigations in adolescent girls with anorexia nervosa. Bone 41:103-110.

22. Pike KM (1998) Long-term course of anorexia nervosa. Response, relapse, remission and recovery. Clin Psychol Rev 18:447-475.

23. Saccomani L, Savoini M, Cirrincione M, Vercellino F, Ravera G (1998) Long-term outcome of children and adolescents with anorexia nervosa: study of comorbidity. J Psychosom Res 44:565-571.

24. Schebendach JE (2008) Nutrition in eating disorders. U: Krause's Food & Nutrition Therapy, (Mahan, L. K., Escott-Stump, S., ured.), Saunders Elsevier, St. Louis, str. 563-586.

25. Sharpe MC, Potts SG (2006) Medical psychiatry. U: Davidson's Principles & Practice of Medicine, (Boon, N. A., Colledge, N. R., Walker, B. R., Hunter, J. A. A., ured.), Elsevier Limited, Edinburgh, str. 227-252.

26. Štraus B, Dodig S (2009) Voda i elektroliti. U: Štrausova medicinska biokemija, (Čvorišćec, D., Čepelak, I., ured.), Medicinska naklada, Zagreb, str. 55-82.

27. Vidović V(1998) Anoreksija i bulimija, 2 izd.,4P, Zagreb.

28. Walsh JME, Wheat ME, Freund K (2001) Detection, evaluation, and treatment of eating disorders. J Gen Intern Med 15:577-590.

29. 2000 CDC growth charts for the United States: Methods and development (2002) National Center for Health Statistics, Washington.

INDICATORS OFNUTRITIONAL STATUSANDNUTRITIONALSUPPORT FORADOLESCENTS WITHANOREXIA NERVOSA

Vedrana Škoro Petranović[1], Orjena Žaja[2], Ines Banjari[3]

[1]Sestre milosrdnice University Hospital Centre, Department of Food, Vinogradska 29, HR-10000 Zagreb, Croatia, vedrana.skoro@kbcsm.hr

[2]Sestre milosrdnice University Hospital Centre, Department of Pediatrics, Vinogradska 29, HR-10000 Zagreb, Croatia

[3]Faculty of Food Technology Osijek, Department of Food and Nutrition Research, F. Kuhača 20, HR-31000 Osijek, Croatia

Original scientific paper

Summary

Anorexia nervosa is a permanent disorder of attitude towards food and image on the appearance of one's own body, which leads to disrupted food intake, significant damage to physical health and psycho-emotional functioning. Associatedmetaboliccomplicationsdue to the physiologicalcharacteristics ofthe period of growthandsexual developmentcan be potentiallyirreversible, certainlyfatalifbeen recognizedlate. The aim was to investigate the value of anthropometric parameters with possible electrolytic deviations, and toexamine thedurationandnumberof hospitalizations depending onthe presence and the frequency ofnutritionalsupport, caloric valueanddurationin 171patientshospitalizedin theDepartment of Pediatrics, Sestre milosrdnice University Hospital Centre of Zagreb. Some of the parameters such as body weight, percentage of ideal body weight and body mass index were significantly lower than expected due to age, and for the level of electrolytes-no larger deviations were found. Girls who have receivedan average1068.7±425.5kcalnutritionalsupport per day werelonger in the hospital, but fewer times hospitalized. With regular andappropriate diagnosticapproach, thiseating disordercan be detectedat an earlier stage, when the function of interventionandmultidisciplinaryprofessional help(specialist doctor, psychiatrist, psychologist, clinicalnutritionist) can preventfurther development ofthe disease.

Key words: Anorexianervosa, adolescents, anthropometry, electrolytes, nutritionalsupport, multidisciplinaryhelp

IS THERE CONNECTION BETWEEN BENZOATES FROM SOFT DRINKS AND HYPERACTIVITY AMONG OSIJEK PRESCHOOL CHILDREN?

Maja Miškulin[1]*, Nika Pavlović[2], Aida Mujkić[3], Jelena Vlahović[1]

[1]University of Osijek, Faculty of Medicine, Josipa Huttlera 4, 31 000 Osijek, Croatia
[2]Institute of Public Health for the Osijek-Baranja County, Franje Krežme 1, 31 000 Osijek, Croatia
[3]University of Zagreb, School of Medicine, Andrija Štampar School of Public Health, Rockefellerova 4, 10000 Zagreb, Croatia

Original scientific paper

Summary

Introduction: Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood-onset psychiatric disorders. The impact of certain food additives, preservatives and artificial colorings on the prevalence of this disorder is still controversial and not fully explored.

Aim: To explore the possible connection between benzoates from soft drinks and hyperactivity among Osijek preschool children.

Materials and methods: This cross-sectional study was conducted during April and May 2007 in Osijek, Eastern Croatia. A special questionnaire was administered to parents of 810 preschool children from kindergartens in Osijek (aged 5.9±0.7 years; 401/810, 49.5% boys and 409/810, 50.5% girls) during April and May 2007. The questionnaire contained questions on the preschool children age, gender, weight, average daily intake of soft drinks, type of soft drink consumed, food allergy and ten-item parents Conner's index for ADHD diagnose. The concentration of benzoates in 50 commercially available soft drinks was determined by the method of high-performance liquid chromatography (HPLC) with UV detector.

Results: Among all preschool children there were only 4.9% (40/810) of them who did not drink soft drinks. The mean concentration of benzoates in all samples was 97.7±26.7 mg/L. The questionnaire revealed that 95.1% (770/810) of study subjects consuming soft drinks were taking a mean of 0.6 L of soft drink per day, containing 58.6 mg of benzoates. Among those who did drink soft drinks there were 8.6% (66/770) of them positive for ADHD and none in group who did not.

Conclusion: ADHD is more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. It is not clear do these benzoates produce ADHD or just further facilitate ADHD that already exists. Further investigations are needed.

Key words: benzoates, soft drinks, hyperactivity, preschool children, Croatia.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common behavioral and neurodevelopmental disorder which is characterized by hyperactivity, impulsivity, and inattention in children and adolescents (Linnet et al., 2003). Prevalence of ADHD among school-aged children in different studies varies between 5 and 12% (Linnet et al., 2003; Grizenko et al., 2008) and the ADHD worldwide-pooled prevalence was estimated to be 5.29% (Polanczyk et al., 2007). It has also been stated that the prevalence of this disorder declines with increasing age (Golmir-

zaei et al., 2013). Concerning the preschool children there are only few studies dealing with the prevalence of this disorder in this age group and they showed prevalence of this disorder ranging from 4.3% to 31.1% (Ardalan et al., 2002; Hebrani et al., 2007; Soma et al., 2009; Meysamie et al., 2011). It is interesting that the results of majority of these studies indicate a marked difference in the prevalence of ADHD symptoms when the evaluation was performed by parents compared to teachers; the prevalence was consistently higher in the parent survey (Soma et al., 2009; Meysamie et al., 2011).
Although, the pathogenesis of ADHD is still un-

known, primary and secondary factors are estimated to be implicated in ADHD pathogenesis. Primary roles are shaped in the cerebral cortex by catecholamine metabolism. Also, etiology of ADHD is attributed to genetic factors in about 80% (Golmirzaei et al., 2013). The secondary roles are created by various environmental factors (Pliszka, 2007; Millichap, 2008). Some of these factors, which are associated with ADHD, are pregnancy and birth related risk factors which are classified into three groups including prenatal, perinatal, and postnatal risk factors. Regarding prenatal risk factors, a large number of studies have shown that maternal exposure to alcohol, tobacco, and cocaine during pregnancy increases the risk of ADHD. On the other hand, some studies showed that prenatal viral infections are associated with increased risk of ADHD (Mann and McDermott, 2011; Golmirzaei et al., 2013). Various studies have demonstrated that preeclampsia, maternal anemia, lower serum level of iron and iodine, and trauma to abdomen during pregnancy are associated with increased risk of ADHD development (Mann and McDermott, 2011; Amiri et al., 2012). Regarding perinatal risk factors, a number of risk factors such as prematurity, low birth weight, and breech delivery are estimated to be associated with increased risk of ADHD (Millichap, 2008). Postnatal risk factors include postnatal viral infections such as measles, varicella, and rubella increasing the risk of developing ADHD (Millichap, 2008). Additionally, several other factors such as breast-feeding, head injury in early childhood and adolescence, encephalitis, convulsion and endocrine disorder are estimated to be risk factors for development of ADHD (Millichap, 2008; Golmirzaei et al., 2013). In addition to the mentioned factors, several sociodemographic factors such as maternal education, family income, male gender, and maternal age at pregnancy are known to be predictive factors for developing ADHD (Millichap, 2008; Amiri et al., 2012). Considering the socioeconomic status it has been concluded that although genetic and neurological determinants may be the primary predictors of the development of ADHD in a child, etiology appears to be influenced by socioeconomic situation of his/her family (Russell et

al., 2014).

Under European regulation (EC) No. 1333/2008, food additives are defined as any substances 'not normally consumed as food itself' which are added to a food to perform a technological purpose e.g. preservation (European Commission, 2008). There are twenty-six categories of food additives outlined in this regulation, which fall broadly into two main categories depending on their purpose (1) safety and prevention of degradation of food by bacteria, oxidation or chemical reactions or (2) improvement of the taste, appearance or mouth-feel of the product (Martyn et al., 2013). Benzoic acid and its salts (benzoate) may naturally be present in food, but with their antimicrobial properties they have a long history of use as food preservatives (Lazarević et al., 2011). Their use as food additives was limited by Joint FAO/WHO Expert Committee on Food Ad¬ditives (JECFA). According to JECFA acceptable daily intakes (ADI) of 0–5 mg/kg body weight for benzoic acid and benzoates have been established (WHO, 1997). Some studies suggested that very high intake of benzoic acid can cause adverse health effects such as metabolic acidosis, hyper¬pnoea and convulsions (WHO, 1997). Few epidemiologic studies reported allergic reactions to benzoic acid and benzoate (urticaria, rinhitis and pruritis) (Nettis et al., 2004; Asero, 2006; Lazarević et al., 2011). Among other products, as widely used food preservatives, benzoates are often used in soft drinks, such as carbonated drinks and various fruit juices. Soft drinks are likely to be the major contributing factor to the intake of benzoates for young children, due to the high levels of consumption of these products by this age group (Food Standards Agency, 2008).

In a recent literature, one of the most frequently investigated potential hazards associated with preschool children and food additives, is the influence of these substances on development of ADHD (Martyn et al., 2013). Until today, there were several studies that have dealt with the issue of the food additives intake and prevalence of ADHD in children but the impact of these substances on the prevalence of this disorder is still controversial and not fully explored because

the results of aforementioned studies are contradictory (Rowe and Rowe, 1994; Bateman et al., 2004; McCann et al., 2007; Connolly et al., 2010; Lok et al., 2013).

The aim of this study was to explore the possible connection between benzoates from soft drinks and hyperactivity among Osijek preschool children.

Materials and Methods

This cross-sectional study was conducted during April and May 2007, among parents of preschool children attending kindergartens in Osijek (Eastern Croatia). Participation in the study was voluntary, and the study was approved by the Ethics Committee of the Institute of Public Health for the Osijek-Baranja County. A total number of 1 378 specially designed anonymous questionnaires were delivered randomly to parents of preschool children attending kindergartens in Osijek. The overall response rate was 58.8% (810/1378), and all of the 810 completed questionnaires, were included in further statistical analyses. The final sample size consisted of 810 preschool children mean age 5.9 ± 0.7 years, 49.5% (401/810) of boys and 50.5% (409/810) of girls.

Questionnaire

The anonymous questionnaire contained questions on the preschool children age, gender, weight, average daily intake of soft drinks, type of soft drink consumed, food allergy and ten-item Conner's abbreviated behavior rating scale for the ADHD diagnose. This ten-item is considered to be reliable instrument for screening purposes performed by parents in order to establish the existence of ADHD in a child (Gross-Tsur et al., 2006). The index consists of ten statements regarding the child's behavior and parents are supposed to express their agreement with the statements using the 4-point Likert scales (ranging from 0 for not at all true to 3 for very much true). The ADHD total score of 16 or above is considered indicative for existence of ADHD in the child.

Analytical methodology

The most popular and most widely available 50 soft drinks in Croatia were collected in grocery stores in the Osijek area. The concentration of benzoates (E210-E213) i.e. benzoic acid (E210), sodium benzoate (E211), potassium benzoate (E212) and calcium benzoate (E213), in 50 samples of commercially available soft drinks was determined by the method of high-performance liquid chromatography (HPLC) with UV detector on a Varian instrument (Walnut Creek, USA, 1993) (Varian Associates Inc, 1993) with a UV/VIS Star 9050 detector and Star 9012 pump. The method was standardized by AOAC; 979.08; Edition 18, Vol.2; Chapter 29. Standard solutions of benzoic acid (dr. Ehrenstorfer) were also prepared in accordance with the same norm.

Operating chromatography conditions were: room temperature; Zorbax C-18 column 5 μL, 150 mm x 4.6 mm, with Superguard LC-18 precolumn (20 mm x 4.6 mm); mobile phase: 20% acetic acid buffered to pH 3 with saturated solution of sodium acetate; flow rate 2 mL/min; wavelength 254 nm; injection volume 20 μL, and limit of detection 2.53 mg/L.

Soft drink samples were homogenized and degassed on „Branson 1210" ultrasonic bath, filtered through a 0.45-μm syringe filter and injected into the system.

Statistical analysis

Statistical analysis included data obtained by the laboratory analysis of soft drinks and data collected through the anonymous questionnaires. Normality of data distribution was tested by the use Kolmogorov-Smirnov test. All data were processed by the methods of descriptive statistics. The proportions were calculated and compared by the use of Fisher's exact test. Spearman's correlation coefficient was also calculated, to test the correlation between the amount of daily consumed soft drink and the presence of ADHD symptoms in a group of children who drank these drinks. $P < 0.05$ was considered statistically significant. Statistical analysis was done by the SPSS Statistical Package for Windows, version

13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The study sample consisted of 810 preschool children mean age 5.9±0.7 years (range 5.0-7.5 years), 49.5% (401/810) of boys and 50.5% (409/810) of girls attending the kindergartens in Osijek (Eastern Croatia). The mean weight of all children was 22.6±4.2 kg, ranging 13.0 to 60.0 kg.

Among all preschool children there were 95.1% (770/810) who drank soft drinks and 4.9% (40/810) of those who did not drink them at all. When looking the amount of daily consumed soft drinks among subjects who drank soft drinks it was discovered that this amount ranged from 0 L to 1.0 L daily, with 73.4% (565/770) of preschool children consuming the amount of 0.5 L or above (Table 1). The mean amount of daily consumed soft drink among children who drank soft drinks was 0.6 L.

Table 1. Preschool children from Osijek (Eastern Croatia) who drank soft drinks according to the daily consumed amount of soft drink and daily intake of benzoates via soft drink

Daily consumed amount of soft drink	Daily intake of benzoates via soft drinks	Preschool children from Osijek who consumed soft drinks N (%)
0 L	0 mg	20 (2.6)
0.20 L	19.5 mg	185 (24.0)
0.50 L	48.9 mg	208 (27.0)
0.75 L	73.3 mg	179 (23.3)
1.00 L	97.7 mg	178 (23.1)
Total		**770 (100.0)**

The results of the questionnaire showed that the preschool children from Osijek had most frequently consumed non-carbonated fruit juices (52.5%), ice teas (30.3%), coke (coca-cola) (12.1%), carbonated soft drink with orange flavor (3.4%) and at least other carbonated soft drinks (1.8%).

The mean concentration of benzoates in all samples of soft drinks was 97.7±26.7 mg/L. When calculating the amount of benzoates that children intake considering the amount of consumed soft drink it is evident that mean value of benzoates is 57.5±29.8 mg, ranging from 0 mg to 97.7 mg daily, depending on the amount of daily consumed soft drink. Considering that questionnaire revealed how 95.1% (770/810) of study subjects consuming soft drinks were taking a mean of 0.6 L of soft drink per day, it can be calculated that mean daily intake of benzoates via soft drinks among 95.1% of study population is 58.6 mg (Table 1). According to the mean body weight of all children (22.6 kg) and mean value of benzoates in all soft drinks (57.5 mg) it is further evident that mean daily intake of benzoates in study popula-

tion is 2.5 mg/kg body weight (50.0% of ADI), ranging from 0 to 4.3 mg/kg body weight (86.0% of ADI), also depending on the amount of daily consumed soft drink.

Among preschool children who drank soft drinks there were 8.6% (66/770) of them positive for ADHD and none (0%; 0/40) in group who did not drink them and this difference was statistically significant (Fisher's exact test; P=0.031).

When looking the children with ADHD symptoms according to the amount of daily consumed soft drink the study has showed that the number of children with the ADHD symptoms grew in parallel with the daily consumed amount of soft drink. Spearman rank correlation between the amount of daily consumed soft drink and the presence of ADHD symptoms in a group of children who drank these drinks was r=0.130; p=0.000 (Table 2).

Table 2. Preschool children from Osijek (Eastern Croatia) with ADHD symptoms according to the amount of daily consumed soft drink

The amount of daily consumed soft drink	Presence of the ADHD symptoms		Total
	No N (%)	Yes (%)	
0 L	20 (100.0)	0	20 (2.6)
0.20 L	178 (96.2)	7 (3.8)	185 (24.0)
0.50 L	192 (92.3)	16 (7.7)	208 (27.0)
0.75 L	159 (88.8)	20 (11.2)	179 (23.3)
1.00 L	155 (87.1)	23 (12.9)	178 (23.1)
Total	704 (91.4)	66 (8.6)	770 (100.0)

This study revealed that soft drinks are important source of benzoate intake among preschool children from Eastern Croatia, because 95.1% of children who drank those drinks in mean amount of 0.6 L daily through those drink intake around 2.6 mg/kg body weight of benzoates daily or 52.0% of ADI of benzoates. This finding confirms the fact that due to the high levels of consumption of these products by this age group, soft drinks are likely to be the major contributing factor to the intake of benzoates for young children, (Food Standards Agency, 2008). Also, this finding confirms results of some studies that have suggested that preschool children may have increased exposure and consumption of certain food additives, in comparison with adults and are therefore an important subgroup that should be addressed by exposure assessments (Goldman and Koduru, 2000; Huybrechts et al., 2011; Martyn et al., 2013). Generally speaking, considering the dietary habits, especially range of foodstuffs consumed during childhood it has been concluded that preschool children are likely to be the population group with the highest exposure to chemicals in the diet (Lawrie, 1998). When assessing the exposure of children to food additives, and other chemicals in diet, it is very important to bear in mind that because of some factors children are more vulnerable to their negative effects in relation to adults (Martyn et al., 2013). Young children may be more vulnerable than adults to chemical exposures as a result of their immature organ systems, rapid physical development and higher metabolic rates (Wilson et al., 2007). Furthermore, metabolic processes may differ between children and adults as, per kilogram body weight, smaller organs usually need more oxygen and nutrients than adult organs (Ginsberg et al., 2004). Finally, the distribution and absorption of chemicals throughout the body can differ, e.g. water-soluble substances are distributed over a relatively greater volume within the body of a child in comparison with that of an adult and can penetrate more easily into tissues and organs from the bloodstream (Martyn et al., 2013).

Considering the influence of benzoate intake on a children behavior this study points to the possible connection between the intake of benzoates through soft drinks and presence of the symptoms of ADHD among Osijek preschool children because ADHD was more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. This finding is in compliance with the results of studies conducted in the United Kingdom (Bateman et al., 2004; McCann et al., 2007), but is in contrast to the results of studies conducted in Ireland and Hong Kong, China (Connolly et al., 2010; Lok et al., 2013). This study also showed that the number of children with the ADHD symptoms grew in parallel with the daily consumed amount of soft drink, which further supports the existence of possible connection between the observed variables.

However, the definite conclusion cannot be drawn because of possible confounding factors that one should take into account when evaluating the results of this study. First of all, the evaluation of the existence and severity of ADHD symptoms among children was done by parents through

ten-item Conner's abbreviated behavior rating scale for the ADHD diagnose and studies dealing with the ADHD among preschool children showed a marked difference in the prevalence of ADHD symptoms when the evaluation was performed by parents compared to teachers with the prevalence's in the parent surveys being consistently higher (Soma et al., 2009; Meysamie et al., 2011). There are several possible explanations for this discrepancy. For example, parents may expect their children to be well-behaved and obedient, thus evaluating their behavior more strictly. Also, parents of preschool children may have difficulty determining whether a child's behavior is abnormal, thus resulting in more false positives in the parent survey (Soma et al., 2009; Meysamie et al., 2011). Second potential limitation of this study is an absence of questions regarding all possible risk factors for ADHD besides the intake of food additives such as genetic factors and other environmental factors, because it is possible that children who scored positive for ADHD were more prone to the development of ADHD symptoms due to some preexisting risk factors in their personal or family history that were not taken into account during this study. Finally, since the study is designed as a cross-sectional study, we cannot draw definitive conclusions about the established cause-effect relationships between the intake of benzoates through soft drinks and presence of the symptoms of ADHD among Osijek preschool children.

Conclusions

ADHD is more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. It is not clear do these benzoates produce ADHD or just further facilitate ADHD that already exists. Further investigations are needed.

Literature

1. Amiri S, Malek A, Sadegfard M et al (2012) Pregnancy-related maternal risk factors of attention-deficit hyperactivity disorder: a case-control study. ISRN Pediatr 2012:458064
2. Ardalan G, Farhud D, Shahmohammadi D (2002) Hyperactivity, attention and concentration deficit in preschool children. Iran J Pediatr 12:53–56
3. Asero R (2006) Sodium benzoate-induced pruritus. Allergy 61:1240–1241
4. Bateman B, Warner J, Hutchinson E et al (2004) The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Child 89:506–511
5. Connolly A, Hearty Á, Nugent A et al. (2010) Pattern of intake of food additives associated with hyperactivity in Irish children and teenagers. Food Add Contam 27:447–456
6. European Commission (2008) Regulation (EC) No. 1333/ 2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives. Off J Eur Union L 365.31.12.2008, p. 16 365/16
7. Food Standards Agency (2008) Survey of benzoates and sorbates in soft drinks. Food Survey Information Sheet No. 06/08. London, Food Standards Agency
8. Ginsberg G, Hattis D, Miller R et al (2004) Paediatric pharmacokinetic data: implications for environmental risk assessment for children. Pediatrics 113:973–983
9. Goldman LR, Koduru S (2000) Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Perspect 108:443S
10. Golmirzaei J, Namazi S, Amiri S et al (2013) Evaluation of attention-deficit hyperactivity disorder risk factors. Int J Pediatr 2013:953103
11. Grizenko N, Shayan YR, Polotskaia A et al (2008) Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. J Psychiatry Neurosci 33:10–16
12. Gross-Tsur V, Goldzweig G, Landau YE et al (2006) The impact of sex and subtypes on cognitive and psychosocial aspects of ADHD. Dev Med Child Neurol 48:901–905
13. Hebrani P, Abdolahian E, Behdani F et al (2007) The prevalence of attention deficit hyperactivity disorder in preschool-age children in Mashhad, North-East of Iran. Arch Iran Med 10:147–151
14. Huybrechts I, Sioen I, Boon PE et al (2011) Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. Arch Public Health 69:1–12
15. Lawrie C (1998) Different dietary patterns in relation to age and the consequences for intake of

food chemicals. Food Add Contam 15:75–81

16. Lazarević K, Stojanović D, Rančić N (2011) Estimated daily intake of benzoic acid through food additives in adult population of South East Serbia. Cent Eur J Public Health 19:228–231

17. Linnet KM, Dalsgaard S, Obel C et al (2003) Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. Am J Psychiatry 160:1028 –1040

18. Lok KY, Chan RS, Lee VW et al (2013) Food additives and behavior in 8- to 9-year-old children in Hong Kong: a randomized, double-blind, placebo-controlled trial. J Dev Behav Pediatr 34:642–650

19. Mann JR, McDermott S (2011) Are maternal genitourinary infection and pre-eclampsia associated with ADHD in school-aged children? J Atten Disord 15:667–673

20. Martyn DM, McNulty BA, Nugent AP et al (2013) Food additives and preschool children. Proc Nutr Soc 72:109–116

21. McCann D, Barrett A, Cooper A et al (2007) Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet 370:1560–1567

22. Meysamie A, Fard MD, Mohammadi MR (2011) Prevalence of Attention-Deficit/Hyperactivity Disorder Symptoms in Preschool-aged Iranian Children. Iran J Pediatr 21:467–472

23. Millichap JG (2008) Etiologic classification of attention-deficit/hyperactivity disorder. Pediatrics 121:e358–e365

24. Nettis E, Colanardi MC, Ferrannini A et al (2004) Sodium benzoate-induced repeated episodes of acute urticaria/angio-oedema: randomized controlled trial. Br J Dermatol 151:898–902

25. Pliszka S (2007) Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 46:894–921

26. Polanczyk G, de Lima MS, Horta BL et al (2007) The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164:942–948

27. Rowe KS, Rowe KJ (1994) Synthetic food colouring and behaviour: a dose response effect in a double-blind, placebocontrolled, repeated-measures study. J Paediatr 125:691–698

28. Russell G, Ford T, Rosenberg R et al (2014) The association of attention deficit hyperactivity disorder with socioeconomic disadvantage: alternative explanations and evidence. J Child Psychol Psychiatry 55:436–445

29. Soma Y, Nakamura K, Oyama M et al (2009) Prevalence of attention-deficit/hyperactivity disorder (ADHD) symptoms in preschool children: discrepancy between parent and teacher evaluations. Environ Health Prev Med 14:150–154

30. Varian Associates Inc (1993) Varian chromatography systems: Operator's Manual. Walnut Creek, Varian Associates Inc

31. Wilson NK, Chuang JC, Morgan MK et al (2007) An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. Environ Res 103:9–20

32. World Health Organization (1997) Evaluation of certain food additives: forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 868. WHO, Geneva

PREVALENCE OF FOOD ALLERGIES IN THE POPULATION OF PRESCHOOL CHILDREN FROM THE CITY OF OSIJEK

Nika Pavlović[1], Jelena Vlahović[2], Maja Miškulin[2]*

[1]Institute of Public Health for the Osijek-Baranja County, Franje Krežme 1, 31 000 Osijek, Croatia
[2]University of Osijek, Faculty of Medicine, Josipa Huttlera 4, 31 000 Osijek, Croatia

Original scientific paper

Summary

Introduction: Food allergy is an immune-mediated hypersensitivity to allergens in food. This allergy affects about 6-8% of children younger than three years, about 4% of school-age children and about 2% of the adult population in the world.
Aim: To investigate the prevalence of food allergies in the population of preschool children from the city of Osijek and to identify the most common causes of these allergies in the study population.
Materials and methods: This cross-sectional study was conducted during April and May 2007, among preschool children (mean age 5.9±0.7 years, 401/810, 49.5% of boys and 409/810, 50.5% of girls) attending kindergartens in Osijek. The 810 parents of mentioned children filled out the specially designed questionnaire. The questionnaire contained questions on age, sex and body weight of the child, the existence of a diagnosis of food allergy and type of food allergy.
Results: In the studied population of preschool children there were 5.4% (44/810) of children diagnosed with food allergies. The most common causes of food allergies in the study population were different food additives (40.9%), eggs (18.2%), peanut (13.7%), milk (9.2%) and honey, pesticides, fish and gluten with the same frequency of 4.5% each.
Conclusion: Food allergies often occur among preschool children from the city of Osijek with the particularly significant prevalence of allergic reactions to various food additives. In children with confirmed allergy to food ingredients, it is necessary to exclude such ingredient from the diet. In this sense, it is essential that manufacturers mark in detail the content of each foodstuff so that consumers can avoid eating those foodstuffs to which they are allergic.

