Coll. Antropol. **42** (2018) 3: ???–???
Short report

Mediterranean Diet in Pregnancy and its Association with Newborns` Body Size in Dalmatia, Croatia – The Preliminary Results of the Croatian Islands Birth Cohort Study

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ABSTRACT

The quality and quantity of pregnant womens' nutrition have an impact on offsprings` birth size. Although health benefits of Mediterranean diet are widely confirmed, the poor compliance with its recommendations was already detected in population of Dalmatia, coastal region of Croatia. The data of 131 mother–newborn pairs, participants in the ongoing Croatian Islands` Birth Cohort Study (CRIBS), were analyzed: (1) to test whether pregnant women in the study consume Mediterranean diet, traditional in this area, and (2) to examine the association of maternal diet in pregnancy with newborns` anthropometric parameters (birth weight, length and head circumference). The preliminary results of the factor analysis of 20 items from Food Frequency Questionnaire resulted in two significant factors, both having positive loading with the components of Mediterranean diet. Factor 1 had highest positive loadings for blue fish, white fish, olive oil and fatty cheese consumption, and Factor 2 had highest loading coefficients for fermented milk products (yogurt), sea food, cereals and legumes. Linear regression of the two obtained factors and newborns` body size parameters showed significant correlation between the weight-at-birth and the Factor 2 ($p<0.05$). Factor analysis of FFQ confirmed that the pregnant women from the CRIBS study mostly follow Mediterranean diet, but, opposite to results of some other studies, the association of the Mediterranean diet and body size parameters at birth was not confirmed in this research.

Key words: *Croatian Islands Birth Cohort Study (CRIBS), cohort, dietary intake in pregnancy, Mediterranean diet, newborns` anthropometry, Croatia*

Introduction

Western world countries recognized 1980s as crucial in the shift in diet toward increased intake of processed food and prepared meals, and greater consumption of edible oils and sugar-sweetened beverages. Worsened quality of food and change in dietary habits, by developing an affinity and fondness towards unhealthy but easily accessible, affordable and tasty food, in combination with drastically reduced physical activity, led to rise in obesity.[1] Obesity, besides being associated with poorer mental health outcomes and reduced quality of life, is also recognized as an important risk factor for many diseases including cardiovascular diseases, type 2 diabetes, stroke, some types of cancer, etc.[2] The obesity pandemic is not a problem only in the United States and Europe, but in low and middle income countries too.[3]

Traditional diet, which includes home-prepared food made of homegrown fruits and vegetables, and livestock products, is still present in smaller rural communities, like in Dalmatia, Croatia, especially on the islands.[4,5] Population of Dalmatia has traditionally been living from agriculture and fisheries. Their diet can be seen as a variant of Mediterranean diet, because it is mostly characterized by the consumption of brown bread, olive oil, fish, red wine, fruits and vegetables.[6] Mediterranean diet is traditionally practiced in countries of the Mediterranean basin, which are Greece, Cyprus, coastal regions of Albania, Montenegro and Croatia, Southern Italy, Southern France, Spain, and Portugal. The most prominent char-

Received for publication May 17, 2108

acteristics of Mediterranean diet are the consumption of olive oil (preferably virgin oil), fruits, nuts, and whole grains daily, with moderate red wine and legumes consumption. Animal products are consumed only occasionally, as a relish, while the priority is given to fish and white meat, over red and processed meat.[7] The numerous investigations of the Mediterranean diet confirmed its beneficial health effects on prevention of cardiovascular diseases and type 2 diabetes[8], reversion of metabolic syndrome[9] (MetS), and different types of cancer.[10] However, although traditions have historically shown strong vitality and resistance to cultural reduction and one-way development, the traditional diet of islanders is changing under influence of modernization.[6,11] These changes have been noted from 1990ies on, on islands Hvar[12], Vis[13,14], and Korčula.[14]

Varied, balanced high quality food and adequate macro- and micronutrient intake in pregnancy are recognized as crucial for the health status of both the mother and child.[15] In addition to that, but if we take into account the developmental origins of health and disease hypothesis[16], nutrition in pregnancy might have long-term consequences on future generations. Recommendations for nutrients in pregnancy differ according to both the eating tradition and nutrition status of the population.[15] Traditional Mediterranean diet has shown a positive impact not only by contributing to conception of women who were undergoing assisted reproduction technology methods[17], but also as beneficial for pregnant women`s health[18], and health of their children in early life.[19,20]

In this paper we present the preliminary results of the CRoatian Islands Birth Cohort Study (CRIBS), an ongoing study which is being conducted in Middle Dalmatia on Eastern Adriatic. Presented analyses were conducted with aims: (1) to determine whether pregnant participants in the study consume Mediterranean diet, (2) to test the association of maternal diet in pregnancy with newborns` anthropometric parameters (birth weight, length and head circumference) using the data from questionnaires and obstetric records.