Key words: food allergy, food additives, preschool children, Croatia.

Introduction

More than 20% of adults believe that they have an allergic reaction to food or food additive (artificial colorings, preservatives) (Turkalj and Mrkić, 2012). Reactions to foods are not new and have been described for two thousand years. The ancient Greek physician, Hippocrates, describes a reaction to milk in the 1st century. Anaphylactic reactions to egg and fish have been described as earlier as the 16th and 17th century (Cianferoni and Spergel, 2009).
Food allergy refers to an abnormal immunologic response to allergens in food, most frequently proteins, that occurs in a susceptible host. These reactions are reproducible each time the food is ingested and they are often not dose dependent. Based on the immunological mechanism involved, food allergies may be further classified in: IgE-mediated, which are mediated by antibodies belonging to the Immunoglobulin E (IgE) and are the best-characterized food allergy reactions; cell-mediated when the cell component of the immune system is responsible for the food allergy and mostly involve the gastrointestinal tract; and mixed IgE-mediated-cell mediated when both IgE and immune cells are involved in the reaction (Lee and Burks, 2006; Nowak-Wegrzyn and Sampson, 2006; Cianferoni and Spergel, 2009; Sicherer and Sampson, 2009; Turkalj and Mrkić, 2012).
The prevalence of food allergies, as well as the

*Corresponding author: miskulin.maja@gmail.com

prevalence of all allergic diseases appears to be rising, during last few decades, especially in developed countries (Cochrane et al., 2009). The incidence of the allergic reactions to food is associated with the type of diet, mode of food preparation, cultural and social factors inherent to particular countries and cultures. Anaphylactic reactions to food are one of the most important medical problems because they are one of the most common causes of systemic anaphylaxis that is life threatening and one of the most important reasons for seeking emergency medical care (Sampson, 2003). Food allergy is the leading cause of anaphylaxis treated in hospital emergency departments in Western Europe and the United States. Food allergy alone in the United States appears to account for approximately 30,000 anaphylactic reactions, 2,000 hospitalizations, and possibly 200 deaths each year. Furthermore, food allergy is the most common cause of anaphylaxis in children (Cianferoni and Spergel, 2009).

In majority of Western countries the most frequently occurring allergies are those to cow's milk protein, eggs, peanuts, nuts, fish, shellfish, flour and soya (Chapman et al., 2006). In children younger than three years the most common allergy is allergy to cow's milk proteins. This allergy in most allergic children (about 80% of them) disappears until the age of five, unlike peanut allergy in which only 20% of allergic children develops tolerance during adulthood. Some recent studies suggest that tolerance to certain food develops significantly slower. Accordingly, at the age of four, only 19% of children have become tolerant to cow's milk protein, 11% of children have become tolerant to egg protein, and only at the age of 16 years, about 80% of children have become tolerant to the protein of cow's milk and eggs (Savage et al., 2007; Skripak et al., 2007). The most common food allergies in children after the allergy to cow's milk proteins are the allergies to eggs, peanuts and flour, and in adults the most common food allergies are those to peanuts, nuts, fishes and shellfishes (Turkalj and Mrkić, 2012). Additionally, there are more and more concerns regarding the food additives as a potential allergens in children and adults although their adverse reactions seem to be very

rare in the general population (0.01-0.23%) but higher in atopic individuals (2-7%) (Randhawa and Bahna, 2009). Food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods (WHO and FAO, 2013).

Food allergy affects about 6-8% of children younger than three years, about 4% of school-age children and about 2% of the adult population in the world (Cianferoni and Spergel, 2009; Kolaček, 2011; Turkalj and Mrkić, 2012). Food allergies are, accordingly, more common in children and the prevalence of allergies is greater among younger children. To our knowledge, to this point, in Croatia there have been no studies aiming to determine the prevalence of food allergies in preschool children as one of the most vulnerable population subgroups concerning this issue.

The aim of this study was to investigate the prevalence of food allergies in the population of preschool children from the city of Osijek (Eastern Croatia) and to identify the most common causes of these allergies in the study population.

Materials and Methods

This cross-sectional study was conducted during April and May 2007, among parents of preschool children attending kindergartens in Osijek (Eastern Croatia). Participation in the study was voluntary, and the study was approved by the Ethics Committee of the Institute of Public Health for the Osijek-Baranja County. A total number of 1 378 specially designed questionnaires were delivered randomly to parents of preschool children attending kindergartens in Osijek. The overall response rate was 58.8% (810/1378), and all of the 810 completed questionnaires, were included in further statistical analyses.

The final sample size consisted of 810 preschool children mean age 5.9±0.7 years, 49.5% (401/810) of boys and 50.5% (409/810) of girls. The questionnaire contained questions on age, gender and body weight of the child, the existence of a diagnosis of food allergy and type of food allergy.

Statistical analysis

Descriptive statistics were used for data processing and analyzed using SPSS Statistical Package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The study sample consisted of 810 preschool children, 49.5% (401/810) of boys aged 5.9±0.7 years and 50.5% (409/810) of girls aged 5.8±0.7 years. The overall prevalence of food allergies in the studied population was 5.4% (44/810).

The most common causes of food allergies in the study population were different food additives (40.9%; 18/44), eggs (18.2%; 8/44), peanut (13.7%; 6/44), milk (9.2%; 4/44) and honey, pesticides, fish and gluten with the same frequency of 4.5% (2/44) each (Table 1).

Table 1. The most common causes of food allergies among six years old preschool children from Osijek (Eastern Croatia).

Causes of food allergy	Preschool children with food allergy N (%)
Food additives	18 (40.9)
Eggs	8 (18.2)
Peanut	6 (13.7)
Milk	4 (9.2)
Honey	2 (4.5)
Pesticides	2 (4.5)
Fish	2 (4.5)
Gluten	2 (4.5)
Total	44

The majority of data currently available on food allergy prevalence among children are only based on self-reporting or parent reporting questionnaire data like the data from this study, although there are some regions of the world such as Central and South America, Africa, Eastern Europe and the Middle East where food allergy prevalence data (of any kind) are still lacking (Prescott et al., 2013).

This study revealed that the overall prevalence rate of food allergy among six years old preschool children from Osijek (Eastern Croatia) was 5.4% that is in compliance with the prevalence established among preschool children from Portugal and Turkey (5.7%) but higher than the prevalence established among preschool children from Korea (3.7%), Japan (3.6% to 4.8%) and Hong Kong (4.8%) (Orhan et al., 2009; Ho et al., 2012; Kusunoki et al., 2013; Gaspar-Marques et al., 2014; Park et al., 2014). Similarly to the latter ones the self/parent-reported prevalence of food allergy in some European countries, such as Austria, Slovenia, Switzerland, Greece and Belgium has also been less than 5.0% in some studies that is also in contrast with the results obtained in this study among Croatian preschool children from Eastern Croatia (Steinke et al., 2007). Also in contrast to the results of this study there are some studies that showed higher prevalence of food allergy in children. In Canada and the USA, for example, survey reports suggest childhood food allergy prevalence rates of around 7-8%, with similar or even higher rates reported in some European countries such as Spain, Poland, Finland and the Netherlands (Brugman et al., 1998; Steinke et al., 2007; Fernández Rivas, 2009; Gupta et al., 2011).

The most common causes of food allergy in the

study population were different food additives (40.9%), eggs (18.2%), peanut (13.7%), milk (9.2%) and honey, pesticides, fish and gluten with the same frequency of 4.5% each. These findings are somewhat different from the results of similar studies conducted elsewhere because in these studies the food additives, pesticides, honey and gluten were not reported as a possible cause of allergic reactions (Steinke et al., 2007; Orhan et al., 2009; Fernández Rivas, 2009; Gupta et al., 2011; Ho et al., 2012; Park et al., 2014).

The prevalence of allergic reactions to eggs ranged between 13.6% and 20.2% (Steinke et al., 2007; Fernández Rivas, 2009; Orhan et al., 2009; Ho et al., 2012; Park et al., 2014) that is similar to the prevalence established in this study. The prevalence of allergic reactions to peanut established in this study was higher than those established in the studies conducted in Korea (9.3%) and Hong Kong (8.5%), but lower than the prevalence established in the United States (25.2%) (Gupta et al., 2011; Ho et al., 2012; Park et al., 2014). When comparing the prevalence of allergy to cow's milk established in this study (9.2%) with those established in similar studies conducted elsewhere it is evident that this allergy is less frequent among six year old children from Eastern Croatia than among children of similar age from Korea (31.4%), the United States (21.1%), Turkey (18.1%) and Spain (13.9%) but similar, although still slightly lower, than the prevalence established among children of similar age from Hong Kong (10.8%) (Fernández Rivas, 2009; Orhan et al., 2009; Gupta et al., 2011; Ho et al., 2012; Park et al., 2014). The above mentioned foodstuffs are especially important bearing in mind that those are the three leading causes of food-induced anaphylaxis among children (Park et al., 2014).

It is interesting that majority of studies conducted elsewhere have recognized fruits as an important potential cause of allergic reactions while that was not case in this study. Accordingly, parent's reported prevalence of allergic reactions to fruits among children from Korea, Austria, Belgium, Denmark, Finland, Germany, Greece, Italy, Poland, Slovenia, Switzerland and Spain ranged between 18.4% and 33.3% (Steinke et al., 2007; Fernández Rivas, 2009; Park et al., 2014). This

is possible explanation for the extremely high reported prevalence of allergy to various food additives among children from Eastern Croatia. Namely, all parents of the children with allergic reactions to foods containing canned fruit and fruit juices have reported that their children are allergic to food additives, and it is likely that the majority of them are actually allergic to some kind of fruit contained in the product that initiated an allergic reaction.

Furthermore it is interesting that studies conducted elsewhere in the world have frequently recognized fish and shellfish as potential causes of allergic reactions in children while none of the children in this study was allergic to shellfish and only 4.5% of them to fish (Fernández Rivas, 2009; Gupta et al., 2011; Ho et al., 2012; Park et al., 2014). This finding can easily be explained with dietary habits in Croatia, because it is well known that the fish consumption in Croatia is relatively low especially in Eastern Croatia whereby the population of Eastern Croatia has worse dietary habits in relation to the population of other regions (Pucarin-Cvetković et al., 2010; Periš et al., 2012).

This study has several limitations that should be considered when evaluating the obtained results. One of the refers to the lack of application of the specially designed questionnaire that would serve as a tool for collecting additional data concerning the subjects' personal and family history and information about possible risk factors for the occurrence of food allergy among each of them. Also because of the sensitivity of parents in regard to some types of foodstuffs and their ingredients such as food additives it is possible that some causes of allergic reactions such as latter ones were unconsciously been more reported than other ones that maybe even have far larger allergic potential.

Nevertheless, this study, as a first study in Croatia dealing with the issue of food allergy in children has certainly provided some valuable data regarding this problem in Croatia. Hopefully, there will be more similar studies in other Croatian regions that will help creating the broader picture of this issue with some new insights. All of this can be important part of promotion of the food allergy awareness for health policy and

health care system to better anticipate the growing impact and growing need for better services, community education and training to cope with this rising global health issue.

Conclusions

The results of this study have confirmed that food allergies are important issue among preschool children in Croatia. Bearing in mind that food allergy continues to increase in prevalence in many countries, one can say that the problem of food allergy is becoming a major public health issue, with practical implications for the food industry, educational establishments and healthcare systems.

In this sense, it is essential that manufacturers mark in detail the content of each foodstuff so that consumers can avoid eating those foodstuffs to which they are allergic. But it is also necessary to further educate consumers regarding the potential of particular foodstuff to initiate the allergic reactions and also to continuously educate heath care workers in order to enable them to timely recognize and adequately treat allergic reactions, especially those that can potentially be life-threatening, such as anaphylaxis.

Literature

1. Brugman E, Meulmeester JF, Spee-van der Wekke A et al (1998) Prevalence of self-reported food hypersensitivity among school children in The Netherlands. Eur J Clin Nutr 52:577–581

2. Chapman JA, Bernstein IL, Lee RE et al (2006) Food allergy, a practice parameter. Ann Allergy Asthma Immunol 96:S1–68

3. Cianferoni A, Spergel JM (2009) Food allergy: review, classification and diagnosis. Allergol Int 58:457–466

4. Cochrane S, Beyer K, Clausen M et al (2009) Factors influencing the incidence and prevalence of food allergy. Allergy 64:1246–1255

5. Fernández Rivas M (2009) Food allergy in Alergológica-2005. J Investig Allergol Clin Immunol 19(Suppl 2):37–44

6. Gaspar-Marques J, Carreiro-Martins P, Papoila AL et al (2014) Food Allergy and Anaphylaxis in Infants and Preschool-Age Children. Clin Pediatr (Phila) 53:652–657

7. Gupta RS, Springston EE, Warrier MR et al (2011) The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics 128:e9–e17

8. Ho MH, Lee SL, Wong WH et al (2012) Prevalence of self-reported food allergy in Hong Kong children and teens--a population survey. Asian Pac J Allergy Immunol 30:275–284

9. Kolaček S (2011) Preosjetljivost na hranu u dječjoj dobi. Acta Med Croatica 65:155–161

10. Kusunoki T, Morimoto T, Sakuma M et al (2013) Effect of eczema on the association between season of birth and food allergy in Japanese children. Pediatr Int 55:7–10

11. Lee LA, Burks AW (2006) Food allergies: prevalence, molecular characterization, and treatment-prevention strategies. Annu Rev Nutr 26:539–565

12. Nowak-Wegrzyn A, Sampson HA (2006) Adverse reactions to foods. Med Clin North Am 90:97–127

13. Orhan F, Karakas T, Cakir M et al (2009) Prevalence of immunoglobulin E-mediated food allergy in 6-9-year-old urban schoolchildren in the eastern Black Sea region of Turkey. Clin Exp Allergy 39:1027–1035

14. Park M, Kim D, Ahn K et al (2014) Prevalence of immediate-type food allergy in early childhood in Seoul. Allergy Asthma Immunol Res 6:131–136

15. Periš D, Miškulin M, Šincek D (2012) Povezanost socio-ekonomskog statusa i indeksa tjelesne mase umirovljenica s područja Osječko-Baranjske županije. Med Jad 42:103–109

16. Prescott SL, Pawankar R, Allen KJ et al (2013) A global survey of changing patterns of food allergy burden in children. World Allergy Organ J 6:21

17. Pucarin-Cvetković J, Kern J, Vuletić S (2010) Regionalne karakteristike prehrane u Hrvatskoj. Acta Med Croatica 64:83–87

18. Randhawa S, Bahna SL (2009) Hypersensitivity reactions to food additives.Curr Opin Allergy Clin Immunol 9:278–283

19. Sampson HA (2003) Food allergy. J Allergy Clin Immunol 111:540–547

20. Savage JH, Matsui EC, Skripak JM et al (2007) The natural history of egg allergy. J Allergy Clin Immunol 120:1413–1417

21. Sicherer SH, Sampson HA (2009) Food allergy: recent advances in pathophysiology and treatment. Annu Rev Med 60:261–277

22. Skripak JM, Matsui EC, Mudd K et al (2007) The natural history of IgE--mediated cow's milk allergy. J Allergy Clin Immunol 120:1172–1177

23. Steinke M, Fiocchi A, Kirchlechner V et al (2007) Perceived food allergy in children in 10 European nations. A randomised telephone survey. Int Arch Allergy Immunol 143:290–295

24. Turkalj M, Mrkić I (2012) Alergijske reakcije na hranu. Liječ Vjesn 134:168–173

25. World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO) (2013) Codex Alimentarius Commission, procedural manual. 21st edition. WHO/FAO, Rome

COW'S MILK ALLERGY IN THE POPULATION OF INFANTS AND EARLY PRESCHOOLERS FROM THE VUKOVAR – SRIJEM COUNTY

Darija Vrdoljak[1], Maja Miškulin[1]*, Jelena Vlahović[1], Nika Pavlović[2]

[1]University of Osijek, Faculty of Medicine, Josipa Huttlera 4, 31 000 Osijek, Croatia
[2]Institute of Public Health for the Osijek-Baranja County, Franje Krežme 1, 31 000 Osijek, Croatia

Summary

Introduction: Cow's milk allergy is the most common allergic reaction present among infants and early preschoolers. The early diagnosis of the condition is crucial for adequate treatment of the affected child.
Aim: To determine prevalence of cow's milk allergy in the population of infants (0-1 years) and early preschoolers (1-4 years) from Vukovar–Srijem County and demographical characteristics of affected children.
Materials and methods: This cross-sectional study was conducted from 1st May 2013 to 30th April 2014 in Vukovar General County Hospital and was approved by the responsible Ethical Committee. It included 59 children - suspected of having cow's milk allergy (38/59, 64.4% boys and 21/59, 35.6% girls; mean age 20.8±16.2 months). The serum concentrations of the specific IgE antibodies to cow's milk protein were determined and demographic data for each patient were collected (gender, age, place of living). Standardized UniCAP fluorescence-enzyme immunoassay (Phadia AB, Uppsala, Sweden) was used to determine serum concentration of the specific IgE antibodies for cow's milk protein.
Results: In observed population there were 35.6% (21/59) of children affected with cow's milk allergy. The allergy was more common with boys (39.5%), and children who lived in villages (56.5%). Average value of serum concentration of the specific IgE antibodies to cow's milk protein among affected children was 9.9±23.8 kU/L, and it was higher for boys (12.6 kU/L) in comparison to girls (3.2 kU/L).
Conclusion: This study indicates that the cow's milk allergy is relatively frequent within the observed population thus there is a need for further evaluation of this issue. In addition, the study has shown that the diagnosis of cow's milk allergy in Croatia is still relatively late and because of that there is a need to make additional efforts for earlier diagnosis of such condition that would also improve the therapeutic approach to affected children.

Key words: cow's milk allergy, child, food allergy, Croatia.

Introduction

The clinical response to cow's milk proteins was first described by the Galen and Hippocrates and at the beginning of the last century, Finkelstein described the first case of anaphylactic shock after intake of milk (Jurčić and Oberiter, 1996). Cow's milk allergy is a hypersensitivity reaction initiated by specific immunologic mechanisms. In most children with cow's milk allergy, the condition can be immunoglobulin E (IgE)-mediated and is thought to manifest as a phenotypical expression of atopy, together with (or in the absence of) atopic eczema, allergic rhinitis and/or asthma. A subset of patients, however, have non-IgE mediated (probably cell-mediated) allergy and present mainly with gastrointestinal symptoms in reaction to the ingestion of cow's milk (Fiocchi et al., 2010).

The perception of milk allergy is far more frequent than confirmed cow's milk allergy. In a large European survey of above 44,000 telephone contacts, 5 million European respondents claimed to be milk-allergic, with adult women as the group making most of these claims. There were also wide national differences ranging from 13.8% of reports from Greece to 52.3% from Finland. In this survey milk was the most often reported offending food in children (38.5% of reports) and the second food most often implicated

*Corresponding author: miskulin.maja@gmail.com

by adults (26%) (Steinke and Fiocchi, 2007). In a group of 600 children less than 4 years, cow's milk allergy was reported by the parents of 18 children (3%) (Kilgallen and Gibney, 1996). Milk reactions were reported by the parents of 2% of children without wheeze and by 16% of wheezers (Sandin et al., 2005). According to age, the prevalence of self-reported cow's milk allergy varies between 1 to 17.5% in preschoolers, 1 and 13.5% in 5 to 16-year-olds, and between 1 and 4% in adults (Roehr et al., 2004). Cow's milk-specific IgE sensitization point prevalence progressively decreased from about 4% at 2 years to less than 1% at 10 years of age (Matricardi et al., 2008). The most reliable data in epidemiology are those from birth cohorts that are free from selection bias (Fiocchi et al., 2010). There are five such challenge-confirmed studies. The cow's milk allergy prevalence during infancy ranged from 1.9% in a Finnish study, 2.16% in the Isle of Wight, 2.22% in a study from Denmark, 2.24% in the Netherlands, and up to 4.9% in Norway (Saarinen et al., 1999; Høst, 2002; Høst et al., 2002; Venter et al., 2006; Kvenshagen et al., 2008). To our knowledge, in Croatia there are no epidemiological data concerning the exact prevalence of cow's milk allergy in children, but according to the reports of pediatricians one can say that the cow's milk allergy is the most frequent allergic reaction in Croatian children aged two years or less (Vrdoljak, 2014).

Cow's milk contains several proteins that could each in principle elicit an allergic reaction in a sensitized individual. The most common cause of cow's milk allergy in children is alpha-lactalbumin, rarely casein, and in adults beta-lactoglobulin. Individuals with cow's milk allergy may present with a wide variety of symptoms. Consequently, knowledge of the various cow's milk allergic disorders and a detailed medical history are essential for the clinician to arrive at the correct diagnosis and the early diagnosis of the condition is crucial for adequate treatment of the affected child (Vrdoljak, 2014).

Allergic (immune-mediated) reactions to cow's milk may be classified as "immediate" (typically IgE-mediated) or "late onset" (typically non-IgE or cell-mediated) reactions. Immediate reactions to cow's milk may present as generalized systemic reactions (anaphylaxis) or IgE-mediated gastrointestinal, cutaneous, and/or respiratory reactions. Patients presenting with IgE-mediated disorders will typically have positive skin tests and/or serum IgE antibodies to milk (Fiocchi et al., 2010; Vrdoljak, 2014). Symptoms of late-onset cow's milk allergy are not IgE-mediated and typically develop one to several hours or after several days of ingesting cow's milk. There are no reliable laboratory tests to diagnose late-onset cow's milk allergy and tests for IgE antibodies are negative. The majority of disorders involving late-onset cow's milk allergy are localized to the gastrointestinal tract, but disorders involving the skin and respiratory tract also occur (Fiocchi et al., 2010; Vrdoljak, 2014).

The aim of this study was to determine prevalence of cow's milk allergy in the population of infants (0-1 years) and early preschoolers (1-4 years) from Vukovar-Srijem County and demographical characteristics of affected children.

Materials and Methods

This cross-sectional study was conducted from 1st May 2013 to 30th April 2014 in Vukovar General County Hospital. Participation in the study was voluntary, and the study was approved by the Ethics Committee of the County General Hospital Vukovar.

The final sample consisted of 59 children from the Vukovar–Srijem County - suspected of having cow's milk allergy (38/59, 64.4% boys and 21/59, 35.6% girls; mean age 20.8±16.2 months). The serum concentrations of the specific IgE antibodies to cow's milk protein were determined and demographic data for each patient were collected (gender, age, place of living).

Standardized UniCAP fluorescence-enzyme immunoassay (Phadia AB, Uppsala, Sweden) was used to determine serum concentration of the specific IgE antibodies to cow's milk protein. According to the UniCAP fluorescence-enzyme immunoassay manufacturer the measuring range of the specific IgE antibodies to cow's milk protein in serum was 0.1 to 100.0 kU/L. According to the manufacturer, the expected normal values of the specific IgE antibodies for cow's milk protein in serum were all values below 0.35 kU/L and these were considered negative, while all values equal or above 0.35 kU/L were considered

positive. All positive values were further classified into classes as follows: Class I: 0.35–0.69 kU/L; Class II: 0.70–3.49 kU/L; Class III: 3.50–17.49 kU/L; Class IV: 17.50–49.99 kU/L; Class V: 50.00–99.99 kU/L and Class VI: 100.0 kU/L or above (Phadia AB, 2012).

Statistical analysis

Statistical analysis included data obtained by the laboratory analysis of participants' blood and collected demographic data. Normality of data distribution was tested by the use of Shapiro-Wilkinson test. All data were processed by the methods of descriptive statistics. The proportions were calculated and compared by the use of Fisher's exact test. The quantitative variables were compared by the use of Mann Whitney U-test. $P<0.05$ was considered statistically significant. Statistical analysis was done by the SPSS Statistical Package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The study sample consisted of 59 children from the Vukovar–Srijem County, 64.4% (38/59) boys mean age 21.1±16.8 months and 35.6% (21/59) girls mean age 20.2±15.4 months. According to the age group all subjects were divided into four groups. In group one (aged 1-12 months) there were 39.0% (23/59) of subjects; in group two (aged 13-24 months) there were 22.0% (13/59) of subjects; in group three (aged 25-36 months) there were 13.6% (8/59) of subjects and in group four (aged 37-48 months) there were 25.4%

(15/59) of subjects. According to the place of living in the study sample there were 61.0 % (36/59) of children who lived in town and 39.0% (23/59) of children who lived in village.

The study revealed that among observed children there were 35.6% (21/59) of children affected with cow's milk allergy, i.e. with the measured serum concentrations of the specific IgE antibodies to cow's milk protein equal or above 0.35 kU/L. The average value of serum concentration of the specific IgE antibodies to cow's milk protein among affected children was 9.9±23.8 kU/L, with the minimal measured value of 0.5 kU/L and maximal measured value of 100.0 kU/L. When looking the measured serum concentrations of the specific IgE antibodies to cow's milk protein according to the gender it was evident that boys had higher average value of these antibodies (12.6 kU/L) in comparison to girls (3.2 kU/L) but this difference was not statistically significant (Z= -0.982; P=0.331).

All children who were, according to the measured values of serum concentrations of the specific IgE antibodies to cow's milk protein, found positive for the presence of cow's milk allergy were further classified into classes of positivity proposed by the manufacturer of the used immunoassay. It was evident that 33.3% (7/21) of allergic children belonged to the class I, 42.9% (9/21) of allergic children belonged to the class II, 14.2% (3/21) of allergic children belonged to the class III, none of the allergic children belonged to the class IV and 4.8% (1/21) of allergic children belonged to the classes V and VI (Table 1).

Table 1. Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein (that imply the existence of cow's milk allergy) according to the classes of positivity proposed by the manufacturer of the used immunoassay.

Classes of positivity to the specific IgE antibodies for cow's milk protein according to the values of the specific IgE antibodies for cow's milk protein in patients' serum (kU/L)	Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein N (%)
Class I: 0.35–0.69	7 (33.3)
Class II: 0.70–3.49	9 (42.9)
Class III: 3.50–17.49	3 (14.2)
Class IV: 17.50–49.99	0
Class V: 50.00–99.99	1 (4.8)
Class VI: 100.0 kU/L or above	1 (4.8)
Total	21 (100.0)

When looking the all children from the Vukovar–Srijem County suspected of having cow's milk allergy according to their gender it was found that among all of them there were 39.5% (15/38) of boys with the confirmed cow's milk allergy and 28.6% (6/21) of girls with the confirmed cow's milk allergy, but this difference was not statistically significant (Fisher's exact test; P=0.571). According to the age of diagnose, the study showed that the mean age of the confirmation of the existence of cow's milk allergy in the study

population was 27.2±15.9 months, 25.5±16.5 in boys and 31.5±14.9 months in girls.

When looking the age of diagnose of the existence of cow's milk allergy according to the age groups of the children it was evident that the diagnose of this allergy was confirmed in 13.0% (3/23) of children aged 1-12 months, 53.8% (7/13) of children aged 12-24 months, 50.0% (4/8) of children aged 25-36 months and 46.7% (7/15) of children aged 37-48 months (Table 2).

Table 2. The age of diagnose of the existence of cow's milk allergy among children from Vukovar–Srijem County allergic to cow's milk according to the age group.

Age group of children	Children from the Vukovar–Srijem County with the positive values of serum concentrations of the specific IgE antibodies to cow's milk protein N (%)	Total
1-12 months	3 (13.0)	23
13-24 months	7 (53.8)	13
25-36 months	4 (50.0)	8
37-48 months	7 (46.7)	15
Total	21 (35.6)	59

When looking the all children from the Vukovar–Srijem County suspected of having cow's milk allergy according to the place of their living the study revealed that 22.2% (8/36) of children with confirmed cow's milk allergy lived in town and 56.5% (13/23) of children with confirmed cow's milk allergy lived in village, and this difference was statistically significant (Fisher's exact test; P=0.012).

This study has shown that cow's milk allergy was confirmed in 35.6% of subjects with suspected allergy, accordingly, this study also confirmed the well-known fact, according to which the prevalence of allergy established by the diagnostic testing is always three to four times lower than those claimed by patients themselves or their parents (Kilgallen and Gibney, 1996; Roehr et al., 2004; Sandin et al., 2005; Rona et al., 2007; Steinke and Fiocchi, 2007; Kolaček, 2011). Data from cross-sectional studies conducted elsewhere in the world demonstrated the prevalence rates of cow's milk allergy from 0.6 to 2.5% in preschoolers, 0.3% at 5 to 16 years of age, and of less than 0.5% in adults (Madrigal

and Alfaro, 1996; Osterballe et al., 2005; Rona et al., 2007). Comparing the results of this study regarding the established prevalence of cow's milk allergy with the above mentioned results of similar studies conducted elsewhere it can be said that the prevalence of this allergy in study population from the Vukovar–Srijem County is much higher.

This study has further shown that the average value of the serum concentration of specific IgE antibodies to cow's milk protein in all children allergic to cow's milk was 9.9 kU / L, where boys had higher average value of these antibodies (12.6 kU/L) in comparison to girls (3.2 kU/L). According to the gender the existence of cow's milk allergy was confirmed in 39.5% of boys and 28.6% of girls. These results are in compliance with the results of the similar studies conducted in Finland, Lithuania and Iran, which also showed how cow's milk allergy is much more common in boys than in girls (Pyrhönen et al., 2009; Kavaliūnas et al., 2013; Teymourpour et al., 2013). In addition, in a study conducted in Iran that examined the incidence of anaphylaxis

caused by cow's milk allergy in the population of children aged five years, it was found that the average value of serum concentrations of specific IgE antibodies to cow's milk protein in patients with anaphylaxis was 19.3 kU/L, where in 59.2% of cases of anaphylaxis occurred among boys. If this value is compared with the values determined in this study, in which the boys had a significantly higher average value of serum concentrations of specific IgE antibodies to cow's milk compared to girls, it can be concluded that in this study, clinical manifestations of allergy in boys were much severe and that boys are consequently more prone to develop anaphylaxis (Teymourpour et al., 2013). With regard to the higher incidence of allergy to cow's milk and all other nutritional allergies in boys, in the available literature there is no unique or exact explanation. As possible causes narrower airways in small boys than girls, increased bronchial tone or possibly higher concentrations of IgE antibodies in the blood of the boys are mentioned and all of them lead to changes in the bronchial lumen in response to exposure to a variety of potentially allergenic stimuli from food (Kavaliũnas et al., 2013).

Results of the study showed that the mean age of diagnosis of cow's milk allergy in the study population was 27.2 months, while it was slightly lower in boys (25.5 months) than in girls (31.5 months). When comparing these results with the results of a similar study conducted in Thailand, where the mean age of diagnosis of cow's milk allergy was 14.8 months, it may be concluded that the confirmation of cow's milk allergy in the studied population of children from the Vukovar–Srijem County was relatively late (Ngamphaiboon et al., 2008).

The study further showed that 56.5% of subjects with confirmed allergy to cow's milk lived in the village and 22.2% in the town, which is consistent with the results of a similar study conducted in Poland in the population of children aged up to three years, which showed that the prevalence of nutritional allergies, including those to cow's milk was higher among children who lived in the village (Malinowska et al., 2002). Possible reasons for the frequent occurrence of cow's milk allergy in children from rural areas compared to

children from the town is the earlier introduction of unprocessed cow's milk in the diet of infants and young children in the village compared to the town which consequently leads to more frequent development of allergic reactions (Vrdoljak, 2014).

This study has several limitations that should be considered when evaluating the obtained results. One of the limitations refers to the small number of children from the Vukovar–Srijem County - suspected of having cow's milk allergy and consequently small number of children with confirmed allergy to cow's milk. Second limitations refers to the lack of application of the specially designed questionnaire that would serve as a tool for collecting additional data concerning the subjects' personal and family history and information about possible risk factors for the occurrence of cow's milk allergy among each of them.

Conclusions

This study indicates that the cow's milk allergy is relatively frequent within the observed population of children from the Vukovar-Srijem County pointing to the need for further evaluation of this issue. In addition, the study has shown that the diagnosis of cow's milk allergy in Croatia is still relatively late. Bearing in mind that early diagnosis of the condition is crucial for the adequate treatment of the affected child one must make additional efforts for earlier diagnosis of such condition that would also improve the therapeutic approach to affected children.

Literature

1. Fiocchi A, Brozek J, Schünemann H et al (2010) World Allergy Organization (WHO) Diagnosis and Rationale for Actions againts Cow's Milk Allergy (DRACMA) Guidelines. Pediatr Allergy Immunol 21(suppl 21):1–125
2. Høst A (2002) Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol 89(Suppl 1):33–37
3. Høst A, Halken S, Jacobsen HP et al (2002) Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. Pediatr Allergy Immunol 13(Suppl 15):23–28
4. Jurčić Z, Oberiter V (1996) Nepoželjne

imunološke (preosjetljivosti) i neimunološke (nepodnošljivost) reakcije pri prehrani kravljim mlijekom. Mljekarstvo 46:41–55

5. Kavaliūnas A, Šurkienė G, Dubakienė R et al (2013) Potential risk factors of food allergy in children: EuroPrevall project results in Vilnius, Lithuania. Health 5:2032–2038

6. Kilgallen I, Gibney MJ (1996) Parental perception of food allergy or intolerance in children under 4 years of age. J Hum Nutr Diet 9:473–478

7. Kolaček S (2011) Preosjetljivost na hranu u dječjoj dobi. Acta Med Croatica 65:155–161

8. Kvenshagen B, Halvorsen R, Jacobsen M (2008) Adverse reactions to milk in infants. Acta Paediatr 97:196–200

9. Madrigal BI, Alfaro AN (1996) Adverse reactions to food in daycare children. Rev Alerg Mex 43:41–44

10. Malinowska E, Kaczmarski M, Wasilewska J (2002) Total IgE levels and skin test results in children under three years of age with food hypersensitivity. Med Sci Monit 8:CR280–287

11. Matricardi PM, Bockelbrink A, Beyer K et al (2008). Primary versus secondary immunoglobulin E sensitization to soy and wheat in the Multi-Centre Allergy Study cohort. Clin Exp Allergy 38:493–500

12. Ngamphaiboon J, Chatchatee P, Thongkaew T (2008) Cow's milk allergy in Thai children. Asian Pac J Allergy Immunol 26:199–204

13. Osterballe M, Hansen TK, Mortz CG et al (2005) The prevalence of food hypersensitivity in an unselected population of children and adults. Pediatr Allergy Immunol 16:567–573

14. Phadia AB (2012) Product/Allergy-testing-products/ImmunoCAP-Lab-Tests/ImmunoCAP-Total-IgE/Test-Principe/ http://www.phadia.com/Products/Allergy-testing-products/ImmunoCAP-Lab-Tests/sIgE/Test-Principle/ Accessed 20 July 2014

15. Pyrhönen K, Näyhä S, Kaila M et al (2009) Occurrence of parent-reported food hypersensitivities and food allergies among children aged 1-4 yr. Pediatr Allergy Immunol 20:328–338

16. Roehr CC, Edenharter G, Reimann S et al (2004) Food allergy and non-allergic food hypersensitivity in children and adolescents. Clin Exp Allergy 34:1534–1541

17. Rona RJ, Keil T, Summers C et al (2007) The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol 120:638–646

18. Saarinen KM, Juntunen-Backman K, Järvenpää AL et al (1999). Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: a prospective study of 6209 infants. J Allergy Clin Immunol 104:457–461

19. Sandin A, Annus T, Björkstén B et al (2005) Prevalence of self-reported food allergy and IgE antibodies to food allergens in Swedish and Estonian schoolchildren. Eur J Clin Nutr 59:399–403

20. Steinke M, Fiocchi A, the REDALL group (2007) Perceived Food allergy in children. A report on a representative telephone survey in 10 European countries. Int Arch Allergy Asthma Immunol 143:290–295

21. Teymourpour P, Shokouhi R, Fazlollahi MR et al (2013) Factors Affecting the Severity of Cow's Milk Anaphylaxis. Iran J Allergy Asthma Immunol 12:190–191

22. Venter C, Pereira B, Grundy J et al (2006) Incidence of parentally reported and clinically diagnosed food hypersensitivity in the first year of life. J Allergy Clin Immunol 117:1118–1124

23. Vrdoljak D (2014) Dijagnostički pristup alergiji na kravlje mlijeko u populaciji djece dojenačke i rane predškolske dobi s područja Vukovarsko – srijemske županije. Završni rad. Medicinski fakultet, Osijek

INFLUENCE OF EXTRACTION TYPE ON THE TOTAL PHENOLICS, TOTAL FLAVONOIDS AND TOTAL COLOUR CHANGE OF DIFFERENT VARIETIES OF FIG EXTRACTS

Stela Jokić[1]*, Ibrahim Mujić[2], Ana Bucić-Kojić[1], Darko Velić[1], Mate Bilić[1], Mirela Planinić[1], Jasmina Lukinac[1]

[1]University of Josip Juraj Strossmayer in Osijek, Faculty of Food Technology Osijek, Franje Kuhaca 20, 31000 Osijek, Croatia
[2]Colegium fluminense Polytechnic of Rijeka, Trpimirova 2/V, 51000 Rijeka, Croatia

Original scientific paper

Summary

The study examined the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous ethanol solution on the total phenolics, total flavonoids and total colour change of extracts from different varieties of figs (Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja). The total phenolic content was determined by using Folin Ciocalteau assay. The content of total flavonoids was measured spectrophotometrically by using the aluminium chloride colourimetric assay. Colour changes were obtained by chromameter CR-400 (Minolta) in L*a*b* colour model. Ultrasound-assisted extraction showed highest total phenolic content (up to 13.72%) and total flavonoid content (up to 18.55%) compared to classic solid-liquid extraction. According to given results total colour changes of extracts were reduced (up to 32.1%) applying ultrasound. Significant difference was found between the total phenolic and total flavonoid content according to different varieties of fig.

Keywords: fig, extraction, phenolics, flavonoids, colour

Introduction

The cultivated fig (*Ficus carica L.*) belongs to botanical family *Moracea*. Figs are a widespread species commonly grown, especially in warm, dry climates and they are mostly concentrated in the Mediterranean. Figs are an excellent source of minerals, vitamins and dietary fibre, fat and cholesterol-free and contain essential amino acids as well as high content of phenolic substances (Slavin, 2006; Solomon et al., 2006).

Phenolic compounds represent an important component of fruit because of their significantly contribution to the taste, colour and nutritional value of fruits (Veberic et al., 2008), and in recent years have been the subject of scientific research for many researchers because of their positive effects on human health, which is attributed to the antioxidation activities.

Extraction is an important step in isolation and in later identification and quantification of phenolic compounds (Cacace and Mazza, 2003). Solid-liquid extraction is a commonly used method of isolation phenolic compounds from plant materi-

als. Selection of solvents is one of the most important steps in the extraction, and in previous studies the most commonly used solvent for the solid-liquid extraction of phenolic compounds from plant materials are methanol, ethanol and their liquid mixtures with different content of water and other organic solvents such as acetone, ethyl acetate (Naczk and Shahid, 2004).

Ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of use of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics. Ultrasound can also reduce the operating temperature allowing the extraction of thermolabile compounds (Wang and Weller, 2006).

The phenol content from fig have been recently analysed by Duenas et al. (2008), Solomon et al. (2006) and Veberic et al. (2008), but literature data about the influence of different extraction methods on the extractability of phenolic compounds from figs are scarce. This study examined the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous

*Corresponding author: stela.jokic@ptfos.hr

ethanol solution on the total phenolics, total flavonoids and total colour change of extracts from five different autochthonous varieties of figs (Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja).

Materials and methods

Material

Fruits from five selected commercial fig (*Ficus carica L.*) varieties: Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja were harvested in region of Istria at the optimal ripening time (given by experts in agronomy) in year 2008. Fig samples were frozen at -20 °C and than freeze dried in the freeze drying equipment (LIO-10P, Kambic d.o.o., Slovenia). Samples were grounded a blender before the extraction. Dry matter content of fig samples were determined by drying of 5 g milled fig samples at 105 °C to constant mass. Analyses were done in duplicate and the average dry matter content was noted as percentage (%). The content of dry matter was in range from 85-88% depending from fig variety of samples used in this study.

Solid-liquid extraction

In the test tubes, 0.5 g of fig sample was mixed with 20 ml of 80% aqueous ethanol solution. The extraction process was conducted in laboratory scale by using water bath (Julabo SW-23, Germany) with shaking (200 rpm) for 15 minutes at 50 °C. All extraction runs were performed in duplicate. Obtained extracts were separated from rough particles after extraction by decantation and were centrifuged (Sigma 2-16, Germany) for 10 minutes on 10 000g. Supernatant was decanted and updated to the known volume (20 ml). Supernatant was stored at +4 °C until further analysis.

Ultrasound-assisted extraction

Fig samples (0.5 g) were extracted by 80% aqueous ethanol solution (20 ml) as a solvent. The extraction process was carried out using ultrasonic bath (Sonorex Super RK 100 SH) at the 50 °C for

15 minutes in two repetitions.
Ethanol extracts were centrifuged at 10 000 g for 10 minutes and pooled with extraction solvent. Supernatant was stored at +4 °C until further analysis.

Total phenolic content (TPC)

The concentration of total phenolic compounds in the extracts was determined by Folin-Ciocalteau micro-method (Waterhouse, 2009) as follows: 40 μl of extract was mixed with 3160 μl of distilled water and 200 μl of Folin-Ciocalteau reagent. After 30 seconds to 8 minutes, 600 μl of 20% of sodium carbonate solution was added. All test tubes with mixture were shaken for 10 seconds on the Vortex and incubated in a water bath at 40 °C. Absorbance was measured after 15 min on UV/VIS spectrophotometer (UV 1700 Shimadzu, Japan) at 765 nm against blank sample. Blank sample was prepared with water instead of extract. Determination of total phenolic compounds was carried out in a duplicate and calculated from the calibration curve obtained with gallic acid, which was used as a standard and final results were recalculated and expressed as gallic acid equivalent per dry basis of fig samples (mg GAE/gdb).

Total flavonoid content (TFC)

The concentration of total flavonoid compounds in the extracts was determined by the aluminium chloride colourimetric assay (Marinova et al., 2005) as follow: 1 ml of extract was added to 10 ml volumetric flask containing 4 ml of distilled water and 0.3 ml 5% NaNO2. After 5 min, 0.3 ml 10% AlCl3 was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Determination of total flavonoid compounds was carried out in a duplicate and calculated from the calibration curve obtained with (+)-catechin, which was used as a standard and final results were recalculated and expressed as (+)-catechin equivalent per a dry basis of fig samples (mg CE/gdb).

Colour measurement

The colour of samples was measured using Minolta CR-400 Chromameter. The figs were milled in a coffee grinder to obtain fine powder. Analyses of colour values were done twenty times for each sample. Three parameters, L (lightness), a (redness) and b (yellowness), were used to study changes in the colour. L refers to the lightness of the samples and ranges from black = 0 to white = 100. A negative value of a indicates green, while a positive one indicates red-purple colour. Positive b indicates yellow and negative blue colour. The total colour difference (ΔE) was calculated as follows (Hunter, 1975):

$$\ddot{A}E = \sqrt{\left[\left(L - L_0\right)^2 + \left(a - a_0\right)^2 + \left(b - b_0\right)^2\right]} \quad (1)$$

where L0, ao and b0 indicate colour parameters of fresh fig samples. Fresh fig samples were used as the reference and a higher ΔE represents greater colour change from the reference material.

Statistical analysis

Statistica 7.0 (Stat Soft Inc., USA) was used for data analyzing. One-way analysis of variance (ANOVA) and multiple comparisons (post-hoc LSD) were used to evaluate the significant difference of the data at $p<0.05$. Data were expressed as means ± standard deviation. Experiments were replicated two times for statistical purpose.

Results and discussion

The aim of this study was to examine the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous ethanol solution as a solvent on the total phenolics, total flavonoids and total colour change of extracts from five different autochthonous varieties of figs: Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja. (Fig. 1).

| BJELICA | TERMENJAČA | ŠARAGUJA | BRUŽETKA BIJELA | CRNICA |
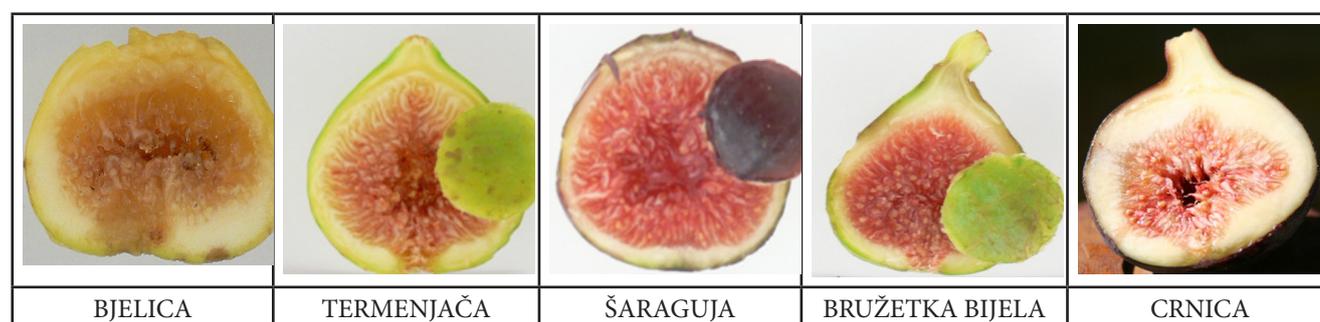
Fig. 1. The autochthonous fig varieties in Istria (Croatia)

Dry matter content in all experimental runs was determined and the results of total phenol and total flavonoid concentrations were expressed on dry basis, which generally provides more accurate and reliable data comparison.