Materials and Methods

Description of the CRIBS Cohort

The ongoing CRoatian Islands Birth Cohort Study (CRIBS) is the first prospective birth cohort study in the South-Eastern Europe, aiming to assess the prevalence of known risk factors (biological, environmental and behavioral) for the Metabolic Syndrome (MetS) in populations from Croatian Dalmatian islands (Hvar and Brač) and mainland population (city of Split with surroundings). This Eastern Adriatic area has high prevalence of the MetS, which is higher on islands than in the neighboring mainland area. Recruitment of pregnant women who conceived naturally, had singleton pregnancies and had no history of chronic diseases began in February 2016 and will last until reaching the aimed number of 500 mother-child pairs (predicted to be reaches by October 2018).

TABLE 1

FACTOR ANALYSIS OF FOOD FREQUENCY QUESTIONNAIRE DATA: SUMMARY RESULTS.

Total Variance Explained

	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative variance %
1	2.917	15.354	15.354
2	1.830	9.630	24.984
3	1.516	7.980	32.965
4	1.446	7.610	40.575
5	1.338	7.043	47.617
6	1.180	6.209	53.826
7	1.032	5.433	59.259
8	1.015	5.343	64.602
9	0.968	5.093	69.695
10	0.878	4.621	74.316
11	0.779	4.097	78.413
12	0.762	4.011	82.424
13	0.631	3.320	85.745
14	0.575	3.027	88.771
15	0.544	2.866	91.637
16	0.465	2.445	94.082
17	0.427	2.249	96.331
18	0.373	1.962	98.293
19	0.318	1.597	99.917
20	0.002	0.110	100.000

Comprehensive data on medical, anthropometric, socioeconomic, nutritional, lifestyle, psychological and other characteristics of both mothers and children, from pregnancy up to the children`s age of two, are being collected. Pregnant women participated in the study voluntarily and they signed written, informed consents for themselves at joining the research, and for their children, right after the birth.

The Ethics Committee of the Institute for Anthropological Research approved the study protocol.

Description of study sample and questionnaire measures

Initial sample consisted of 131 mother – newborn pairs from Split (N = 71) and islands of Brač and Hvar (N = 60) but since nine children were born preterm, 9 mother – newborn pairs were excluded from further analyses. During pregnancy mothers filled out two specially designed questionnaires, which contained questionnaire measures about maternal demographic, obstetrics, and lifestyle characteristics (diet included), well-being and mental state. Only a subset of the collected data is presented here.

TABLE 2

SCORE COEFFICIENTS DERIVED FROM PRINCIPAL COMPONENT ANALYSIS OF FOODS OR FOOD GROUPS CONSUMED BY PREGNANT WOMEN IN THE CRIBS STUDY.

Variables	Components (Factors)							
	1	2	3	4	5	6	7	8
blue fish	**0,776**							
white fish	**0,752**	0,230						
olive oil	**0,524**					0,300	-0,466	
fatty cheese	**0,485**		0,383			-0,381	0,419	
fermented milk products (yogurt)		**0,760**						
sea food	0,342	**0,690**						
cereals and legumes		**0,492**	0,271	0,228	0,217			0,233
dark bread			0,779	0,236				
seasonal fruits			0,493	-0,279	0,351	-0,309		
butter		0,304	0,461	-0,298				
milk				0,732		0,206		
potatoes				-0,659	0,307		0,206	
eggs					0,797			
wine				0,358	0,479		0,265	0,380
pulses						0,812		
white bread		0,289	-0,401		0,339	-0,452	0,213	0,295
non-fatty cheese							0,772	
vegetables	0,264	0,253			0,405	-0,264	0,454	
wine								0,896
pasta							0,747	

*Variables defining first two factors are highlighted in bold.