Fig. 2 showed the total colour change (ΔE) of freeze dried fig samples. It can be seen that fig sample variety Crnica showed the smallest total colour changes, while fig sample variety Termenjača showed the highest colour changes according to fresh fig sample. An ANOVA analysis showed the existence of four groups which differed significantly (p < 0.05) from one to another according to different fig varieties. Represented results show that there are no statistically significant differences according to colour changes between freeze dried fig samples variety Bjelica and Bružetka bijela.
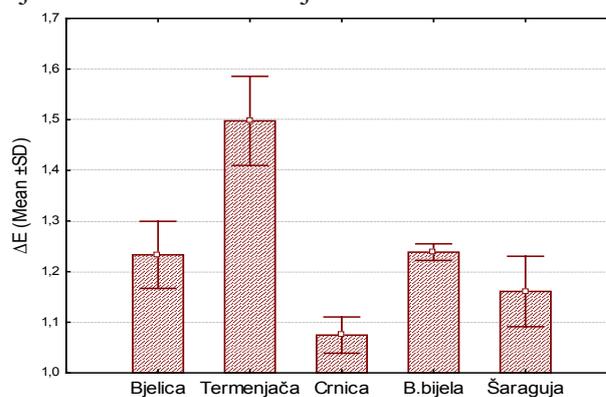
Fig. 2. Total colour change (ΔE) of freeze dried fig samples

Fig. 3 showed the total colour change (ΔE) of extracts obtained by ultrasound-assisted extraction and solid-liquid extraction with 80% aqueous ethanol solution from different fig varieties. It can be seen that total colour changes of extracts were significantly reduced applying ultrasound up to 31.1% in fig variety Bjelica, 6.2% in fig variety Termenjača, 15.2% in fig variety Crnica, 26.6% in fig variety Bružetka bijela and up to 3.1% in fig variety Šaraguja compared to classic solid-liquid extraction.
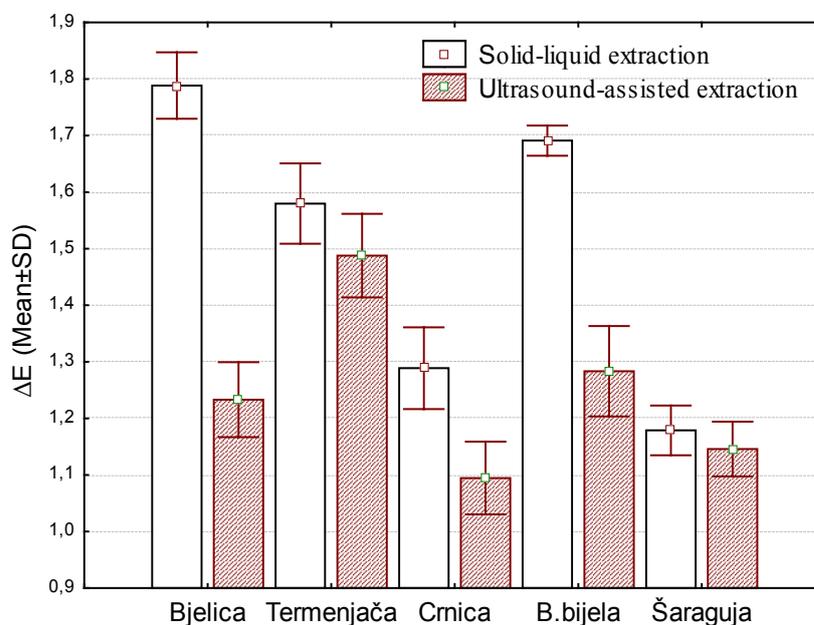
Fig. 3. Total colour change (ΔE) of extracts from different fig varieties

Fig. 4 and Fig. 5 showed the influence of two investigated extraction methods on total phenolic content and total flavonoid content in different fig extracts, respectively. The aqueous ethanol solution was selected as solvent due to environmental safety, low cost and less toxicity, unlike the other solvents (e.g. methanol).
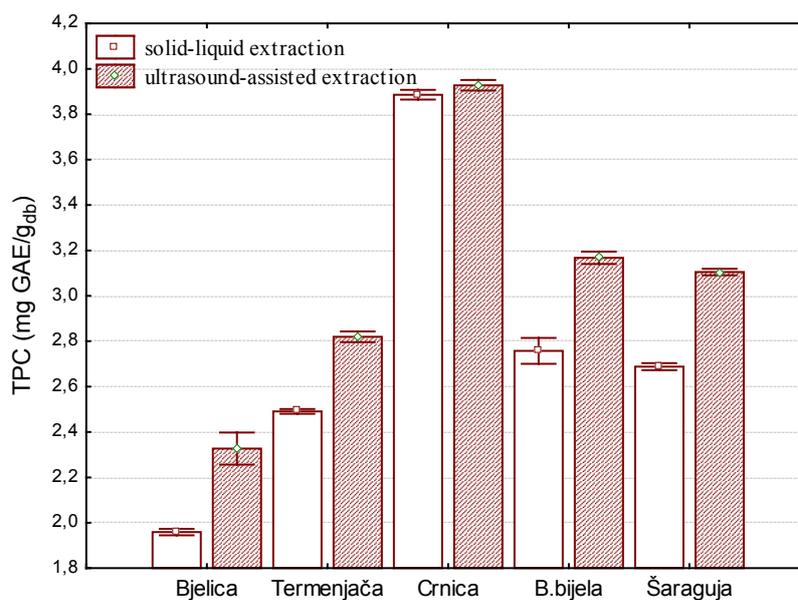
Fig. 4. Effect of extraction type on total phenolic content in different fig extracts

Although for the food purposes water represented the best solvent selection, as a polar solvent (such as the phenolic compounds) water extracted the other undesirable macromolecules as well (protein, polysaccharide, etc.) especially at higher temperatures and pressures (Rostagno et al., 2003; Tsao and Deng, 2004). Spigno et al. (2007) found that a higher content of water in the ethanol/water solution (concentration of aqueous ethanol solution lower than 50%) reduced the extraction of polyphenols. Also, Rostango et al. (2004) found that it is necessary to add a certain amount of water in the extraction solvent in order to improve the extraction of phenolic compounds.

The water content higher than 60% resulted in a reduction of the extraction yield the same components. Using pure ethanol as solvent reduced the extraction efficiency since the polyphenols, due to a number of hydroxyl groups (such as flavonoids, glycosides), are hydrophyl, and as such generally more soluble in water-ethanol solutions than in pure alcohol. The reason for low content of phenolic substances in water extracts can also be the increased activity of enzymes (polyphenol oxidase, PPO) that degraded phenolic substances unlike alcoholic media in which the same is inactive (Lapornik et al., 2005).
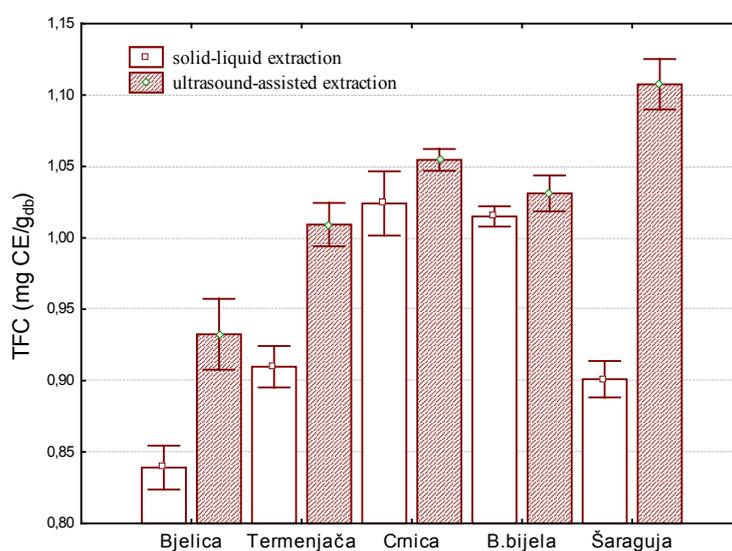

Fig. 5. Effect of extraction type on total flavonoid content in different fig extracts

Represented results (Fig. 4 and Fig. 5) showed that ultrasound-assisted extraction increased the total phenolic content (up to 13.72%) as well as the total flavonoid content (up to 18.55%) in fig samples. The reason for this is the mechanical effects of ultrasound which caused a greater penetration of solvent into cellular materials and improved mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. The efficient cell disruption and effective mass transfer can increased the extraction yield and, furthermore, ultrasound can even improved the quality of extracts (Mason et al., 1996; Wang and Weller, 2006). The highest concentration of total phenols was obtained from fig extracts variety Crnica, while the lowest con-

centration of total phenols was achieved from fig variety Bjelica. Statistical analysis (ANOVA, post-hoc LSD, $p<0.05$) showed that applied extraction methods had no statistically significant influence only on total phenolic content in the fig samples variety Crnica. The darker varieties of figs resulted in the highest content of phenolics and flavonoids compare to white type of figs such as analyzed variety of fig Bjelica. The similar results have been published by Veberic et al. (2008), where the phenol content in the white variety of fig ("Škofjotka") was significantly lower than in the darker varieties ("Miljska figa" and "Črna petrovka").

Similar data for the extraction yield of phenolic compounds from figs had been published by

other authors, where specific differences exist because of different methodologies of experimental work, sample diversity (climate, variety, etc.) or part of the sample from which extraction was carried out (fruit with or without skin, pulp, leaves) and ways of expression the content of phenolic compounds (expressed on the mass of fresh sample, or on the mass of dry matter) in the sample. Marinova et al. (2005) were determined the phenolic content from freeze-dried fig samples with 80% aqueous methanol solution at room temperature. The extraction yield of total phenolic content from fig sample was expressed as mg gallic acid equivalents GAE/100g fresh weight (TPC = 59.0 mgGAE/100g fresh mass) and total flavonoid content was expressed as mg catechin equivalents CE/100g fresh weight (TFC = mgCE/100g fresh mass).

Conclusions

According to experimental results in this study, ultrasound-assisted extraction showed the highest total phenolic content (up to 13.72%) and total flavonoids content (up to 18.55%) compared to classic solid-liquid extraction. Represented results show that total colour changes of extracts were reduced (up to 32.1%) applying ultrasound. Significant difference was found between total phenolic and total flavonoids content according to different varieties of fig. The darker varieties of figs resulted in the highest content of phenolics and flavonoids compare to white type of figs. Ultrasound can increase the extraction yield and improved the quality of extracts. Additional research is required to investigate the chemical composition of these phenolic compounds in fig samples.

Acknowledgements

References

Cacace J E, Mazza G (2003) Mass transfer process during extraction of phenolic compounds from milled berries. J Food Eng 59:379-389

Duenas M, Pérez-Alonso J J, Santos-Buelga C, Escribano-Bailón T (2008) Anthocyanin composition in fig (Ficus carica L.). J Food Compos Anal 21:107-115

Hunter RS (1975) Scales for the measurements of colour difference. In: Measurement of Appearance, John Willy & Sons, New York, USA, pp 133–140

Lapornik B, Prošek M, Golc Wondra A (2005) Comparison of extracts prepared from plant by-products using different solvent and extraction time. J Food Eng 71: 214-222

Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Chem Technol Metall 40: 255-260

Mason TJ, Paniwnyk L, Lorimer JP (1996) The uses of ultrasound in food technology. Ultrason Sonochem 3: 253–260

Naczk M, Shahidi F (2004) Extraction and analysis of phenolics in food. J Chromatogr A 1054: 95-111

Rostango MA, Palma M, Barroso CG (2004) Pressurized liquid extraction of isoflavones from soybeans. Anal Chim Acta 522: 169-177

Slavin JL (2006) Figs: Past, Present and Future. Nutr Today 41: 180-184

Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, Altman A, Kerem Z, Flaishman MA (2006) Antioxidant activities and anthocyanin content of fresh fruits of common fig (Ficus carica L.). J Agric Food Chem 54: 7717-7723

Spigno G, Tramelli L, De Faveri DM (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J Food Eng 81: 200-208

Tsao R, Deng Z (2004) Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatogr B 812: 85-99

Veberic R, Colaric M, Stampar F (2008) Phenolic acids and flavonoids of fig fruit (Ficus carica L.) in the northern Mediterranean region. Food Chem 106: 153-157

Wang L, Weller CL (2006) Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Techn 17: 300–312

Waterhouse AL (2009) Determination of total phenolics. In: Current Protocols in Food Analytics Chemistry, Wrolstad, R.E. (ed.), : John Wiley & Sons Inc., New York

EFFECT OF VARIETY, GROWING SEASON AND STORAGE ON POLYPHENOL PROFILE AND ANTIOXIDANT ACTIVITY OF APPLE PEELS

Ante Lončarić*, Vlasta Piližota

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, HR-31000 Osijek, Croatia

Original scientific paper

Summary

Identification and quantification of the major polyphenols, and antioxidant activity (AOA), in the peel of apple varieties, Granny Smith (GS) and Gold Rush (GR), during storage and over two growing seasons (2011 and 2012) were examined.GR had higher amount of flavan-3-ols, dihydrochalcones, and flavonols compared to GS. Of all polyphenolic groups, flavonols were the most influenced by the climacteric conditions during the growing season. The higher amount of phenolic acids was detected in 2012 for the both apple varieties. Dihydrochalcones were influenced more by the variety than by the climacteric conditions during growing season. Changes in polyphenol content (TPC) and AOA during storage depended on the variety. Samples of apple peel powder, after storage, preserved the most of the antioxidants and functional properties, suggesting that apple peel powder may be used in a various food products to add phytochemicals and promote good health.

Keywords: Apple by-product, Phenolics, Freeze-drying, Antioxidant activity

Introduction

Recently, a general consensus has been achieved to sustain the hypothesis that the specific intake of foods and beverages, containing relatively high concentrations of phytochemicals such as flavonoids, may play a meaningful role in reducing cardiovascular disease (CVD) risk through an improvement in vascular function and a modulation of inflammation (Habauzi and Morand, 2012): Apples are generally considered "healthy food" with the existing saying "An apple a day keeps the doctor away". Like in other fruits and vegetables, polyphenols are the main ingredients that are considered to have a positive impact on health (Boyer and Liu, 2004; Xiuzhenet al.,2007; Hyson, 2011):The phytochemical composition of apples varies greatly between different varieties of apples, and it was found that there are also small changes in phytochemicals during the maturation and ripening of the fruit (Kondo et al.,2002; McGhieet al.,2005; Wojdyło et al.,2008):Ceymannet al.(2012) conducted study on 104 European apple varieties for 12 polyphenols by UHPLC–MS. This study is one of the more comprehensive evaluations of the polyphenol content and profile of different apple varieties

so far. They identified two main classes of apples based on their polyphenol profile: those rich in flavan-3-ols and those rich in phenolic acids. From this and other studies it could be concluded that polyphenol profile of apples are highly variety dependent (Łata and Tomala, 2007; Łata, 2007; Matthes and Schmitz-Eiberger, 2008; Wojdyło et al., 2008; Neveuet al., 2010; Ceymannet al., 2012): The concentration of polyphenols is influenced by the environmental factors and by the geographic region, storage, and growing season. Various authors reported seasonal effect on antioxidant capacity and polyphenols (Matthes and Schmitz-Eiberger, 2008; Mainlaet al., 2011; Keverset al., 2011):Strackeet al., (2009) reported growing season variations in the antioxidant capacity and the polyphenol content up to 20%. In conflict to this van Der Sluiset al. (2001) and Guyotet al. (2003) observed little or no seasonal effect on antioxidant capacity and polyphenol concentration in apple varieties when they compared the results of different growing seasons. However, at the present time, the influence of pre-harvest factors on polyphenol profiles has only been investigated in relation to growing season, and the impact of the other pre-harvest factors on polyphenol profile remains unknown.

*Corresponding author: ante.loncaric@ptfos.hr

Polyphenols seem to be stable during storage. Regarding apple peel, it was reported that phenolic metabolism in apple peel is relatively stable, and the health benefits of phenolics in apple peel should be maintained during long-term storage, while some studies reported increase of TPC (first 60 days) and decrease after 100 days (Lattanzioet al.,2001; van der Sluiset al.,2001; Golding et al.,2001; Napolitano et al.,2004): In contrast, some researchers found that epicatechin, quercetin glycosides and procyanidins in GS apples generally decreased during storage (Pirettiet al.,1994): The majority of researchers reported that no change occurred in the concentrations of simple phenols (mainly chlorogenic acid), flavonoids and anthocyanin during storage (Perez-Ilzarbeet al.,1997; Awad and de Jager, 2000; Golding et al.,2001):

In 2000, it was estimated that 2.7 million deaths (4.9%) and 26.7 million disability adjusted life years (DALYs; 1.8%) were attributable to low fruit and vegetable intake globally. Manny diseases could be prevented by increasing dietary intake of fruits and vegetables to the minimum recommended daily intakes established by the WHO (Lock et al.,2004): One way of increasing the intake of phytochemicals from fruit and vegetable is restoration and enrichment of food products with by-products obtained from fruits and vegetables processing industry.

During apple juice, sauce and canned apple manufacture, the antioxidant - rich peels of apples are discarded. It is known that apples and especially their skins have high concentration of phenolic compounds, dietary fiber, and minerals and may assist in the prevention of chronic diseases (Wolf et al., 2003; Biedrzyckaet al., 2008; Denis et al., 2013): Some authors found that on average 46% of the polyphenolics in the whole apples were in the skin (McGhieet al., 2005): It is well known that freeze–drying is superior process of dehydration. Freeze-drying utilizes the principle that under high vacuum, frozen water is directly removed by sublimation of ice without passing through intermediate liquid stage, those providing product with no damage, little or no loss in sensory qualities, and a porous honeycomb structure (Sethiet al.,2007):The aim of this work was to investigate how rich is the freeze-dried apple skin in polyphenols in order to be used in a various food products.

Materials and methods

Chemicals

Folin-Ciocalteu reagent was purchased from Kemika (Zagreb, Croatia), 2,6-dichlorophenol indophenols, phloretin, catechin, epicatechin, rutin, quercitin, chlorogenic acid, caffeic acid from Sigma Chemical Co. (St. Louis, USA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), 2,2-diphenyl-1-picrylhydrazyl, procyanidin B2 from Fluka (St. Louis, USA) phlorizin from Aldricht (St. Louis, USA), methanol (HPLC gradient grade), and o-phosphoric acid (85%) from Panareac (Barcelona, Spain):

Apple varieties used for experiment

Actual apple varieties, Granny Smith (GS), and Gold Rush (GR) were harvested from the Agricultural Institute (Osijek, Croatia) in 2011 and 2012. Both apple varieties were stored in the commercial storage facility under the normal atmosphere at 2-6 oC before they were purchased for study. Physical and chemical analysis was carried on the whole apples (flesh and peel): Total polyphenol content (TPC) and antioxidant activity (AOA) of the apple peels were evaluated immediately after harvesting and after freeze–drying, and after 1 and 6 months of storage.

Physical and chemical analysis

Apples were held at room temperatures for cca 1 h before preparing for analysis. Before the apple fruits were disintegrated (Braun Multiquick Professional 600 Watt Turbo) for the analysis the core was removed. Content of soluble solids of apples were measured with table Abbe refractometer and is given in Brix (°Brix): Acids were measured by titration with 0.1 M NaOH and phenolphthalein as an indicator and given in g/100 g, as malic acid. Reducing and total sugar content was determined by Luff Schoorl's method (Eganet al., 1981), and vitamin C (L-ascorbic acid) by volumetric method - titration with di-

chlorophenol indophenol (DCPIP):

Freezing and freeze – drying

Before freeze-drying apple peels were firstly disintegrated and then placed in a plastic bags and frozen at -18 °C for 12 h before freeze-drying in laboratory freeze-dryer (Christ Freeze Dryer, Gamma 2-20, Germany): Drying conditions were as follows: freezing temperature: -55 °C; the temperature of sublimation: -35 °C to 0 °C; and the vacuum level: 0.220 mbar. The temperature of isothermal desorption varied from 0 °C to 22 °C under the vacuum of 0.060 mbar. Freeze-drying lasted about 48 h until the total content of solids was 94-98%.

Determination of total polyphenol content

The extraction of polyphenols from prepared apple peels was carried out with acidified methanol (1 g apple purée in 10 mL acidified methanol): The samples were held at the ambient temperature for 1 h. After 1 h mixture was filtered through pleated filter paper. The extracts were used for the determination of TPC and AOA. Extraction of phenolics for identification of phenolics were performed as follows: phenolics were extracted from freeze-dried samples (250 mg) using 80% aqueous methanol (5 mL): The mixture was sonicated for 15 min and centrifuged at the room temperature for 15 min. After extraction samples were filtered through 0.45 μm poly(tetrafluoroethylene) syringe-tip filter and extracts were used for HPLC analysis.

The TPC was determined by the modified colorimetric Folin-Ciocalteu method (Ough and Amerine, 1988): 0.2 mL of apple extract and 1.8 mL of deionizer water were added to a 23 mL test tube. 10 mL of Folin-Ciocalteu reagent (1:10) was added to the solution, and finally 8 mL of 7.5% of sodium carbonate (Na_2CO_3) solution was transferred into the test tubes. The color was developed in 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK): The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as g of gallic acid per kg of sample equivalents (g GAE/kg) of sample.

Antioxidant activity determination

2,2′-Azino-bis-3ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS) scavenging activity
ABTS assay followed the method of Arnaoet al.(2001) with some modifications. The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured ABTS value was over the linear range of the standard curve.
1, 1-Diphenyl-2-picryl-hydrazil (DPPH•) scavenging activity
For DPPH assay 0.2 mL of the apple extract was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min the absorbance was measured at 517 nm (Brand-Williams et al.,1995): The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve.

Identification of phenolics

The analytical HPLC system employed consisted of a Varian LC system (USA) equipped with a ProStar 230 solvent delivery module, and ProStar 330 PDA Detector. Phenolic compounds separation was done in an OmniSpher C18 column (250 x 4.6 mm inner diameter, 5 μm, Varian, USA protected with guard column (ChromSep 1 cm x 3 mm, Varian, USA): The data were collected and analyzed on IBM computing system equipped with Star Chromatography Workstation software (version 5.52): The same solvents and gradient elution program were used in determination of phenolic acids and flavonols. Solvent A was 0.1% phosphoric acid and solvent B was 100% HPLC grade methanol. The elution conditions were as follows: 0-30 min from 5% B to 80% B; 30-33 min 80% B; 33-35 min from 80%, B to 5% B; with flow rate=0.8 mL/min (Jakobeket al.,2007): Phenolic standards were used to generate characteristic UV – vis spectra and calibration curves. Individual phenolics in the sam-

ple were tentatively identified by comparison of their UV – vis spectra and retention times with spiked input of polyphenolic standard. Three replicated HPLC analyses were performed for each sample.

Statistical analysis

All measurements were done in triplicate and data were expressed as mean ± standard deviation. The experimental data were subjected to an one-way analysis of variance (ANOVA) and Fisher's LSD were calculated to detect significant difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program MS Excel (Microsoft Office 2007 Professional):

Results and discussion

Effect of variety

Apples, and especially their skins, contain the polyphenol groups (flavonols, flavan-3-ols, phenolic acids, dihydrochalcones) of which the main compounds are: epicatechin, procyanidin B2, phloretin xyloglucoside, phloridzin, and chlorogenic acid. GS and GR apple peel contained an additional six quercetin glycoside (glucoside-galactoside, rhamnoside, xyloside, arabinoside, and rutinoside): Differences in phenolic profile between GS and GR apple peel were only in levels of certain phenolic compounds. Apple variety GS contained the lower sum of polyphenols determined by HPLC than GR.

Table 1. The physicochemical parameters of GS and GR apple varieties

	Granny Smith		Gold Rush	
Parameters	2011	2012	2011	2012
The average fruit weight (g)	206.83 ± 11.2	168.51 ± 17.5	192.74 ± 12.6	107.95 ± 9.3
Hardness (kg/cm2)	9.9 ± 0.170	9.64 ± 0.090	10.33 ± 0.123	10.02 ± 0.206
Moisture (%)	83.76 ± 0.104	81.49 ± 0.111	82.84 ± 0.034	82.89 ± 0.014
Soluble solids (° brix)	12.03 ± 0.150	14.63 ± 0.060	15.00 ± 0.000	14.13 ± 0.120
L-ascorbic acid (mg/100 g)	5.47 ± 0.240	7.80 ± 0.201	8.42 ± 0.450	4.75 ± 0.000
Acids (g/100 g of malic acid)	0.48 ± 0.010	0.63 ± 0.000	0.51 ± 0.040	0.38 ± 0.019
pH	3.33 ± 0.010	3.31 ± 0.014	3.72 ± 0.02	3.80 ± 0.016
Sugars				
Reducing	6.45 ± 0.24	7.25 ± 0.034	6.21 ± 0.12	7.35 ± 0.018
Total	9.19 ± 0.180	9.86 ± 0.028	8.08 ± 0.14	9.27 ± 0.072
TPC (g EGA/kg)				
Peel	3.11 ± 0.138	2.88 ± 0.062	3.53 ± 0.059	3.57 ± 0.072
Peel + Flesh	1.06 ± 0.034	1.29 ± 0.068	1.07 ± 0.024	1.14 ± 0.007
Flesh	0.76 ± 0.015	0.90 ± 0.046	0.97 ± 0.049	1.05 ± 0.019
AOA				
ABTS (mmol TE/100ml)				
Peel	47.89 ± 0.07	49.28 ± 1.127	54.08 ± 0.686	54.83 ± 0.259
Peel + Flesh	7.00 ± 0.242	7.35 ± 1.068	11.99 ± 0.484	12.78 ± 0.880
Flesh	4.26 ± 0.348	3.35 ± 0.816	9.06 ± 0.461	8.92 ± 0.432
DPPH (mmol TE/100ml)				
Peel	3.89 ± 0.299	4.03 ± 0.116	4.73 ± 0.064	4.61 ± 0.095
Peel + Flesh	1.69 ± 0.226	2.19 ± 0.052	1.79 ± 0.045	3.04 ± 0.070
Flesh	1.62 ± 0.008	2.04 ± 0.122	1.77 ± 0.048	2.66 ± 0.094

Epicatechin with 8.6 – 14.1 mg/100 g and procya-nidin B2 with 4.3 – 8.0 mg/100 g contributed the most to the total flavan-3-ol content. The phenol-ic acids were dominated by chlorogenic acid with <lod-13.6 mg/100 g. Dihydrochalcones (phlorid-zin and phloretin-xyloglucoside) were found in both varieties, but amount of dihydrochalcones in GS were relatively lower compared to those in GR (0.3 – 1.4 mg/100 g and 0.4 – 1.3 mg/100 g, respectively): Flavonols were present in the range of 6.6 to 14.5 mg/100 g, among which rutin accounted for approximately half of the flavonols (4.0 - 5.6 mg/100 g): Comparing the polyphenol patterns of GS and GR, it can be seen that the to-tal levels of flavan-3-ols and flavonols are similar in GS (2012) and GR (2011), but both varieties had slightly higher amounts of flavan-3-ols than flavonols. In samples GS (2011) and GR (2012) dominant polyphenol group was flavan-3-ols. Dihydrochalcones and phenolic acids contribute with proportions of approximately 4% and 20%, respectively to the polyphenol pattern of both analyzed varieties (Table 2): Summing up these polyphenol groups, analyzed by HPLC (Table 2), the amounts were between 27 and 50 mg/100 g FM (depending on growing season) which is lower than the TPC determined by the Folin-Ciocalteu method with 288 to 357 mg gallic acid equivalents/100 g (Table 1): This comparison be-tween these two methods showed that the latter was several times higher than the sum of poly-phenols calculated from HPLC data. This trend was found in both apple varieties. The difference can be explained by poor specificity of the Folin-Ciocalteu assay, which is known to measure addi-tional components present in the extract (Single-ton et al.,1999):Vrhovseket al.(2004) excluded these substances by an additional cleanup step on a C-18 cartridge. The consequence of this addi-tional clean up step is that the TPC reported by Vrhovseket al.(2004) are lower than reported by other studies. For example, Sanoneret al.(1999) reported 128 mg epicatechin equivalents/100 g in the cortex area of Golden Delicious using epicat-echin as a reference, compared to 86 mg catechin equivalents/100 g in the same variety reported by Vrhovseket al.(2004): GR had also higher AOA (Table 1): Higher AOA is associated with higher levels of procyanidin B2 and epicatechin, which is in accordance to results reported by Tsaoet al. (2005) for procyanidin B2 and epicatechin, which were the major contributors to the AOA of apple. Besides, GS apple fruits were bigger in size, with higher acid content, but of similar hardness and moisture content as GR apples.