Assessment of dietary intake in pregnancy was determined using Dietary adequacy assessment questionnaire for adults[21] (DAAQA), the food frequency questionnaire adapted from the Harvard Semiquantitative Food Frequency Questionnaire[22].

The DAAQA consisted of 101 food items divided into 9 groups: cereals and grain products (14 items), vegetables (17 items), fruit (11 items), pulses and legumes (5 items), meat, fish and eggs (17 items), milk and dairy products (14 items), fat (5 items), sweets (4 items) and beverages (14 items) questions, with 7 available answers regarding the frequency of consumption (never, once a month, 2-3 times a month, once a week, 2-3 times a week, 4-6 times a week, and every day). Due to the recommendations that subjects-to-variables ratio should be no lower than five[23], 20 food variables (foods or food groups) characteristic for the Mediterranean diet were selected for the factor analysis (listed in Table 2). Information on birth weight, length, head circumference and infant sex were taken from obstetric records.

Statistical analyses

Statistical analysis included the factor analysis of food items with a factor loading coefficient >0.20, and the associations between the food items were measured using Pearson`s correlations. A single linear regression analysis for each factor and mothers` characteristics (age, employment status, level of education, and smoking) was conducted to assess independent associations. The estimated factor scores, age, level of physical activity, smoking and socioeconomic status were further used as independent variables in the logistic regression with the weight-at-birth, height-at-birth and head circumference-at-birth, for the lowest and the highest 15% of newborns as dependent variables. Anthropometric parameters of newborns were Z-standardized according to the WHO referent values[24] and compared between sexes using t-test.

Statistical analyses were performed using SPSS 11.0 and p values ≤ 0.05 were considered statistically significant.

Results

The factor analysis of the 20 selected food variables resulted in two significant factors (Table 1). Factor 1, with an eigenvalue of 2.917, had highest positive loadings for blue fish, white fish, olive oil and fatty cheese consumption, and it explained 15.354% of variance. Factor 2, which

TABLE 3

PEARSON`S CORRELATION FOR FOOD CONSUMPTION IN PREGNANCY, FOR FOOD INCLUDED IN FACTOR ANALYSIS.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
white bread	1																			
dark bread	**-0.225**	1																		
wine	**0.174**	**0.160**	1																	
fatty cheese	**0.087**	0.276	**0.204**	1																
non-fatty cheese	0.096	0.134	0.085	**0.271**	1															
sea food	0.154	**0.050**	-0.080	**0.188**	0.143	1														
pulses	0.298	**0.140**	**0.184**	**0.385**	**0.267**	**0.281**	1													
eggs	0.152	**-0.066**	**0.221**	0.126	0.078	0.117	**0.250**	1												
white fish	0.019	-0.096	-0.068	**0.286**	0.103	**0.343**	**0.168**	**0.042**	1											
blue fish	0.014	0.059	-0.041	**0.293**	0.103	**0.192**	**0.225**	**0.125**	**0.461**	1										
olive oil	**-0.113**	**0.077**	-0.095	0.002	-0.105	0.028	-0.014	0.019	**0.217**	**0.261**	1									
butter	**-0.028**	0.156	**0.175**	0.079	**0.176**	0.042	**0.109**	0.022	0.102	-0.010	-0.041	1								
wine	-0.271	-0.028	-0.086	-0.163	0.169	0.015	-0.089	-0.071	0.061	0.075	0.111	0.026	1							
cereals and legumes	0.114	0.097	0.202	**0.221**	0.136	**0.303**	**0.211**	**0.163**	**0.191**	0.076	0.014	**0.140**	0.041	1						
potatoes	0.204	0.148	0.045	0.100	0.056	-0.050	0.131	**0.120**	-0.094	-0.014	-0.086	0.004	0.019	0.170	1					
milk	0.127	**-0.072**	0.095	0.055	**0.189**	0.060	0.111	**0.166**	0.064	**0.009**	0.108	**0.181**	-0.019	-0.049	0.051	1				
seasonal fruits	**0.152**	**0.069**	**0.129**	**0.126**	**0.106**	0.082	-0.046	0.027	-0.017	0.091	0.030	0.033	0.059	**0.134**	**0.122**	0.120	1			
vegetables	0.044	0.164	-0.137	0.041	-0.024	0.131	0.053	0.060	0.049	0.063	-0.007	-0.019	**0.124**	**0.110**	**0.177**	-0.233	-0.001	1		
fermented milk product (yogurt)	**0.169**	**0.083**	0.118	-0.016	**0.185**	**0.352**	**0.205**	0.027	0.066	0.093	0.038	**0.165**	**0.116**	**0.159**	-0.037	0.162	**0.052**	0.060	1	
pasta	0.129	**0.030**	-0.047	**0.107**	0.012	0.003	**0.035**	**0.018**	-0.043	**0.128**	0.182	0.095	**0.051**	**0.030**	**0.110**	0.126	0.106	**0.082**	-0.046	1