Table 2. Polyphenol profile of apple peels of GS and GR apple varieties (mg/100g fw) in two apple seasons.

Polyphenols		Granny Smith		Gold Rush	
		2011	2012	2011	2012
Procyanidin B2		4.25 ± 0.024^c	4.31 ± 0.081^c	5.68 ± 0.032^b	7.97 ± 0.062^a
Phloridzin		0.50 ± 0.163^c	0.26 ± 0.016^d	1.28 ± 0.016^a	1.38 ± 0.081^b
(-)Epicatchin		8.86 ± 0.260^c	$8.60 \pm 0.1.65^c$	9.60 ± 0.098^b	14.13 ± 0.227^a
Phloretin xyloglucoside		0.37 ± 0.054^c	0.37 ± 0.022^c	1.31 ± 0.013^a	1.02 ± 0.217^b
Chlorogenic acid		1.56 ± 0.193^c	10.24 ± 0.010^b	<lod	13.63 ± 0.044^a
Caffeic acid		1.24 ± 0.035^a	0.18 ± 0.001^c	<lod	0.18 ± 0.004^b
Quercetin* glycoside	23.517	0.70 ± 0.038^b	<lod	2.51 ± 0.029^a	<lod
	24.280	0.89 ± 0.025^c	1.72 ± 0.034^a	0.82 ± 0.005^d	1.48 ± 0.050^b
	24.605	1.07 ± 0.034^b	0.91 ± 0.019^c	3.29 ± 0.035^a	<lod
	24.848	<lod**	2.34 ± 0.036^b	2.73 ± 0.018^a	1.76 ± 0.058^c
	25.605	<lod	1.44 ± 0.008^b	0.37 ± 0.003^c	2.52 ± 0.088^a
Rutin		$3.96 \pm 0.322d$	5.47 ± 0.089^b	4.78 ± 0.338^c	5.54 ± 0.020^a

*Quercetin glycoside retention times.
**<lod: below limit of detection (<0.15 mg/100 g). Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at p ≤ 0.05, (ANOVA, Fisher's LSD).

Effect of growing season

All apples are collected from the orchard of the Agricultural institute Osijek near Osijek (45° 32.041', 18° 45.121'): Harvest dates, localization, tree cultivation technique were the same in both growing seasons. Weather conditions are presented in Fig. 1.
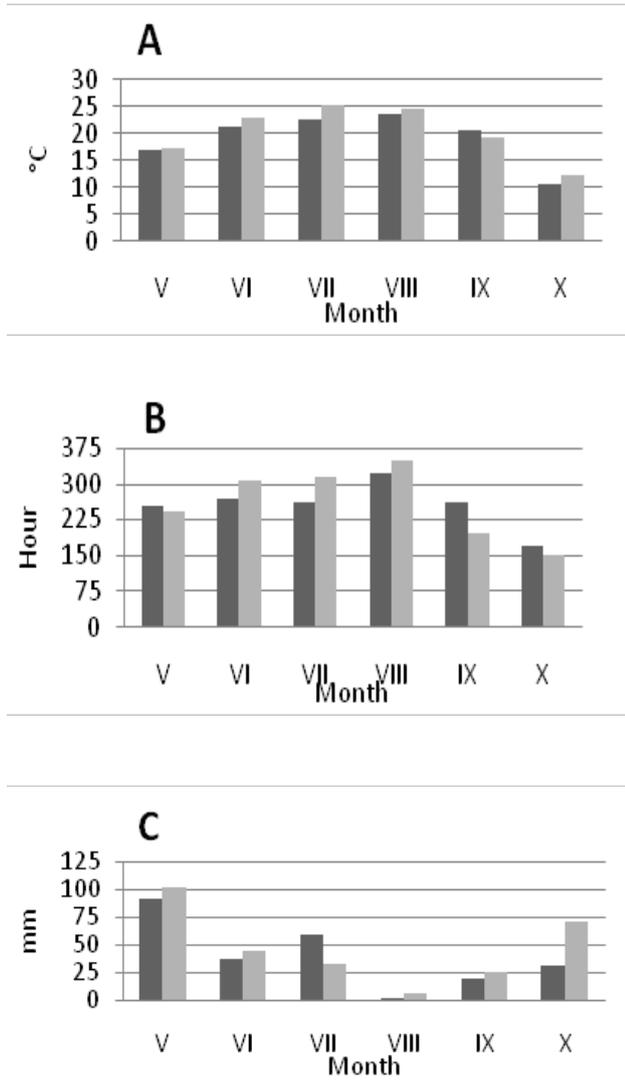

Fig. 1. The weather conditions for growing season 2011 and 2012. A - Mean monthly air temperature (°C); B – Sunshine (h); C –Precipitation (mm) in growing season.

According to data from 1960th to 2012, in both growing season, the mean air temperature was above average except for September (2011) 10.7 °C, average is 11.2 °C. During June, July and August (2011, 2012) the weather was fairly dry 1.7 - 90.46% of the average precipitation for these

three months fell. Only in May (2011, 2012) and September (2012), the precipitation was more than average for this period. Sufficient water supply may result with higher fruit weight; in arid years the fruit weight may be reduced including a lower dilution of the polyphenols in fruits, in contrast to a high fruit weight with a greater dilution. Sunlight hours in both growing seasons were longer than the average; however duration was slightly higher in 2012. Jackson and Lombard (1993) reported that high content of polyphenols is induced during ripening at mean air temperatures between 9° and 29°C or high radiation level. This report is consistent with our results since the higher polyphenol content was measured in 2012 compared to 2011. From these results we can also see that depending on growing season the level of polyphenol classes, flavan-3-ols and flavonols was inversely proportional. GS had higher amount of flavan-3-ols in 2011 and lower amount of flavonols compared to 2012, where we observed higher amount of flavonols and lower amount of flavan-3-ols. We have noticed opposite effect for GR where we found higher amount of flavonols and lower amount of flavan-3-ols compared to 2012, where we observed a higher amount of flavan-3-ols and lower amount of flavonols. Flavonols were the more influenced by the growing season. The extent of sun exposure could be a reason for those findings, because flavonols are located in the skin of the apple and their production is induced by sunlight (Awadet al.,2001): Additionally, the elevated light level provides more energy for carbon assimilation and thus more carbon resources for biosynthesis of polyphenols (Treutter, 2010): The highest amount of phenolic acids was detected in 2012 for both varieties. Polyphenol profiles remained fairly constant over the two years for both varieties, but amount of dihydrochalcones were influenced more strongly by the variety than by the growing year. That is in agreement with Guyotet al.(2003) who concluded an overall stability of polyphenol composition from one year to another, but also found significant year-to-year variations in the levels of individual polyphenol compounds, comparable to our own results. The significant difference between apples, in two growing seasons, was in the fruit size. Mean fruit

weight varied from 206.83 ± 11.2 g to 168.51 ± 17.5 g per fruit for GS, and 192.74 ± 12.6 g to 107.95 ± 9.3 g per fruit for GR in 2011 and 2012, respectively. The level of soluble solids, L-ascorbic acid and acids was higher in 2012 for GS, but in contrast to these levels they were lower for GR in 2012 (Table 1):Considering sugars both varieties had higher amount of reducing and total sugars in 2012.

Effect of storage

Freeze-dried apple peel was stored (180 days) in hermetically closed glass dish, and kept at room temperature. The TPC of GS was increased during the storage, while TPC of GR was decreased. Same trend was observed with AOA.

Table 3. Total phenol content and antioxidative activity of apple peel powder of GS and GR apple variety.

Apple variety		Granny Smith		Gold Rush	
Growing season		2011	2012	2011	2012
TPC (g EGA/kg)	0 day	1.79 ± 0.040	2.30 ± 0.106	3.19 ± 0.080	2.76 ± 0.087
	30 day	2.04 ± 0.030	2.49 ± 0.060	2.91 ± 0.050	2.40 ± 0.056
	180 day	2.06 ± 0.020	2.67 ± 0.121	2.77 ± 0.060	2.47 ± 0.261
ABTS (mmol TE/100ml)	0 day	10.74 ± 0.640	26.73 ± 0.766	46.00 ± 0.170	36.29 ± 0.216
	30 day	31.79 ± 0.360	29.99 ± 0.998	31.50 ± 0.050	34.41 ± 0.727
	180 day	30.79 ± 0.450	28.80 ± 0.116	29.89 ± 0.160	25.18 ± 0.369
DPPH (mmol TE/100ml)	0 day	2.51 ± 0.150	1.49 ± 0.029	2.37 ± 0.040	1.85 ± 0.058
	30 day	2.78 ± 0.080	2.88 ± 0.086	1.30 ± 0.005	2.71 ± 0.066
	180 day	1.79 ± 0.050	1.27 ± 0.133	0.52 ± 0.040	0.64 ± 0.044

An increase of TPC during storage was also reported by Mareczeket al. (2000); Awad and de Jager (2000); Lattanzioet al.(2001): After storage the levels of phloridzin, (-) epicatechin and phloretin xyloglucoside of GS peel remained approximately at the same levels. However, there was significant increase of procyanidin

B2 and quercetin glycosides. After the storage, flavan-3-ols, dihydrochalcones and flavonols, in GS peel, were present in the range of 19.7 – 20.6 mg/100 g; 1.1 – 1.3 mg/100 g; 11.5 – 15.7 mg/100 g in 2011 and 2012, respectively. This increase also increased AOA of GS peel (Table 3): Procyanidin B2, quercetin and its glycosides are

Table 4. Polyphenol profile of apple peel powder of GS and GR apple varieties (mg/100g fw) after storage.

Polyphenols		Granny Smith		Gold Rush	
		2011	2012	2011	2012
Procyanidin B2		4.72 ± 0.098[c]	4.59 ± 0.048[c]	5.93 ± 0.204[b]	8.22 ± 0.275[a]
Phloridzin		0.46 ± 0.011[d]	0.78 ± 0.044[c]	1.23 ± 0.147[b]	1.36 ± 0.115[a]
(-)Epicatchin		8.88 ± 0.030[b]	9.06 ± 0.054[b]	9.58 ± 0.108[b]	14.35 ± 0.149[a]
Phloretin xyloglucoside		0.62 ± 0.051[c]	1.09 ± 0.022[a]	0.89 ± 0.036[b]	0.91 ± 0.086[b]
Chlorogenic acid		<lod*	<lod	<lod	4.11 ± 0.020[a]
Caffeic acid		<lod	<lod	<lod	<lod
Quercetin* glycoside	23.517	<lod	<lod	<lod	<lod
	24.280	1.82 ± 0.002[bc]	3.03 ± 0.018[a]	1.72 ± 0.010[c]	1.95 ± 0.004[b]
	24.605	1.17 ± 0.063[a]	1.16 ± 0.008[a]	<lod	<lod
	24.848	2.77 ± 0.048[b]	4.48 ± 0.028[a]	1.84 ± 0.016[d]	2.27 ± 0.006[c]
	25.605	2.30 ± 0.004[c]	2.66 ± 0.018[b]	2.36 ± 0.022[c]	3.37 ± 0.013[a]
Rutin		3.61 ± 0.046[b]	4.39 ± 0.1.88[a]	3.36 ± 0.008[c]	4.43 ± 0.143[a]

*Quercetin glycoside retention times.
**<lod: below limit of detection (<0.15 mg/100 g).Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at p ≤ 0.05, (ANOVA, Fisher's LSD).

considered to be a strong antioxidant due to their ability to scavenge free radicals. The increase of AOA can also be explained by the presence of polyphenols with an intermediate oxidation state which can exhibit higher radical scavenging activity than the non-oxidized ones. Storage also influenced the level of dihydrochalcones and phenolic acids of GR, in both experimental years, while the level of flavonols was lower in 2012. However, levels of flavan-3-ols were higher after storage. Lattanzioet al.(2001), also, reported that after 60 days of storage the concentration of total phenolics in the skin of Golden Delicious apples increased. After the storage, flavan-3-ols, dihydrochalcones and flavonols, in GR peel, were present in the range of 18.5 – 22.6 mg/100 g; 2.1 – 2.3 mg/100 g; 9.3 – 12.0 mg/100 g in 2011 and 2012, respectively. Phenolic acids were the most susceptible to changes during storage. After the storage there was a complete loss of phenolic acids, in GS peel, only in the case of GR harvested in (2012) the loss was approximately 70%.

Conclusion

Differences in phenolic profile between compared apple varieties were only in levels of phenolic compounds. GR had higher amount of flavan-3-ols, dihydrochalcones, and flavonols compared to GS. Of all the polyphenol groups, flavonols were more influenced by the growing year. The extent of sun exposure, during growing period, could be a reason, because flavonols are located in the skin of the apple and their production is induced by sunlight. For phenolic acids the differences between the growing seasons were significant and the highest amounts were detected for both varieties harvested in 2012. Dihydrochalcones were influenced more by the variety than by the growing season. During storage changes in TPC and AOA depended on the apple variety. The greatest difference was observed in levels of procyanidin B2 and flavonols. Apple peel powder, stored for 1-6 months, preserved the most of antioxidants and functional properties, suggesting that apple peel powder may be used in a various food products to add phytochemicals and promote good health.

References

Arnao, M.B., Cano, A., and Acosta, M. (2001): The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73, 239-244.

Awad, M.A. and de Jager, A. (2000): Flavonoid and chlorogenic acid concentrations in skin of 'Jonagold' and 'Elstar' apples during and after regular and ultra low oxygen storage. Postharvest Biology and Technology 20, 15-24.

Awada, M.A., Wagenmakers, P.S., and de Jager, A. (2001): Effects of light on flavonoid and chlorogenic acid levels in the skin of 'Jonagold' apples. Scientia Horticulturae 88, 289-298.

Biedrzycka, E. and Amarowicz, R. (2008): Diet and health: Apple polyphenols as antioxidants. Food Reviews International 24, 235-251.

Boyer, J., Liu, R.H. (2004): Apple phytochemicals and their health benefits. Journal of Nutrition 3:5, 1-15.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995): Use of A Free-Radical Method to Evaluate Antioxidant Activity. Food Science and Technology-Lebensmittel-Wissenschaft and Technologie 28, 25-30.

Ceymann, M., Arrigoni, E., Scharer, H., Nising, A.B., and Hurrell, R. F. (2012): Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile. Journal of Food Composition and Analysis 26, 128-135.

Denis, M. C., Furtos, A., Dudonne, S., Montoudis, A., Garofalo, C., Desjardins, Y. (2013): Apple Peel Polyphenols and Their Beneficial Actions on Oxidative Stress and Inflammation. Plos One, 8.

Egan, H., Kirk, R., Sawyer, R. (1981): The Luff Schoorl method. Sugars and preserves. In: Pearson`s chemical analysis of foods. 8th edition, Longman Scientific and Technical: Harlow UK, 152-153.

Golding, J.B., McGlasson, W.B., Wyllie, S.G., and Leach, D.N. (2001): Fate of apple peel phenolics during cool storage. Journal of Agricultural and Food Chemistry 49, 2283-2289.

Guyot, S., Marnet, N., Sanoner, P., and Drilleau, J. F. (2003): Variability of the polyphenolic composition

of cider apple (Malus domestica) fruits and juices. (vol 51, pg 6240, 2003). Journal of Agricultural and Food Chemistry 51, 7522.

Habauzit, V., Morand, C. (2012): Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: an update for clinicians. Therapeutic Advances in Chronic Disease 3:(2), 87-106.

Hyson, D.A. (2011): A Comprehensive Review of Apples and Apple Components and Their Relationship to Human Health. Advances in Nutrition 2, 408-420.

Jackson, D.I., and Lombard, P.B. (1993): Environmental and Management Practices Affecting Grape Composition and Wine Quality - A Review. American Journal of Enology and Viticulture 44:4, 409-430.

Jakobek, L., Seruga, M., Novak, I., and Medvidovic-Kosanovic, M. (2007): Flavonols, phenolic acids and antioxidant activity of some red fruits. Deutsche Lebensmittel-Rundschau 103, 369-378.

Kevers, C., Pincemail, J., Tabart, J., Defraigne, J.O., and Dommes, J. (2011): Influence of Cultivar, Harvest Time, Storage Conditions, and Peeling on the Antioxidant Capacity and Phenolic and Ascorbic Acid Contents of Apples and Pears. Journal of Agricultural and Food Chemistry 59, 6165-6171.

Kondo, S., Tsuda, K., Muto, N., and Ueda, J. (2002): Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. Scientia Horticulturae 96, 177-185.

Lata, B. (2007): Relationship between apple peel and the whole fruit antioxidant content: Year and cultivar variation. Journal of Agricultural and Food Chemistry 55, 663-671.

Lata, B. and Tomala, K. (2007): Apple peel as a contributor to whole fruit quantity of potentially healthful bioactive compounds. Cultivar and year implication. Journal of Agricultural and Food Chemistry 55, 10795-10802.

Lattanzio, V., Di Venere, D., Linsalata, V., Bertolini, P., Ippolito, A., and Salermo, M. (2001): Low temperature metabolism of apple phenolics and quiescence of Phlyctaena vagabunda. Journal of Agricultural and Food Chemistry 49, 5817-5821.

Lock, K., Pomerleau, J., Causer, L., McKee, M. (2004): Low fruit and vegetable consumption in Comparative Quantification of Health Risks Global and Regional Burden of Disease Attributable to Selected Major Risk Factors Volume 1. Eds. Ezzati, M., Lopez, A. D., Rodgers, A., Murray, C. J. L. WHO, Geneva, 597-729.

Mainla, L., Moor, U., Karp, K., and Pussa, T. (2011): The effect of genotype and rootstock on polyphenol composition of selected apple cultivars in Estonia.

Zemdirbyste-Agriculture 98, 63-70.

Mareczek, A., Leja, M., and Ben, J. (2000): Total phenolics, anthocyanins and antioxidant activity in the peel of the stored apples. Journal of Fruit and Ornamental Plant Research 8:59-64.

Matthes, A. and Schmitz-Eiberger, M. (2009): Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions. Journal of Applied Botany and Food Quality-Angewandte Botanik 82, 152-157.

Mcghie, T.K., Hunt, M., and Barnett, L. E. (2005): Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. Journal of Agricultural and Food Chemistry 53, 3065-3070.

Napolitano, A., Cascone, A., Graziani, G., Ferracane, R., Scalfi, L., Di Vaio, C. (2004): Influence of variety and storage on the Polyphenol composition of apple flesh. Journal of Agricultural and Food Chemistry 52, 6526-6531.

Neveu V, Perez-Jiménez J, Vos F, Crespy V, Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford) 2010; 2010: bap024. Published online 2010 January 8. doi: 10.1093/database/bap024.

Ough, C.S., Amerine, M.A. (1998): Phenolic compounds. In Methods for analysis of musts and wines. (C. S. Ough and M. A. Amarine, ed.) Wiley NY, 196-221.

PerezIlzarbe, J., Hernandez, T., Estrella, I., and Vendrell, M. (1997): Cold storage of apples (cv Granny Smith) and changes in phenolic compounds. Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology 204, 52-55.

Piretti, M. V., Gallerani, G., Pratella, G. C. (1994): Polyphenol fate and superficial scald in apple. Postharvest Biology and Technology 4(3), 213–224.

Sanoner, P., Guyot, S., Marnet, N., Molle, D., and Drilleau, J. F. (1999): Polyphenol profiles of French cider apple varieties (Malus domestica sp.): Journal of Agricultural and Food Chemistry 47, 4847-4853.

Sethi, S. (2007): Principles of Food Processing In Horticulture, Post Harvest Technology, New Delhi, 110-012, 1-33.

Singleton, V.L., Orthofer, R. (1999): "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent." Methods in Enzymology 299, 152-178.

Stracke, B.A., Rufer, C.E., Weibel, F.P., Bub, A., and Watzl, B. (2009): Three-Year Comparison of the Polyphenol Contents and Antioxidant Capacities

in Organically and Conventionally Produced Apples (Malus domestica Bork. Cultivar 'Golden Delicious'): Journal of Agricultural and Food Chemistry 57, 4598-4605.

Tee, E.S., Siti-Mizura S., Kuladevan, R., Young, S.I., Khor, S.C., Chin, S.K. (1987): Laboratory Procedures in Nutrient Analysis of Foods; Tee, E.S., Siti-Mizura, S., Kuladevan, R., Young, S.I., Khor, S.C., Chin S.K. Ed. Division of Human Nutrition, Institute for Medical Research: Kuala Lumpur 47-54.

Treutter, D. (2005): Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biology 7, 581-591.

Tsao, R., Yang, R., Xie, S., Sockovie, E., and Khanizadeh, S. (2005): Which polyphenolic compounds contribute to the total antioxidant activities of apple? Journal of Agricultural and Food Chemistry 53, 4989-4995.

van der Sluis, A.A., Dekker, M., de Jager, A., and Jongen, W.M.F. (2001): Activity and concentration of polyphenolic antioxidants in apple: Effect of cultivar, harvest year, and storage conditions. Journal of Agricultural and Food Chemistry 49, 3606-3613.

Vrhovsek, U., Rigo, A., Tonon, D., and Mattivi, F. (2004): Quantitation of polyphenols in different apple varieties. Journal of Agricultural and Food Chemistry 52, 6532-6538.

Wojdylo, A., Oszmianski, J., and Laskowski, P. (2008): Polyphenolic compounds and antioxidant activity of new and old apple varieties. Journal of Agricultural and Food Chemistry 56, 6520-6530.

Wolfe, K., Wu, X. Z., and Liu, R.H. (2003): Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry 51, 609-614.

Xiuzhen, H., Tao, S., Hongxiang, L. (2007) Dietary Polyphenols and Their Biological Significance. Intrnational Journal of Molecular Science 8:950-988.

INFLUENCE OF EDUCATION ON BODY WEIGHT REDUCTION

Almir Azabagić[1], Daniela Čačić Kenjerić[2], Midhat Jašić[3], Drago Šubarić[2], Ines Banjari[2]

[1]Salus Tuzla, Family medical practice, Dragodol 25, 75000 Tuzla, Bosnia and Herzegovina
[2]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
[3]University of Tuzla, Faculty of Technology, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina

Summary

Elevated body mass is an important risk factor of many diseases like hypertension, diabetes, hyperlipidaemia, coronary heart disease etc. To reduce body weight various medicament, surgical, behavioural, dietetic and other approaches and methods are used. Education of patients is one of the key points in success, and besides solely implementation it is often used in combination with other methods. The aim of this study was to determine the influence of education in overweight and obese healthy and sick patients on the weight reduction. Study was conducted on the sample of 77 participants of which 39 were overweight and obese but otherwise healthy, 9 were patients with hypertension, 8 were diabetics, 11 patients with gastrointestinal problems and 10 patients with food allergies. During the study observation period all participants were educated on the importance of balanced nutrition via the flyer, weekly menu and individual interview. The observation period was 3 months per person. Basic anthropometric measurements were conducted and BMI calculated for each participant at the beginning and at the end of the observation period. Average reduction in BMI was 3.26 units in overweight and obese but otherwise healthy participants, 3.40 units in patients with hypertension, 5.08 units in diabetics, 0.12 units in patients with gastrointestinal problems and 0.10 in patients with food allergies. Greatest average reduction in BMI was noticed in diabetics. Education on balanced diet and healthy lifestyle via the flyer, weekly menu and individual interview resulted in significant body mass reduction in healthy as well as in patients with diagnosis. Education of overweight and obese persons could be an important tool in weight management programs.

Keywords: obesity, body weight, hypertension, diabetes, allergies, gastrointestinal diseases, education, body weight reduction

Introduction

Balanced diet, physical activity and optimal body eight are important prerequisites in wellbeing and health promotion of each person.

In assessment of the status of the nourishment simple anthropometric measurements like body weight and height from which body mass index (BMI) is calculated, skin fold and muscles circumference, waist circumference, underwater weighing, bioelectrical impedance and other similar techniques are used (Jašić, 2008). The most often used criterion is BMI. According to the BMI, persons with values between 25 and 30 are considered as overweight, those with values between 30 and 35 as obese, while values above 35 imply morbid obesity (Metelko, 2012).

According to the data from the World Health Organization (WHO) 1.5 billion people is overweight and more than 500 million of them are obese. Furthermore, projections indicate continuous raise in those numbers in the future period (Medanić and Pucarin-Cvetković, 2012; Ng et al., 2014).

36.9 % of males and 38.0 % of women globally is overweight or obese. Prevalence of overweight and obesity is higher in developed than in undeveloped and transitional countries, but the continuous raise trend is visible in both of them. According to estimations cumulative number of

obese and overweight adults in Bosnia and Herzegovina is 57.3 % in males and 51.9 % females, while obese rates are 15.4 % in males and 20.4 % in females (Ng et al., 2014). Health expenses due to the obesity caused problems in Europe contribute with 7 % to total health costs which is the same as the costs of the all carcinoma types together (Wilborn et al., 2005).

WHO defines obesity as a disease in which excess fat is accumulated to an extent that health may be adversely affected (Poirier et al., 2011). Obesity is a public health problem correlated with risk factors for the development of diseases like elevated blood pressure, diabetes, heart diseases, dyslipidaemia, some carcinoma types, infertility, spine diseases, skin infections, ulcers, gall stone and others (Steinberger and Daniels, 2002; Wilborn et al., 2005; Gomes et al., 2010; SIGN, 2010; Wiltink et al. 2013). By reducing body weight symptoms of those diseased are reduced as well as the risk of their development (SIGN, 2010).

Patient education is one of the possible approaches and one of the most efficient methods in body weight reduction.

Healthy weight reduction demands professional diagnostics of underlying overweight causes, nourishment status assessment as well as the evaluation of other risk factors like socioeconomic status, employment, marital status etc. Individualisation of diet based on patient's dietary needs, preferences and habits is very important for healthy weight reduction. All nutrients have to be represented in adequate amounts and gained from foods of low caloric value but with high shares of vitamins, minerals, dietary fibres and phytochemicals, e.g. high nutritional density foods (Mahan et al., 2008). Additionally, weight reduction diets which alter macronutrient contribution to the total energy intake should be avoided due to their potentially negative effects on health. For example, long term high protein diet can cause ketoacidosis, kidney and liver diseases, and bone mass reduction (Štimac and Turk, 2008).

Nutritional planning encompasses compiling the list of foods and meals for one or more days in line with dietary needs of a person (Šatalić and Alebić, 2008).

Physical activity varies in types and intensity among persons. To describe physical activity of a person metabolic equivalents (MET) are used. MET represents the ratio of work metabolic rate to a standard resting metabolic rate of 1 kcal/kg/h. 1 MET is considered a resting metabolic rate obtained during quiet sitting. In comparison to quiet sitting, moderate physical activity takes 3-6 MET and vigorous physical activity more than 6 MET (Ainsworth et al., 2005). Precise energy needs of each physical activity type depend on the body weight.

Positive impacts of the physical activity are multiplied with additional time and variability in physical activity types. The highest positive impact is achieved in persons who change their activity level from completely inactive to moderately active. Recommended total training time is 150 minutes of moderate or 75 minutes of vigorous physical activity on a weekly basis, and single short blocks of a 10 minutes are encouraged (US DHHS, 2008).

The aim of this study was to estimate the influence of education on the balanced nutrition and physical activity on the body weight reduction. Subjects were males and females of 20 up to 65 ages, from rural and urban area, and study was conducted in „Salus" private family practice in Tuzla.

Subjects and Methods

Study encompassed data collection, systematisation and classification and at the end analysis of data gathered by anthropometric measurements and data on health status of the patients on the first and final session.

On the first session initiated by the patient, personal information and data on dietary habits and lifestyle were gathered, and anthropometric measurements and medical diagnostics were conducted to determine nourishment and health status of the patient. Education of the patient was delivered through face to face individual interview during which irregularities which patent makes in its diet are indicated and stressed to a patient. At the same occasion principles of the balanced diet aimed at the body weight reduction were highlighted to each patient. As a result

of the session, each patient received an individualised weekly diet plan which should be conducted for the period of the three month. Follow up of the patients was continued after the three month period. On that occasion anthropometric measurements as well as all medical diagnostic conducted at the beginning of the cycle were repeated.

Study design was based on a retroactive insight into the medical records of the "Salus" Tuzla patients.

Participants

This study encompassed 77 subjects. Study subjects were males (16 subjects) and females (61 subjects), 20 up to 65 years old, partly from rural and partly from urban area. Some of the subjects were, aside from being overweight or obese, healthy, while others had additional health problems (Table 1).

Study encompassed only those subjects (patients) who returned for the control check-up session after the 3 month period of assigned diet and confirmed its adherence. Due to that fact

an assumption was made that nourishment status (overweight, obesity) was purported, among others, by the patient's lack of knowledge on the observed subject, and that the result after the 3 month treatment period was due to the conducted education and counselling.

Study relied exclusively on the anthropometric data from the medical records for which patients as well as the physician's approval were gained.

Anthropometric measurements

Body weight measurement was conducted with an Omron Body Composition monitor BF511 with the precision of ± 0.1 kg. Measurements were conducted in the morning, before meal, with clothes but without shoes.

Body height measurement was conducted with a clinical altimeter KaWe with the precision of ± 0.1 cm. During the measurement patients were without shoes, and the measurement itself was conducted with head in Frankfurt position.

Body mass index (BMI) was calculated from the gained data on weight and height.

Table 1. Characteristics of the study participants

Participants	Number of participants	Share (%)
All participants	77	100
GENDER		
Males	16	20.77
Females	61	79.22
LIVING AREA		
Rural	33	42.85
Urban	44	57.14
HEALTH STATUS		
Healthy patients with high body weight	39	50.65
Patients with allergies	10	12.99
Patients with gastrointestinal diseases	11	14.28
Patients with hypertension	9	11.69
Patients with diabetes	8	10.39

Menu for body weight reduction

With the aim of patient's education weekly menu was created in line with up to date dietary guide-

lines (food pyramid) and guidelines for management of obesity in adults (Tsigos et al., 2008). Besides guidelines for healthy nutrition created weekly menu respected patients individual pref-

erences for various types of food. In menu design specially developed software and U.S. Food and Drug Administration (U.S. FDA) food composition tables were used to estimate caloric and nutritive value (FNB, 2005).

Importance of stress avoidance, sleep quantity and quality as well as the physical activity were also stressed to each patient. Furthermore, each patient received recommendations for the appropriate physical activity (Tsigos et al., 2008; Haggis et al., 2013).

Patients were advised to follow prescribed menu for the following three month period.

Data analysis

Gathered data were analysed with respect to patient health status and gender.

Data analysis was conducted with MS Office Excel software (version 2003, Microsoft Corp., USA).

Results and Discussion

Nourishment status, prior and after the three month diet, of those study subjects which were treated only due to the elevated body weight

Average body mass index of study participants treated only due to the elevated body mass prior to education was 32.05 kg/m2. After the treatment average body mass index reduction (ΔBMI) was 3.26 BMI units and average body mass index value of the whole group was 28.79 kg/m2 (Table 2). Average intensity of BMI reduction (ΔBMI/Δt) was 0.22 units higher in females than in males, what can be explained by higher motivation in females than in males. Influence of motivation is visible from the profile of study participants e.g. the fact that group consisted of only 16 males and 66 females.

Results of the study conducted in British adults confirmed that although most of the obese people

are aware of their excessive body weight, only a small part of them is concerned about it and are trying to lose weight and only a minority had participated in a programme of weight control. Men´s awareness was lower than women´s (Wardle and Johnson, 2002).

Results presented on the Fig. 1 show that women who were besides elevated body mass of good health tend to react on it and try to manage body weight earlier than men. At the same time, parallel position of the trend lines for both genders indicates that the efficiency of body weight reduction is the same in men and women.

Higher self-criticism towards the self perceived body image in women than in men, as well as the practice of earlier decision to start on a reduction diet, has been confirmed earlier. According to the results of the study conducted in Dutch men and women, 53 % of men and 39 % of women were overweight or obese but 56 % of men and 52 % of women described their weight status as appropriate (Blokstra et al., 1999). 73.5 % of overweight British women correctly perceives them self as overweight, and additional 15.7 % of them perceives them self as very overweight. In total 94.5 % of obese British women are aware of their weight problem but even 50.5 % of them perceives themselves as only overweight instead of very overweight. More than 60 % of obese and overweight women are trying to lose weight, but about 50 % of them for this purpose chooses own diet, and only 5.9 % of overweight and 17.1 % of obese asks advice from the professional. At the same time, just 67.6 % of overweight British men perceives themselves as overweight or obese, although correct self perception in obese subjects is almost as high as in women (92.7 %). 32.2 % of overweight and 56.0 % of obese men is trying to lose weight, and as same as women, they most often choose to achieve that by their own diet while advice from the professional seeks only 3.9 % of overweight and 14.3 % of obese persons (Wardle and Johnson, 2002).

Table 2. Body mass indexes before (BMI 1) and after (BMI 2) the education and three month diet period for individual participants and average for the whole group of patients treated only for body mass reduction

| Participant	BMI1	BMI2	% change	Participant	BMI1	BMI2	% change
1.	34.00	30.70	9.71	21.	37.00	35.00	5.41
2.	36.21	32.30	10.80	22.	16.00	16.00	0
3.	39.40	32.10	18.53	23.	18.50	19.00	2.70
4.	36.00	30.60	15.00	24.	24.00	24.00	0
5.	47.90	36.00	24.84	25.	27.00	25.00	7.41
6.	34.00	32.80	3.53	26.	27.00	24.00	11.11
7.	34.00	32.70	3.82	27.	28.00	27.60	1.43
8.	37.00	33.00	10.81	28.	29.00	27.00	6.90
9.	34.00	28.00	17.65	29.	29.00	25.60	11.72
10.	35.00	29.00	17.14	30.	30.00	25.00	16.67
11.	35.00	32.00	8.57	31.	30.00	26.10	13.00
12.	35.00	33.50	4.29	32.	30.00	28.00	6.67
13.	37.00	32.00	13.51	33.	31.00	27.00	12.9
14.	27.00	26.00	3.70	34.	33.00	30.50	7.58
15.	28.70	22.50	21.60	35.	33.00	28.30	14.24
16.	30.00	29.00	3.33	36.	34.00	31.20	8.24
17.	30.00	29.00	3.33	37.	34.20	30.30	11.40
18.	31.00	29.00	6.45	38.	36.00	33.00	8.33
19.	31.00	27.00	12.90	39.	39.00	35.00	10.26
20.	32.00	28.00	12.50	Average	32.05	28.79	9.55
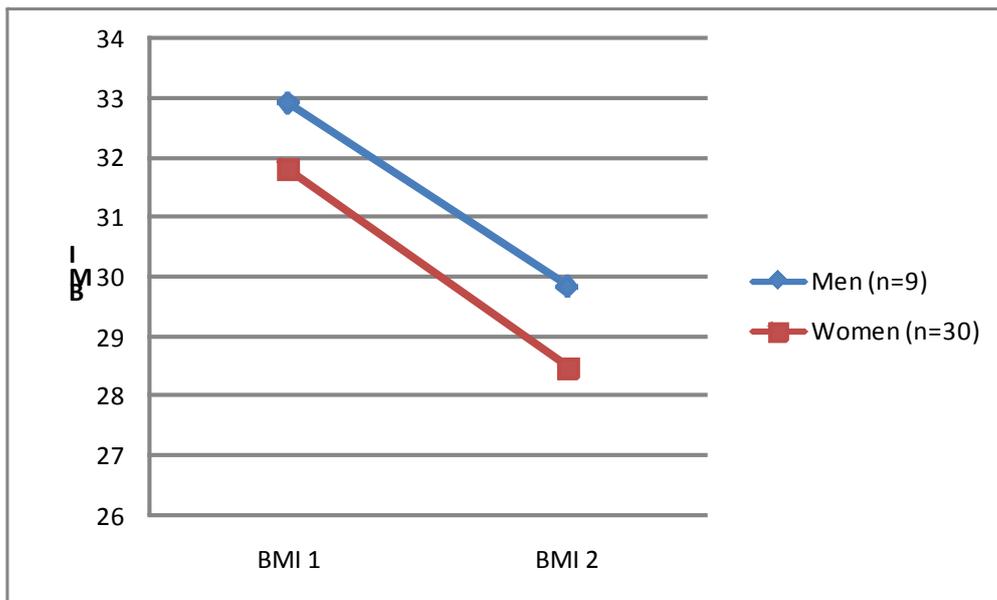
Fig. 1. Average body mass index before (BMI 1) and after (BMI 2) the education and three month diet period for males and females in the group of patients treated only for body mass reduction

In spite of the general opinion that women perceive excessive body weight earlier than men it should be considered that the ability of self-perception varies among ethnical groups. Due to that fact, public nutritional programs should be ethnic-specific and adjusted for the targeted population by addressing food and health in the context of their culture and taking in account the socioeconomic status of the group (Metcalf et al., 2000).

Nourishment status, prior and after the three month diet, of those study subjects which were treated primarily for their diagnosed health problems

Besides healthy overweight and obese participants, this study encompassed 8 participants with diabetes, 9 participants with hypertension, 11 participants with gastrointestinal problems and 10 participants with food allergies (Table 1).
Average BMI value of the participants who were

motivated to visit the physician primarily due to their diagnosed health problems was at the beginning of the study 32.06 kg/m2. By the end of the three month treatment and observation period average BMI of the group decreased 4.60 %, and average BMI value was 30.19 kg/m2.

Average BMI of this group matches with the average values of overweight and obese but otherwise healthy participants whose results are mentioned above. In despite to the fact that this group had an additional motive to reduce weight (health status improvement) they had not managed to achieve the same average body weight reduction.

Unlike in the group of overweight and obese but otherwise healthy participants, in the group of participants with diagnosed health problems BMI value at the beginning of the study was same in men and women. Higher interest in women is visible via more intense body weight reduction in women than in men (Fig. 2).
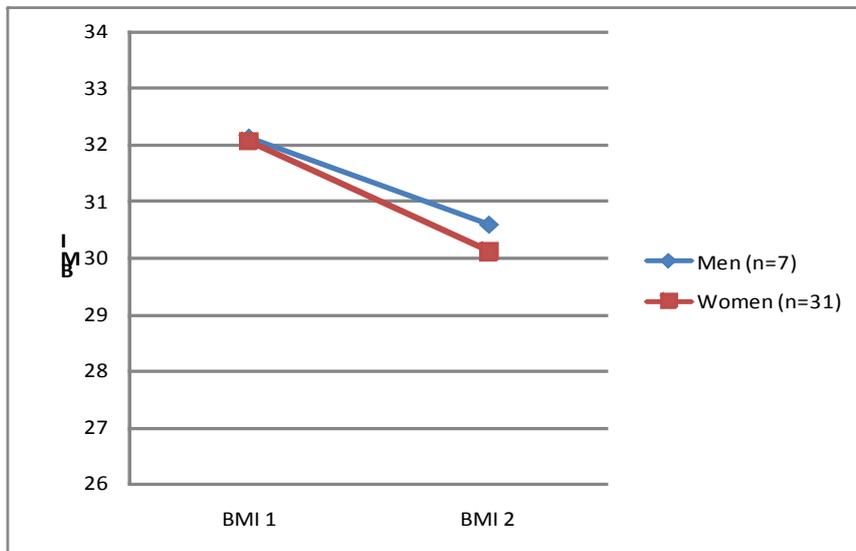

Fig. 2 Average body mass indexes before (BMI 1) and after (BMI 2) the education and three month diet period for males and females in the group of patients treated only for diagnosed medical problems

From 11 participants in the subgroup of patients who have contacted the physician due to diagnosed gastrointestinal problems 7 have managed to reduce body weight (63.64 % of the subgroup), while 4 remaining participants gained weight during the observed period. Considering the treated health issue, this subgroup encompassed

one participant of the normal body weight status. In this patient dietary guidance resulted with gaining weight but in such intensity to remain in the same body weight status group – normal.

Considering the specificity of their health problem, from 10 participants in the subgroup of patients with allergies 6 were overweight or

obese while 4 were of normal weight status. In this subgroup, education on balanced nutrition, as well as the epistemology on the foods which causes their allergy combined with the individualised menu resulted in body weight reduction in 6 participants (60 % of the subgroup), while remaining 4 patients gained weight. 2 out of 4 who gained weight remained throughout the study in the normal weight category.

All 8 patients treated primarily for diabetes on the beginning of the observation period were obese according to the BMI ($\geq$ 30 kg/m2) (SIGN, 2010). Considering the fact that elevated body weight is the risk factor in diabetics, as expected, body weight reduction was observed in all 8 patients (100 % of the subgroup). High intensity of BMI reduction was expected in this subgroup. ΔBMI values imply that the reduction is significant in all but one patient. Namely, diabetics undergo various educations in the public health system and therefore are aware that body weight reduction and after that body weight control is one of the most important elements of diabetes treatment. Results of the studies conducted in diabetics have confirmed that education on balanced nutrition plays an important role in disease control but the motivation for healthy behaviour must be patient driven. Furthermore, it is important that patients awareness, motivation and decision making comes first, and information and skills being considered as essential components to be "available on demand" rather than to be "imposed" (Maldonato et al., 2010). Education

participants of this study in family practice Salus encompassed patients education on balanced nutrition but at the same time provided practical and individualised guide (weekly menu) which patients could follow during the study period. This probably contributed to such high success in body weight reduction of the study participants. As same as the diabetics, all participants from the subgroup of the patients with diagnosed hypertension were obese (BMI $\geq$ 30 kg/m2) (SIGN, 2010). Also, as same as the diabetics, all patients in this subgroup (100 % of the subgroup) have managed to reduce weight during the observation period and ΔBMI was the highest from all observed subgroups and varied from -1.20 up to -6.00.

Effectiveness of education on the body weight reduction among healthy in comparison to those among persons with diagnosed health problems
Average BMI reduction was smaller in the group of patients with diagnosed health problem than in the group of overweight or obese but otherwise healthy patients. To avoid misleading judgements, great differences among subgroups based on the health issues should be considered. Namely, in spite of the fact that relatively high share of patients in the subgroups with gastrointestinal problems and allergies reduced weight the intensity of reduction was not so strong, and additionally in some cases weight gain was perceived (Figures 3 and 4).
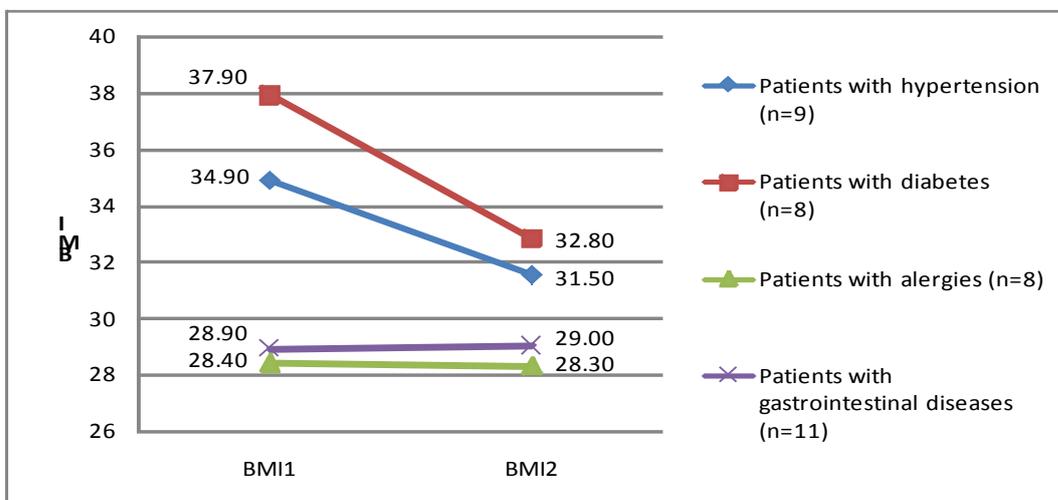

Fig. 3 Body mass indexes before (BMI 1) and after the education and three month diet period (BMI 2) for
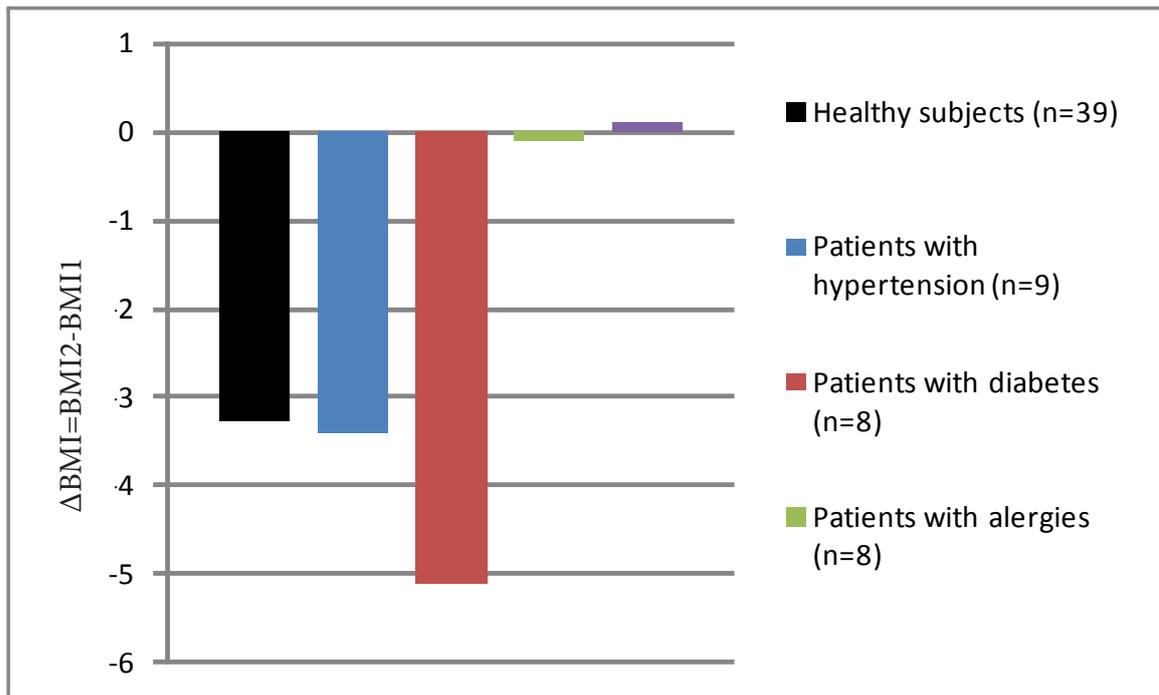
Fig. 4 Average intensity of change in body mass index (ΔBMI) for subgroups based on health status of participants

In the subgroups of patients with diabetes and hypertension BMI reduction intensity was stronger (Figures 3 and 4) which can be explained by the awareness of the patients on the role of excessive body weight in the disease development. Also, fear of consequences of their primary disease elevates the motivation for weight reduction.

Positive impact of the therapeutic patient education in chronic diseases (diabetes, hypertension) and obesity was previously reported and varies from 50 – 80 % (Lagger et al., 2010).

In data analysis and concluding process it should be considered that the observation period of three months is rather short and that for realistic conclusions relating success rate patients follow up should be conducted during the future period. Namely, even results of the study conducted in diabetic population which is highly motivated for positive change in dietary habits to achieve necessary blood glucose control show that benefit declines 1 – 3 months after the intervention ceases (Norris et al., 2002). In populations in which dietary influence is not so direct and measureable and as a result patient does not respect it as

important, decline in learned behaviours would be even faster without continuous professional guidance and motivation.

Considering the nourishment status throughout the study (at the beginning and at the end of the observation period) it is noted that in spite of the body weight reduction patients with gastrointestinal problems and diabetics remained in the same nourishment status category based on the BMI as a criteria (SIGN, 2010) while in other studied subgroups such positive change occurred. The most intensive change with respect to BMI nourishment status categorisation was noted in overweight and obese but otherwise healthy patients.

Although the official criteria of the nourishment status are good orientation point for the professionals dealing with patients in weight management programs, and could also be a motivation source for the patient itself in cases in which starting values are close to the lower boundary value, in our study in which starting values were closer to the upper boundary value they were less important than the weight reduction itself.

An analysis of the evidence from 300+ studies

shows that nutrition education is more likely to be effective when it focuses on behaviour/action (rather than knowledge only) and systematically links theory, research and practice (Contento, 2008).

Conclusions

Results of this study have confirmed the starting hypothesis. The results have confirmed that education leads to weight reduction in more than 50 % of all subjects, and with the success rate of 100 % in some of the specific subgroups in which motivation is high (patients with diabetes and hypertension).

To achieve long term positive effect, sessions should be continued with the patients for the future period of at least one year during which implemented diet could become dietary habit of a person for the rest of the life. Patient's motivation should additionally be supported through the creation of at least one diet plan for each season.

References

Ainsworth, B.E., Haskell, W.L., Whitt, M.C., Irwin, M.L., Swartz, A.M., Strath, S.J., O'Brien, W.L., Bassett, D.R., Schmitz, K.H., Emplaincourt, P.O., Jacobs, D.R., Leon, A.S. (2000): Compendium of physical activities: an update of activity codes and MET intensities, Medicine & Science in Sports & Exercise 32(9 Suppl):S498-S516.

Blokstra, A., Burns, C.M., Seidel, J.C. (1999): Perception of weight status and dieting behaviour in Dutch men and women. International Journal of Obesity and Related Metabolical Disorders 23(1):7-17.

Contento, I.R. (2008): Nutrition education: linking research, theory, and practice. Asia Pacific Journal of Clinical Nutrition 17(1):176-179.