* Correlation coefficients highlighted in BOLD have a p value ≤0.05

TABLE 4

CHARACTERISTICS OF CRIBS MOTHERS BY LIVING LOCATION.

Variables	Mainland population	Island population	*P
Marital status - N (%)			
Married	61 (50)	51 (41.8)	ns
Not married-living with partner	4 (3.3)	5 (4.1)	
Single	1 (0.8)	0	
Employment status - N (%)			
Permanent job	49 (40.2)	43 (35.2)	ns
Occasional	2 (1.6)	1 (0.8)	
Unemployed	15 (12.3)	12 (9.8)	
Level of education - N (%)			
Secondary	26 (21.3)	36 (29.5)	<0.01
Tertiary	40 (32.8)	20 (16.4)	
Smoking in pregnancy - N (%)			
Yes, just like before the pregnancy	0	1 (0.8)	ns
Yes, but less than before pregnancy	16 (13.1)	13 (10.7)	
Quit smoking in pregnancy	14 (11.5)	12 (9.8)	
Quit smoking before pregnancy	10 (8.2)	10 (8.2)	
Never smoked	26 (21.3)	20 (16.4)	
Physical activity in pregnancy – N (%)			
Sedentary-to-light	13 (10.7)	6 (5.0)	ns
Moderate	43 (35.5)	35 (28.9)	
Heavy-to-competitive	10 (8.3)	14 (11.6)	
Meals per day – N (%)			
2	0	2 (1.6)	ns
3	21 (17.2)	20 (16.4)	
4	31 (25.4)	26 (21.3)	
≥5	14 (11.5)	8 (6.6)	

* The qualitative differences between groups were tested using by χ^2-test.

** Level of education - Educational system in Croatia has three levels; primary education (1st-8th grade), secondary education (gymnasiums and art schools last four years, while vocational schools can last three or four years) and tertiary education, after which person obtains bachelor of science, master of science, master of education or doctor of science (or arts) degree.

explained additional 9.630% of variance and had an eigenvalue of 1.830, had highest loading coefficients for fermented milk products (yogurt), sea food, cereals and legumes (Table 2). Scree plot from the Principal Component Analyses of the FFQ is presented in Figure 1, while Pearson`s correlations between 20 selected food variables are presented in Table 3.

The characteristics of pregnant women from the CRIBS study are presented in Table 4. They were between 19.6 and 41.7 years of age (mean age 30.5±4.4 years, no significant differences between islands Brač and Hvar, and the mainland), and most of them were married (92.4%). Almost all unmarried women said that they were living with their partners unwed or that they had a support (financial/emotional) of their partners, future fathers. Three-fourths of women reported being permanently employed, 3.1% being seasonal, occasional or substitute employees, while 22% of women reported being unemployed. One half of both permanently employed and unemployed women completed higher education (BSc, MSc or PhD), just like did all 4 occasionally employed pregnant women. More educated women (with tertiary education) were found on mainland than on islands (p<0.01). Smoking in pregnancy was self-reported by 24.6% of women, although 96.7% of them said that they decreased number of cigarettes per day since they found out they were pregnant. Most of the women reported being moderately active in pregnancy.