FNB, Food and Nutrition Board: Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (Macronutrients). The National Academies Press, Washington DC, 2005. http://www.nap.edu/catalog.php?record_id=10490 [20.09.2013.]

Gomes, F., Telo, D.F., Souza, H.P., Nicolau, J.C., Halpern, A., Serrano, C.V. Jr. (2010): Obesity and coronary artery disease: role of vascular inflammation. Arquivos Brasilieros de Cardiologia 94(2):255-261.

Haggis, C., Sims Gould, J., Winters, M., Gutteridge, K., McKay, H.A. (2013): Sustained impact of community-based physical activity interventions: key elements for success. BMC Public Health 13:892-890.

Jašić M: Ocjena (procjena) prehrambenog statusa. 2008. http://www.hranomdozdravlja.com/?do=osnove [09.07.2012.]

Lagger, G., Pataky, Z., Golay, A. (2010): Efficacy of therapeutic patient education in chronic disease and obesity. Patient Education and Counseling 79:283-286.

Mahan, L.K., Arlin, M.T., Krause, M.V.: Krause's Food and Nutrition Therapy, W.B. Saunders Elsevier, Philadelphia, 2008.

Maldonato, A., Piana, N., Bloise, D., Baldelli, A. (2010): Optimizing patient education for people with obesity: Possible use of the autobiographical approach. Patient Education and Counseling 79:287-290.

Medanić, D., Pucarin-Cvetković, J. (2012): Pretilost – javnozdravstveni problem i izazov. Acta Medica Croatica 66:347-355.

Metcalf, P.A., Scragg, R.K.R., Willoughby, P., Finau, S., Tipene-Leach, D. (2000): Ethnic differences in perceptions of body size in middle-aged European, Maori and Pacific People living in New Zealand. International Journal of Obesity 24:593-599.

Metelko, Ž. (2012): Exercise and metabolic syndrome. Arhiv za higijenu rada i toksikologiju 63(Supplement 3): 23-32.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Ferede Abera, S., Abraham, J.P., Abu-Rmeileh, N.M.E., Achoki, T., AlBuhairan, F.S., Alemu, Z.A., Alfonso, R., Ali, M.K., Ali, R., Alvis Guzman, N., Ammar, W., Anwari, P., Banerjee, A., Barquera, S. (2014): Global, regional, and national prevalence of owerweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384(9945):766-781.

Norris, S.L., Lau, J., Smith, S.J., Schmid, C.H., Engelgau, M.M. (2002): Self-management education for adults with type 2 diabetes. A meta-analysis of the effect on glycemic control. Diabetes Care 25:1159-1171.

Poirier, P., Cornier, M.A., Mazzone, T., Stiles, S., Cummings, S., Klein, S., McCullough, P.A., Ren, F.C., Franklin, B.A. (2011): Bariatric surgery and cardiovascular risk factors: A scientific statement from The American Heart Association. Circulation 123:1683-1701.

Scottish Intercollegiate Guidelines Network (SIGN): Management of obesity - A national clinical guideline. SIGN, Edinburgh, 2010.

Steinberger, J., Daniels, S.R. (2003): Obesity, insulin

resistance, diabetes, and cardiovascular risk in children: an American Heart Association scientific statement from the atherosclerosis, hypertension, and obesity in The Young Committee (Council on cardiovascular disease in the young) and The Diabetes Committee (Council on nutrition, physical activity, and metabolism). Circulation 107:1448-1453.

Šatalić, Z.,. Alebić, I.J. (2008): Dijetetičke metode i planiranje prehrane. MEDICUS 17(1):27-36.

Štimac, D., Turk, T. (2008): Debljina i redukcijske dijete. MEDICUS 17(1):81- 85.

Tsigos, C., Hainer, V., Basdevant, A., Finer, N., Fried, M., Mathus-Vliegen, E., Micic, D., Maislos, M., Roman, G., Schutz, Y., Toplak, H., Zahorska-Markiewicz, B. (2008): Management of obesity in adults: European clinical practice guidelines. Obesity Facts 1:106–116.

U.S. Department of Health & Human Services (US DHHS): Physical Activity Guidelines for Americans. US DHHS, 2008. http://www.health.gov/paguidelines/pdf/paguide.pdf [09.07.2012.]

Wardle, J., Johnson, F. (2002): Weight and dieting: examining levels of weight concern in British adults. International Journal of Obesity 26:1144-1149.

Wilborn, C., Beckham, J., Campbell, B., Harvey, T., Galbreath, M., La Bounty, P., Nassar, E., Wismann, J., Kreider, R. (2005): Obesity: prevalence, theories, medical consequences, management, and research directions. Journal of the International Society for Sports Nutrition 2(2):4-31.

Wiltink, J., Michal, M., Wild, P.S., Zwiener, I., Blettner, M., Münzel, T., Schulz, A., Kirschner, Y., Beutel, M. (2013): Associations between depression and different measures of obesity (BMI, WC, WHtR, WHR). BMC Psychiatry 13:223-230.

NUTRITIONAL STATUS AND DIETARY HABITS OF MENOPAUSAL WOMEN

Samir Tursunović[1], Midhat Jašić[2], Azijada Beganlić[3] Nadia Hot[4]

[1]JZU Dom zdravlja Srebrenika, Zlatnih ljiljana 75350 Srebrenik, Bosna i Hercegovina
[2]Farmaceutski fakultet Univerziteta u Tuzli, Univerzitetska 8, 75000 Tuzla, Bosna i Hercegovina
[3]Edukativni centar Porodične medicine Tuzla
[4]Farmavita doo Sarajevo, Igmanska 5a, Vogošća 71320

Original scientific paper

Summary

Introduction: The majority of menopausal women is changing nutritional status. The causes of this may include: hormonal changes, bad eating habits, heredity, lifestyle etc. The most common symptoms of menopause are hot flushes, sweats and mood swings. Women entering menopause unprepared to cope with the changes of this period of life and with insufficient knowledge of dietary habits that lead to over-supply or lack of nutrients.

Objective: The aim of this study was to determine the degree of nutritional status in postmenopausal women and to determine the degree of correlation between nutritional status and dietary habits of women with menopausal symptoms and the frequency of certain health disorders.

Methodology: The study was carried out collecting, systematization and statistical analysis of data on the nutritional status and dietary habits of a sample of 300 women aged 45 to 55 years old. For women, the absence of a menstrual cycle longer than three consecutive months, it was considered to be in menopause.The survey was conducted in five ambulance of family medicine. To collect the data is used standardized list of questions about eating habits, based on the Likert scale.

Results and discussion: According to the body mass index (BMI), 12:33% of the patients were within the normal range of nutrition, while 87% of respondents were from overweight and obese.

BMI was significantly higher in patients with an increased intake of: soft drinks, sweets and white bread. Soy as a food is present in the diet in only 18% of respondents. Women who have never had symptoms of menopause and menopausal disorders have significantly lower BMI ($p < 0,05$).

The most common diseases in the period of menopause in a patient were examined: hypertension (57%), diabetes (12%), depressive disorders (25%) and cancer (1%).

Conclusion: Food habits of the respondents indicate insufficient knowledge of nutritional needs and recommendations, which resulting in the consumption of food that is not adapted to this period of life. The subjects are not feed buy the standards and recommendations of the WHO, and among them there is an increased risk of diseases typical for menopause, and as a result of the effects of improper nutrition. Nutrition according to the standards and recommendations of the WHO resulting in improved nutritional status of women in menopause, and as result of that is a reduction in the occurrence of diseases and negative symptoms of menopause.

Recommendation: It's needed a systematic and organized education in menopausal women in order to achieve proper nutrition and normal nutritional status, which may contribute to a better quality of life, reducing related diseases and negative symptoms of menopause.

Keywords: menopause, eating habits, nutritional status

Introduction

In the most of menopausal women is changing nutritional status. The causes may be: hormonal changes, bad eating habits, heredity, lifestyle, frequent use of alcohol and tobacco, etc. Many studies point to the importance of nutrition during certain periods of life. In the life of a woman, three important periods related to the endocrine function of the ovary are: puberty, pregnancy

*Corresponding author: samirself04@yahoo.com

and menopause. Menopause, as a period of a woman's life, is marked by changes in endocrine secretion, which marks the end of her menstrual cycle and fertile years. Menopause represents the end of the reproductive period, the average age of onset of menopause is about 50 years (Mc Nagny, 1999). This means that an average woman will spend one-third of her life in menopause. The most common symptoms of menopause in women are sweating, heart palpitations, mood swings, hot flashes, dizziness, fatigue, irritability, anxiety, loss of self-esteem, depression and many others. That is an individual experience, that rarely passes without symptoms. Also, menopause can have an impact on the overall quality of life of women as one of the main causes of osteoporosis and cardiovascular diseases (Ossewaarde ME, 2005). Besides these diseases, obesity and high BMI contributes to morbidity and mortality, leading to some forms of cancer and chronic diseases, such as osteoarthritis, liver and kidney diseases, sleep apnea, and depression (Pi-Sunyer, 2009). Women entering menopause unprepared to cope with changes of this period of life and with insufficient knowledge of dietary habits which lead to oversupply or lack of nutrients. A healthy diet should be balanced, safe and protective. A balanced diet prevents obesity and diseases that arise due to lack of energy and essential nutrients. Protective activity involves the protection of man-made and other diseases (Pokorn, 2003). An unbalanced diet, low physical activity and emotional stress can intensify the symptoms of menopause. Menopausal symptoms may vary in their strength, so some women have not problems, while others suffering during this period of life. About 75% of women going through menopause with minimal discomfort, but 25% of women have expressed problems that affect the ability to work and reduce the vitality and quality of life. Previous studies have proved that between 8% and 15% of menopausal women suffering from depression, although there is no strong association between hormonal changes at menopause and the occurrence of depression (Matthew, 2010). The results showed that menopausal status has a significant effect in response to antidepressants, because it slows down the recovery of depression, and the main reason is

the increased basal levels of the FSH, which interferes with metabolism of antidepressants (Chi-Un et al., 2009). There are many sources of stress for women in menopause: changes in physical appearance, fear of aging, weight gain and many others (Salčić, 2010). This can lead to depressive conditions and loss of life energy. It is known that with a reduced amount of estrogen skin and vagina become drier, which can cause painful intercourse and loss of the woman's libido (Radakovic, 2004). In menopause, lower level of estrogen reduce their protective role, and increase incidence of cardiovascular diseases (Barret-Connor, 1991). Persons with reduced secretion of progesterone and anovulatory have decreased bone density (Prior, 1990). This study seeks to determine the contribution of quality nutrition and physical activity on nutritional status, expressed as BMI of menopausal women, to determine correlation between the level of nutrition and the symptoms of menopause and to define the relationship between dietary habits and incidence of diseases that are typical for menopause.

Methodology

Cross-sectional study was carried out for collection, systematization and statistical analysis of data on nutritional status and dietary habits of 300 women aged 45 to 55 years of age. The survey was conducted in five ambulances of family medicine. Patients were interviewed and measured anthropometric.

Anthropometric Measurements

For all patients were conducted anthropometric measurements that include measurement of body height (BH), body weight (BW), waist circumference (WC) and calculate nutritional status using body mass index (BMI).
BH – was measured with medical altimeter, expressed in centimeters. Measured person standing on a flat surface, without shoes, with placed heels and the head is in the Frankfurt horizontal, which is the line that connects the upper edge of the tragus and the lowest point of the lower edge of the orbit, in parallel with the bottom edge of the plate (Stojanović et al., 2007). The measure-

ment is performed to the nearest 0.1 cm.

BW - was measured with decimal medical scales, expressed in kilograms. Measured person standing in the center of the scales with placed heels and dressed in underwear (weight clothing must be removed). The measurement is carried out to the nearest 100 grams.

WC – was measured with elastic centimeter, expressed in centimeters. Measured person standing freely, and heels are spaced 25 to 30 inches. The measurement is performed in the middle, between the bottom edge of the last rib and the iliac crest in the horizontal plane. The measurement is performed to the nearest 0.1 cm.

BMI is calculated by dividing the BH (body height) expressed in kilograms, with BW expressed in meters squared:

$$BMI\ (kg/m^2) = BW\ (kg)/\ BH\ (m)^2$$

Malnutrition is considered a BMI of less than 18.5, range of the body mass index 18.5 to 24.99 is defined as the normal body weight. People with a body mass index above 25 is considered overweight, while individuals with a BMI over 30 are considered obese. In case of further classification of obesity, BMI over 40 used the term extreme, or morbid obesity (Ogden et al., 2006). The obtained values for BMI will be interpreted according to the classification of nutritional status WHO: malnutrition <18:50, physiological nutritional status 18.50-24.99, 25.0-29.99 overweight, level I obesity 30.00-34.99, 35.00-39.99 level II obesity, level III obesity > 40.00.

Questionnaire

Information about dietary habits of menopausal women, the representation of certain foodstuffs in the diet, physical activity and socio-economic status, will be obtained by questionable list. Questionnaire was adapted to eating habits and type of consumed food. Food standards, recommendations and guidelines are used as a criterion for testing scientifically substantiated claims which define this issue. Questionnaire has 28 questions, which provide answers about nutrition in menopausal women, their health problems that may occur during the period before and during menopause. It also determines the symptoms of menopause among our patients, using of dietary supplements and hormone therapy, and environmental habits.

The survey was conducted using a standardized questionnaire, drawn up on the dietary recommendations, standards and guides (DRI) given by the Committee on Food and Nutrition of the American Academy of Sciences (Anonymous, 2005b), and the World Health Organization (Anonymous, 2010). In preparing the questionnaire were considered guides of American Association for Nutrition (USDA, 2005; USDA, 2010; Guenther et al. 2008), as well as the recommendations of the EU institutions (EFSA 2010).

Principles of Likert scales were used for the formation of the questionnaire (J. Dawes, 2008) to examine the frequency of food consumption and food quality. Most of the questions are formed so that the gradation of possible answers is from 1 to 5, where 5 is the preferred response and represents the highest level of quality food according to the recommendations, standards and guides. ''Questionnaire on eating habits of women in menopause'' was used to prove the objectives and hypotheses.

Questionnaire about eating habits contains questions based on frequency of use certain food, characteristic for dietary habits in Bosnia and Hezegovina. The first part of the questionnaire describes the general information of an anonymous respondent, such as the level of consumption of cigarettes, alcohol, and physical activity. The rest of the questionnaire contains questions about the eating habits of the respondents, in terms of desirable and undesirable foods during this period of life, while the final section provides information on the health status and their behaviors related to these conditions and usage of certain medications. These questions is related to the following entities of food quality:

frequency of food consumption,

- drinking water and beverages,
- consumption types of bread and kind of fat,
- consumption of fresh fruits and vegetables,
- consuming protein-lipid foods
- frequency of consumption foods that have adverse effects on health,
- presence of the disease in patients and
- consuming dietary supplements

Statistical analysis

Non-parametric and parametric methods were used to calculate statistical significance. Distribution of values was determined by D'Agostino and Pearson omnibus normality test. Student's t-test, Mann-Whitney test, Fisher's test and χ2 test were used to calculate the difference between the groups.

ANOVA test was used to calculate the relative difference distribution of variance between variables. Statistical hypotheses were tested at the level of α = 0.05, and the difference between groups in the sample was considered significant when p<0.05 or less. Statistical significance is in-

dicated as: (*) p<0.05 (**) p <0.01 and (***) p <0.001. All data were analyzed using GraphPad Prism version 5 (San Diego, California, USA).

Reuslts and discussion

According to the body mass index (BMI), 12:33% of the respondents were within the normal range of nutrition, while 87% of respondents were overweight and obese. Only 0.33% was malnourished, and about 13.6% belonged to the level II and III of obesity. The largest percentage of respondents in the group was with obesity grade I, what is about 38.67% of the respondents.
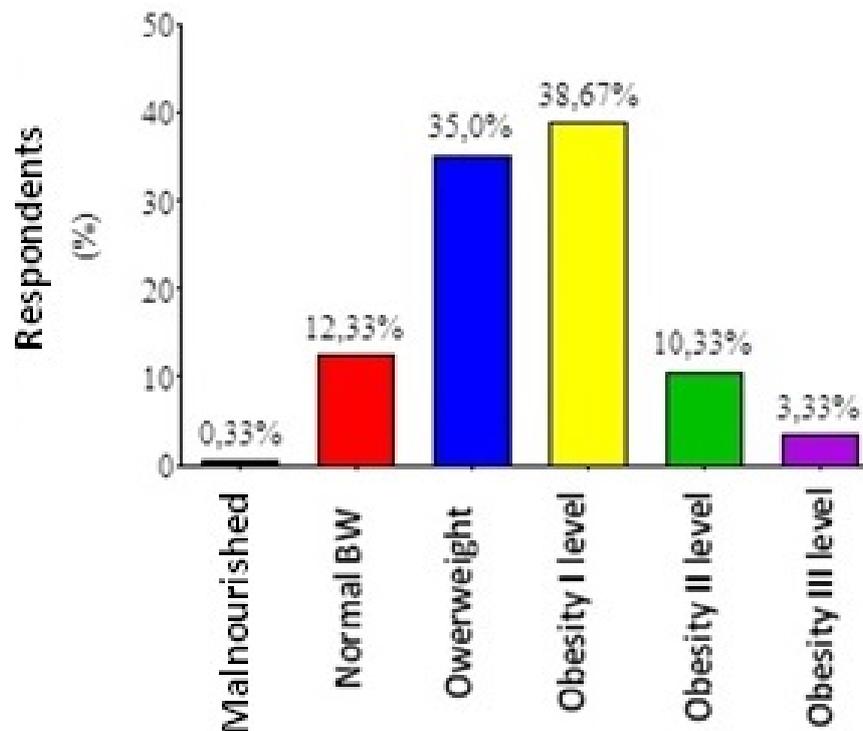

Fig. 1. Representation of the body mass index of subjects according to the World Health Organization

After stratifying to the respondents who mostly sitting or performing household activities, and respondents who walk, it was noted that the BMI of respondents who walk, was significantly lower than in the first group (p <0.01), which indicated the significance of physical activity for body

weight regulation. The appearance of the menopausal symptoms every day, is directly linked to higher BMI value. Subjects with symptoms of menopause every day, generally had higher BMI values.
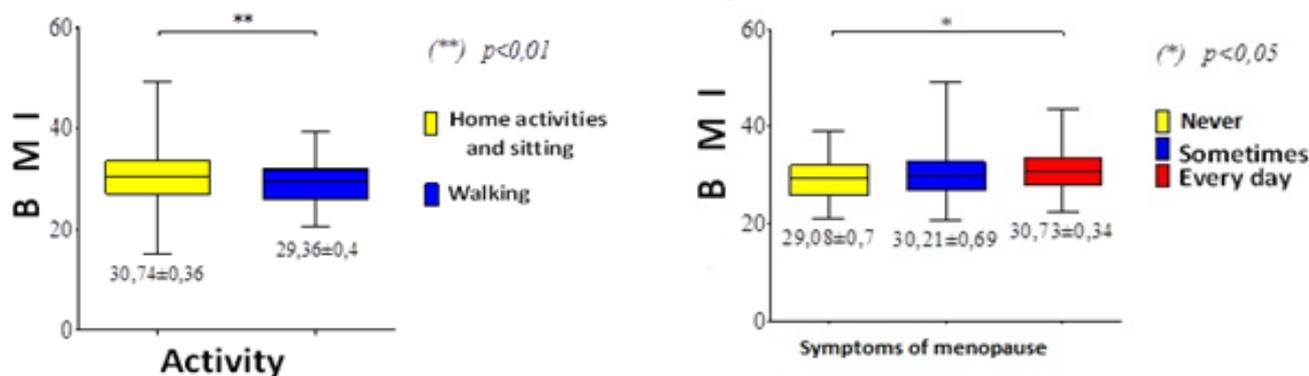
Fig. 2. The relationship of body mass index in the patients with different frequency of menopausal symptoms and the different types of activities

BMI was significantly higher in the patients with an increased intake of: soft drinks, sweets and white bread. Higher BMI was associated with an increase in the daily amount consumed carbonated beverages, or increasing the amount consumed beverages, and result was remarkably higher BMI. (p<0.05; overall Spearman's correlation coefficient: r = 0.12; 95% CI from -0.002600 to 0.2484). There was a significant positive correlation between body mass index and weekly amounts consumption of sweets (p <0.01); overall Spearman's correlation coefficient: r = 0.19; 95% CI 0.07174 to 0.3103).
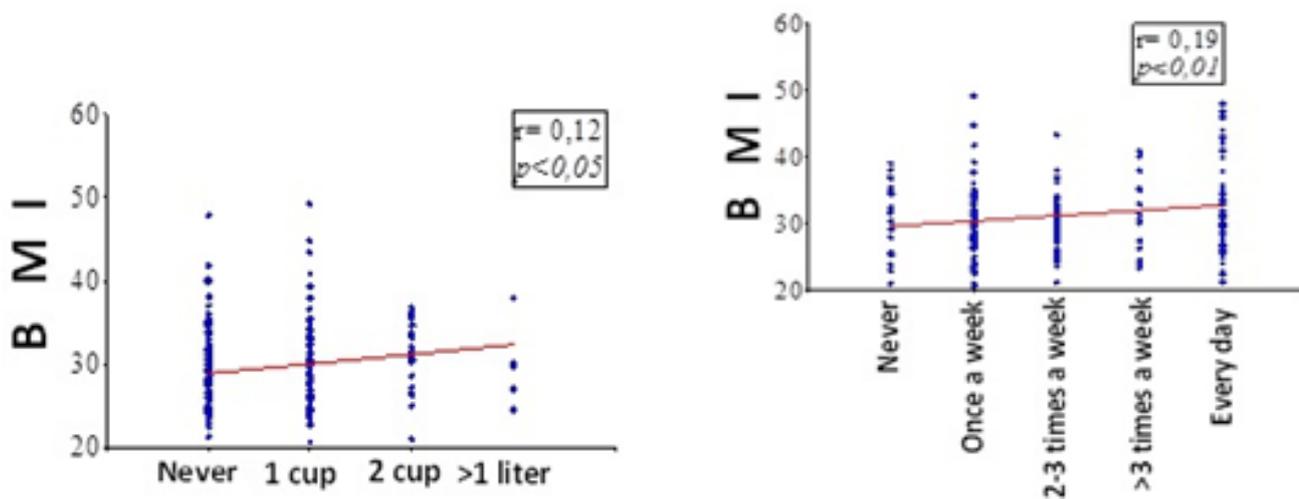

Fig. 3. A) Correlation between body mass index and the daily amount consumed carbonated drinks
B) Correlation between body mass index and weekly amounts consumed delicacy

A significant decrease in body mass index was correlated with the number of daily meals (p<0.05). It's not considered the amount of food intake, and we assumed that the amount of meals is not overabundant, and that the frequent intake of food as result had the acceleration of metabolism, which resulted in a reduced BMI.
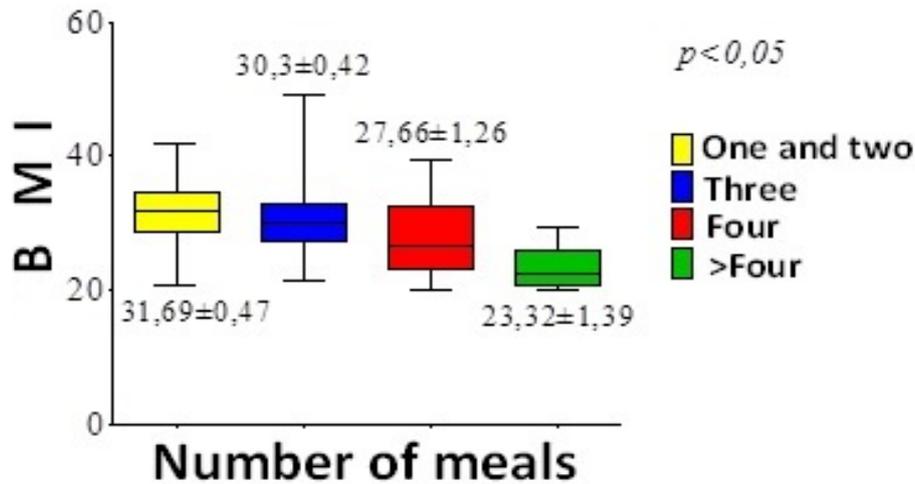
Fig. 4. The relationship of body mass index and number of daily meals.

Quality of life during menopause, mainly depends on the frequency of negative symptoms of menopause and the occurrence of certain diseases of menopause. Respondents who have never had symptoms and diseases in menopause, have a significantly lower BMI ($p<0.05$). The most common diseases in menopausal women were: hypertension (57%), diabetes (12%), depressive disorders (25%) and cancer (1%). The subjects, who had menopausal symptoms every day make up 31.2% of the total, 32.8% had symptoms sometimes, at 24% of the women the symptoms are there 2-3 times a week, while those who have had symptoms of 1-2 times week were in the lowest percentage ($p<0.05$). The application of hormonal therapy in patients with severe menopausal symptoms, resulted in a higher body mass index than female respondents who have not received this treatment ($p <0.05$).
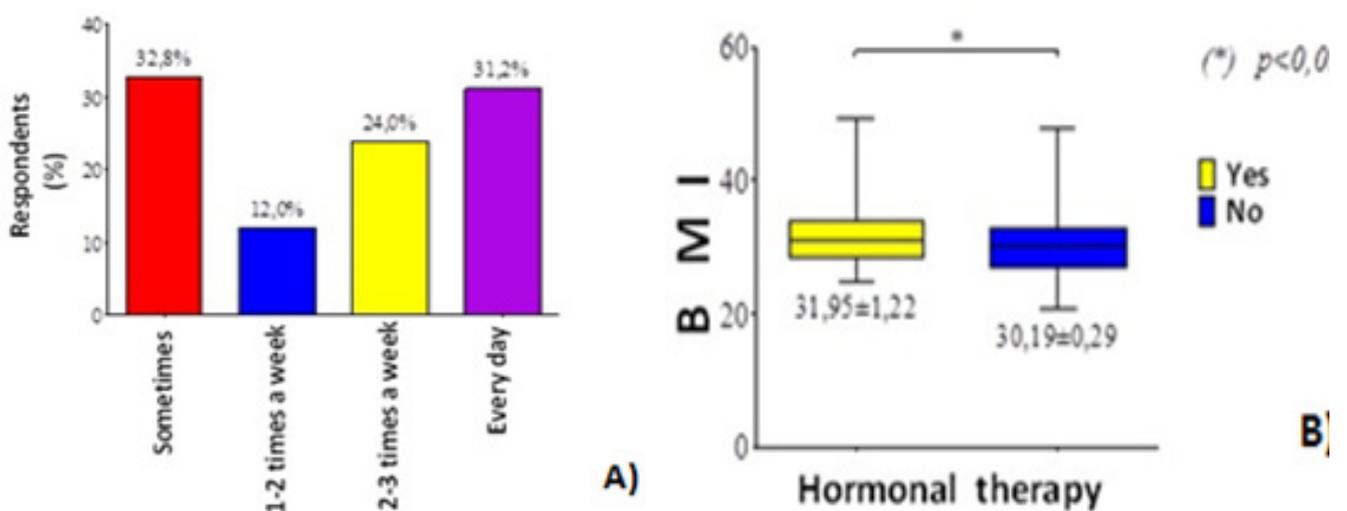

Fig. 5. A) The incidence of symptoms in women who have symptoms of menopause
B) Correlation between body mass index and the use of hormonal therapy

121

It was examined the relationship of eating habits of respondents and frequency of certain diseases that occur during menopause (hypertension, diabetes mellitus, depression and intestinal diseases). Some diseases are significantly associated with wrong eating habits and increased values of body mass index. BMI in patients with depression, hypertension, or diabetes mellitus was significantly higher than women without these diseases (p<0.05, p<0.001 and p<0.001), while body mass index patients with and without intestinal disease was equally distributed.
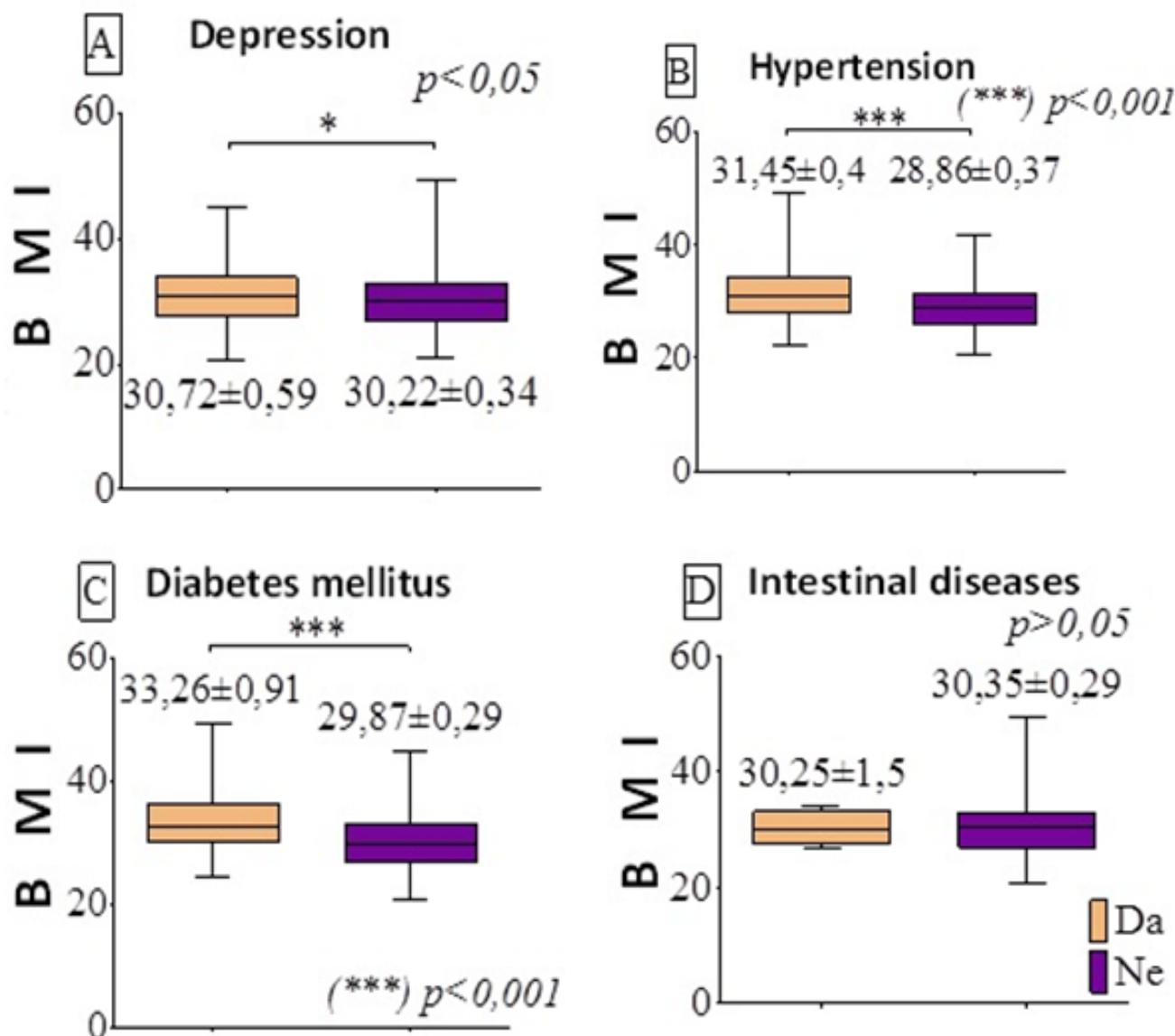

Fig. 6. (A, B, C and D) Body mass index in postmenopausal subjects with and without depression (A), hypertension (B), diabetes mellitus (C), and intestinal diseases (D).

Hypertension and diabetes mellitus were more common in women who consume a greater amount of table salt (p<0.05 for both measurements). It has been noted that there is no connection between salinity and incidence of depression and intestinal diseases. Diabetes is more prevalent in the patients with a higher consumption frequency of carbonated beverages. There was no significant correlation between daily amounts of carbonated beverages and the incidence of hypertension, depression, and intestinal diseases.
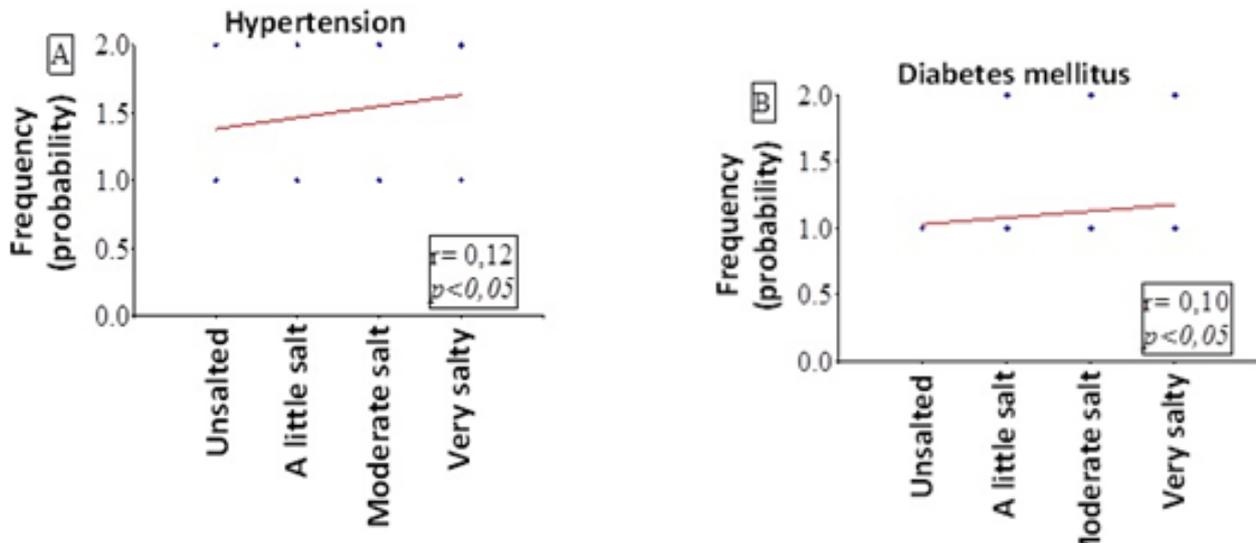
Fig. 7. Correlation between food salinity and frequency of hypertension (A), diabetes mellitus (B)

Discussion

Bad eating habits have resulted in a significant deviation of the body mass index and physiological nutritional status. Only 12.33% of respondents had a physiological nutritional status, underweight was one patient, all other respondents were overweight or obese. Most respondents fall into the category of obesity grade I, even 38.67%. Respondents who mostly sitting or do household activities than women who walk, had significantly higher body mass index. About 25% of respondents had a positive smoking status.

Most respondents do not abide by dietary recommendations. It is observed a significant decrease in body mass index in women who often had a breakfast, and it was observed an increase in body mass index in the patients with lower intake of water in the body, less than one glass per day. Hypertension, diabetes mellitus and depression were significantly less common in the patients who walked more often than respondents with household activities or sitting (hypertension: $p < 0.05$). The body mass index of patients with depression, hypertension, or diabetes mellitus was significantly higher than women without these diseases.

Obesity, high blood pressure, high triglycerides and glucose levels, along with menopause make a set of risks for women, that are commonly called metabolic syndrome. In the treatment of metabolic syndrome, in addition to medications, it is necessary to change the nutritional and lifestyle habits and increase physical activity.

However, comparative studies in adults have shown that the effect of physical activity on weight control is limited and generally lower than those obtained by restriction diet (Hill and Wyatt, 2005; Atlantis et al., 2006; Epstein and Goldfield, 1999; Garrow and Summerbell, 1995; Wing, 1999; Donnelly et al., 2009). It was found that the relative effectiveness of diets and physical activity depends on the degree of obesity and to the maximal effect of diet and physical activity is achieved at low levels obesity (Garrow, 1986). A large number of patients do not follow the recommendations on the intake of carbonated beverages and they are at risk of entry of excessive amounts of carbohydrates and additives that have a negative consequences for the health. It was observed a significant increase in body mass index with the increase in the daily amount consumed carbonated drinks. Also, respondents who consumed higher amounts of carbonated drinks per day were significantly higher incidence of diabetes ($p < 0.05$). 82.67% of respondents had some symptoms of menopause, every day or occasionally, and 25.33% of the total number of respondents had symptoms of menopause every day. Subjects with symptoms of diseases in the menopause had a significantly higher body mass index than women without symptoms.

Less than 10% of the respondents consumed olive oil. Sunflower oil consumed almost 90% of

respondents. However, although the body mass index of subjects who consumed olive oil was lower, than women who consumed margarine or sunflower oil, the differences between those groups were not statistically significant.

Soybeans as an essential food, especially for menopause, is used by about 17.3% of the respondents, which is a small percentage, considering the importance of soybeans to feed menopausal women as a source of phytoestrogens, which are very essential for the regulation of menopausal symptoms. However, research has not demonstrated a statistically significant difference in the occurrence of menopausal symptoms in women who consume and do not consume soybeans, probably due to small sample size and lack of initial amount of soy.

8.3% of respondents have used or now using hormonal therapy for menopausal symptoms and generally, this therapy, and nutritional supplements were prescribed and advised by a physician.

Conclusion

Dietary habits of respondents indicate on insufficient knowledge of nutritional needs and recommendations, which resulting in the consumption of food that is not adapted to this period of life. Respondents do not feed by the standards and recommendations of the WHO, and among them there is an increased risk of diseases typical for the menopause, as a result of the effects of improper nutrition. All these mistakes in diet and other lifestyle habits have yielded results in an increased body mass index, which is also associated with many diseases of our time, as the examined population of women, but also for all other categories of the population. Nutrition according to the standards and recommendations of the WHO results in better physiological nutritional status of menopausal women, which resulting in reduced morbidity and negative symptoms of menopause.

Considering the results of the research, the main recommendation would be seeking to achieve a physiological nutritional status, which will certainly contribute to a more comfortable menopause with less intensity of menopausal discom-

forts, and also reducing the disease in menopausal women.

Literature

Anonymous (2005b). Dietary reference intake for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and aminoacids (macronutrients), Food and Nutrition Board, Institute of Medicine, National Academic Press, Washington DC

Anonimus. (2010). A framework to monitor and evaluate implementation: Global Strategy on Diet, Physical Activity and Health, World Health Organization, Rome. http://www.who.int/dietphysicalactivity/ publications/en/ pristupljeno dana 09.09.2010. god.

Atlantis E, Barnes EH, Singh MA (2006) Efficacy of exercise for treating overweight in children and adolescents: a systematic review. Int J Obes 30: 1027–1040

Barret-Connor E, Bush TL.(1991). Estrogen and Coronary Heart Disease in Women. JAMA 265: 1861-1867

Chi-Un Pae, Laura Mandelli, Tae-Suk Kim, Chang-su Han, Prakash S. Masand, David M. Marks et al. (2009). Effectiveness of antidepressant treatments in pre-menopausal versuspost-menopausal women: A pilot study on differential effects of sex hormones on antidepressant effects. Biomedicine& Pharmacotherapy 63 : 228-235

Dawes, J. (2008).Do data characteristics change according to the number of scale points used?, International Journal of Market Research 2008; 50 (1), pages 61.-77

Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK (2009) Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 41:459–471

Epstein LH, Goldfield GS (1999) Physical activity in the treatment of childhood overweight and obesity: current evidence and research issues. Med Sci Sports Exerc 31(11):553–559

Garrow JS, Summerbell CD (1995) Meta-analysis: effect of exercise, with or without dieting, on the body composition of overweight subjects. Eur J Clin Nutr 49:1–10

Guenther, P.M., Reedy, J., Krebs-Smith, S.M., & Reeve, B.B. (2005). Evaluation of the Healthy Eating Index-2005. Journal of the American Dietetic Association, 108, 1854-1864

Hill JO, Wyatt HR (2005) Role of physical activity in preventing and treating obesity. J Appl Physiol 99:765–770

Matthew A. Karen et al.(2010). Are there bi-directional associations between depressive symptoms and C-reactive protein in mid-life women?. Brain, Behavior, and Immunity; 24: 96–101

McNagny SE. (1999). Prescribing hormone replecement therapy for menopausal. Review of Public Health Vol. 26:115-40

Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM (2006). Prevalence of Overweight and Obesity in the United States, 1999-2004., JAMA 2006 295:1549-1555

Ossewaarde ME, Bots ML, Verbeek AL, Peeters PH, van der Graaf Y,Grobbe DE, et al. (2005). Age at menopause, cause-specific mortality and total life expectancy Ann Intern Med. 19;131 (8):605-16

Pi-Sunyer X (2009) The Medical Risks of Obe-sity. Postgrad Med. November 121(6): 21–33

Pokorn D (2003). Prehrana v različnih življenskih obdobjih. Ljubljana. Marbona

Prior JC, Vinga YM, Schechter MT, Burgess AE.(1990). Spinal Bone Loss and Ovulatory Disturbances. N Engl J Med 323: 1221-1227

Radaković B. (2004).Transdermalna terapija estrogenima u hormonskom nadomjesnom liječenju žena u postmenopauzi.Gynaecol Perinatol 13:16–19

Salčić S. (2010). Kvaliteta života u menopauzi. Apotekar 38: 33-35

Stojanović Z, Milić J, Nikolić P (2007). Rendgen-kefalometrijska procena linearnih i angularnih parametarana bazi lobanje kod dece sa III skeletnom klasom, Vojnosanitetski pregled, 2007; 64: 604-610

USDA (2010). US Department of Agriculture. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, Report of the DGAC on the Dietary Guidelines for Americans, 2010 Press Release Washington D.C.

USDA (2005). U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary Guidelines for Americans 2005. 6-th Edition, Washington D.C.,U.S. Government Printing Office

Wing R (1999) Physical activity in the treatment of the adulthood overweight and obesity: current evidence and research issue. Med Sci Sports Exerc 31:547–552

Instructions to authors

Authors are kindly asked to read the following instructions while preparing the manuscript for publishing in the journal *Food in health and disease.*

Food in health and disease is a scientific-professional journal, published by the Faculty of Pharmacy, University of Tuzla with Co-Publisher Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek.

Food in health and disease publishes o*riginal scientific papers, preliminary communications, scientific notes, reviews* and *professional papers*. All papers need to be written and submitted in English. All contributingmanuscripts will be subjected to critical peer review.

Difference between *scientific* and *professional* papers is in the originality of methods, results and conclusions. Although a professional paper may imply more tangible applications it is generally not considered a new scientific contribution.

Original scientific papers report unpublished results of original research. They must contain significant and original observations to be critically evaluated. Experimental data should be presented in a way that enables reproduction and verification of analyses and deductions on which the conclusions are based.

Preliminary communications include short information on the results of scientific research which require immediate publication.

Scientific notes include reports on shorter but completed research or descriptions of original laboratory techniques (methods, apparatus etc.) and should be concise.

Reviews are original, critical and up-to-date surveys of an area in which, preferably, the author himself/herself is active. They should include recent references from international publications.

Professional papers present new possibilities of improvement within the area of food technology. The emphasis is on the application of known methods and facts as well as on broadening the knowledge in the particular area. The acquired knowledge is applied to the object of research.

Procedure

All contributions are evaluated according to criteria of originality and quality of their scientific and professional content. All manuscripts received for consideration will be acknowledged by the Editorial office. All manuscripts are sent to at least two referees. Based on their opinion, the Editor and the Editorial Board bring a decision about the acceptance of the manuscripts. Contributions may be rejected without reviewing if considered inappropriate for the journal.

Copyright

The authors bear the sole responsibility for the content of the contributions. The Editorial Board assumes that by submitting their papers the authors have not violated any internal rules or regulations of their institutions related to the content of the contributions and that they have not submitted the paper somewhere else. The acceptance of the paper obliges the authors not to publish the same material elsewhere.

Manuscript preparation

The manuscript should consist of max. 15 type written pages including tables, graphs, schemes and photographs. Itshould be written with 1.5 spacing on one side of the paper and margins 2.5 cm. For the text should be used normal plain font (Times New Roman, font size 12). The title of the manuscript and the

title of the chapter should be short and written in bold. The title of subheading should be written in italic. Under the title, author/s full name/s and surname/s should be written, with asterisk next to the name of the corresponding author. Footnote at the bottom of the first page should contain information about the corresponding author (address and e-mail). The affiliations for all authors must be given in the following sequence: University/ Institution, Faculty/Department, Postal address, City, Country. When authors have different affiliations, should be used superscripted Arabic numbers after last name of the author.

Manuscript has to be written without spelling mistakes, impersonal style. It is the author's responsibility to ensure that papers are written in clear and comprehensible English. Authors whose native language is not English are strongly advised to have their manuscripts checked by an English-speaking colleague prior to submission.

The first word in the paragraph must not be retracted. Paragraphs have to be separated by clicking on enter key. Pages have to be numerated (on the bottom right). For decimal numbers in text and tables dot should be used.

Latin words, phrases and abbreviations, including generic and specific names, should be written in italic. Manuscripts should be submitted by e-mail, as attached document, and/or by mail, on CD-ROM, to the Editor's office. The manuscripts should be sent to the following address:

Josip Juraj Strossmayer University of Osijek
Faculty of Food Technology Osijek
Franje Kuhaca 20, HR-31000 Osijek, Croatia
Phone: ++385 (0)31 224 300; Fax.: ++385 (0)31 207 115
e-mail address: ivana.pavlekovic@ptfos.hr

Faculty of Technology
University of Tuzla
Univerzitetska 8, 75000 Tuzla, Bosnia nad Herzegovina
Phone: +387 35 320 745; Fax.: +387 35 320 740
e-mail address: midhat.jasic@untz.ba

For clearness the paper should be divided into the following sections: **Summary,** *Keywords,* **Introduction, Materials and Methods, Results and Discussion, Conclusions and References.**

Summary

Summary must not exceed 300 words and has to show relevant data, methodology, main results and conclusion. Itshould not contain abbreviations or references. After summary, authors are asked to list several keywords.

Keywords

Keywords include the main topic of the paper and should not contain more than 5 words or phrases, which shouldbe separated by commas.

Introduction

Introduction should refer to previous research results and explain the purpose of the investigations.

Materials and methods

Experimental part should be written clearly and in sufficient detail to allow the work to be repeated. Materials andMethods should indicate instruments, samples, subjects, chemicals, etc., giving enough details only if new methodsand/or procedures and/or principles are used. For the well-known methods and techniques an adequate reference(s)citation will suffice.

Results and discussion

The information given in tables and figures should not be repeated, and only relevant data discussed and explained. Combining the results with discussion can simplify the presentation.

Tables and figures should be completely understandable without reference to the text. For publishing reasons, they have to be delivered in graphical formats (*.xls, *.tif or *.jpg) and at the end of the paper.

All illustrations (graphs, schemes, diagrams, pictures, etc.) should be named as figures. Refer to figures by the abbreviation "Fig.". All figures and tables should be cited in the text and numbered consecutively throughout. Preferred program for preparing figures and tables is Microsoft Office Excel.

Be sure to use lettering, data lines, and symbols sufficiently large and thick to be clearly legible when the figure is reduced to the normal published size. In graphs and charts, curves should be identified by using different symbols for points (•, ◊, ○, □, ■, ▲, etc.) and not by line width or colour. Several figures should be grouped in a plate on one page. Do not place figures into bordered frames. Figure caption and legend should be placed at the bottom of each figure, while table headings should appear above the tables. The text into the figures and tables should be written in the same language as the body text.

Whenever possible formulae and equations are to be written in one line, centred on the page, and consecutively numbered in Arabic numbers between round brackets at the right margin of the paper. Refer to equations by the abbreviation "Eq.".

SI (System International) units should be used. Only symbols (not their subscripts, superscripts or description in brackets) of physical quantities should be written in italic. All physical quantities given in table columns or rows and corresponding table headings with units, or graphical plots and corresponding table headings with units, or graphic plots and corresponding axis labels should conform to the algebraic rules, i.e.

$$physical\ quantity\ /\ unit = numerical\ value.$$

It is preferred to write the measurement units as "kg/m3".

Conclusions

Conclusions have to briefly explain significance of the research results.

References

References should be selective rather than extensive (with the exception of review articles). Literature citations inthe text should be referred by author's last name and year, in brackets, such as (Knowles, 2007), or with the lastname of the author as part of the sentence, immediately followed by the year of publication in brackets, such as("Smith (1963) reported growth on media."). If there are more than two authors, mention the first author and add etal., followed by the year.

In the reference list which has to be written at the end of the paper, all authors have to be listed (surname and nameinitials – capitalizing only the initial letters) in alphabetical order, and paper of the same author by chrono-logicalorder. If there are more papers from the same author published in the same year, references have to be differentiated by letters in the text (a, b, c, d) behind the year of publishing. In case of multi author papers, in reference list all the authors have to be written (not the first author and mark "et al.").

Italicize only those words that were underlined or italicized in the original, such as names of microorganisms. Alsotitles of journals have to be written in italics.

For papers published on the web, it is necessary to write the source and the date when the paper was downloaded,besides basic information (title, author's name, etc.).

Abbreviations for periodicals should be in accordance with the latest edition of the Thomson ISI List of Journal Abbreviations (Thomson Scientific, USA). Full stop should be used after each abbreviation.

Reference list should be written as shown:
Journals:
Kopjar, M., Piližota, V., Hribar, J., Nedić Tiban, N., Šubarić, D., Babić, J., Požrl, T. (2008): Influence of trehalose

addition on instrumental textural properties of strawberry pastes, Int. J. Food Prop. 11 (3), 646-655.

Books:

Doyle, M.P., Beuchat, L.R., Montville, T.J. (2001): Food Microbiology: Fundamentals and Frontiers, Washington, USA: ASM Press, pp. 572-573.

Chapter in book:

Varoquaux, P., Wiley, R.C. (1994): Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables. In: Minimally Processed Refrigerated Fruits and Vegetables, Wiley, R.C. (ed.), New York, USA: Chapman, pp. 226-268.

Conference proceedings:

Babić, J., Šubarić, D., Ačkar, Đ., Kopjar, M. (2008): Utjecaj hidrokoloida na reološka svojstva voštanog kukuruznog škroba. In: 43rd Croatian and 3rd International Symposium on Agriculture, Pospišil, M. (ed.), Zagreb,HR, pp. 558-562.