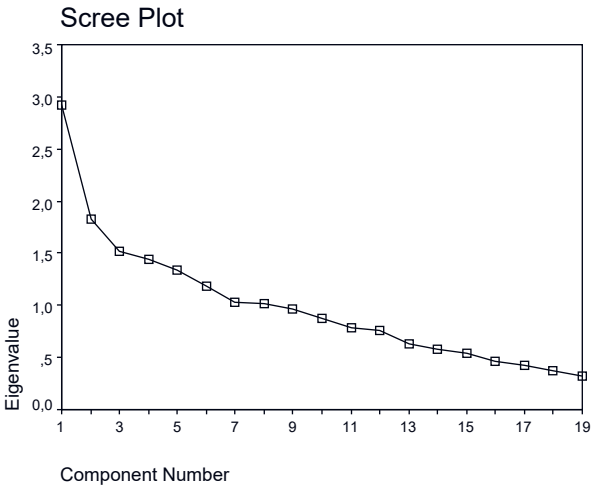

Fig. 1. Scree plot from the principal component analysis of the Food Frequency Questionnaire data with factor loading coefficient >0.20.

The CRIBS pregnant women gave birth to 69 female and 53 male newborns. In comparison with newborn females, newborn males from the CRIBS had significantly higher mean length-at-birth (51.28±1.75 cm vs. 50.48±1.77 cm, p<0.05) and head circumference-at-birth (35.41±0.90 cm vs. 34.97±1.05 cm, p<0.05), while mean weight-at-birth did not differ significantly between sexes (3.64±0.43 kg vs. 3.49±0.44 kg, p=ns). Still, Z-values standardized according to WHO referent values did not differ between genders (Table 5).

Neither tobacco use nor levels of physical activity of mothers were significantly associated with body size parameters in the CRIBS newborns. Although more than 98% of women reported having 3 or more meals per day, 13.9% reported not eating breakfast every day. The habit of having lunch daily was reported by 90.8% of the participants.

Single-linear regression analysis separately for each of two factors and mothers` characteristics (age, employment status, level of education and smoking) showed significant

TABLE 5

THE COMPARISON OF Z-STANDARDIZED VALUES OF WEIGHT-AT-BIRTH, LENGTH-AT-BIRTH AND HEAD CIRCUMFERENCE-AT-BIRTH BETWEEN FEMALE AND MALE NEWBORNS FROM THE CRIBS STUDY.

Anthropometric variables	Gender		*P
	Female (n=69)	Male (n=53)	
Weight	0.5242±0.9153	0.5475±0.8504	ns
Length	0.7133±0.9503	0.7392±0.9245	ns
Head circumference	0.9358±0.8764	0.7477±0.7051	ns
BMI	0.6328±1.0140	0.1120±0.8840	ns
Weight-for-length	0.0307±0.8638	0.0138±0.8907	ns

*Thedifferences between groups were tested by t-test.

regression model only for the level of education of pregnant women and the Factor 1 ($F(1,120) = 7.484$, $p<0.01$), with an R^2 of 0.059. Multiple linear regression for two factors and newborns` body size parameters (weight-, length- and head circumference-at-birth) did not result in any regression model, although weight-at-birth and the Factor 2 significantly correlated ($p<0.05$, Figure 2). Logistic regression model for the lowest and the highest 15% of the CRIBS newborns as dependent variables included Factors 1 and 2, mothers` age at birth, level of physical activity, smoking status, level of education and employment status; no significant regression model was found.

Discussion

Early-life factors influence fetal programming, birth and child health outcomes later in life.[25]

Diet and lifestyle of mothers are important determinants of health of both mother and offspring, starting from the preconceptional period.[26] In particular, the first 1000 days of life (counting from conception up to two years of age) are found to be crucial for the prevention of adulthood diseases.[27]

Mediterranean diet in pregnancy was found to affect favorably not only on maternal hypertension/preeclampsia, but also on intrauterine growth and certain acute and chronic complications of prematurity.[28] This type of diet is typical for the coastal part of Croatia, although changes in dietary habits and rather poor compliance with the current recommendations on Mediterranean diet in general population on Eastern Adriatic were noted on Hvar in 1990ies[12] and confirmed on Korčula[14] and Vis[13,14] in 2014. However, our results suggest that women in pregnancy follow the Mediterranean diet and most of them eat regularly (3 or more meals per day). Pregnancy is recognized as an ideal time to encourage healthy lifestyles.[29] It is possible that our pregnant women would not follow Mediterranean diet that strictly if they were not pregnant, but in pregnancy they may be more receptive to health messages and wish to ensure optimal nutrients for future child. CRIBS women already demonstrated awareness of desirable healthy lifestyle habits in pregnancy by reporting that 46.4% of all smokers quit smoking after finding out that they were pregnant, and the rest reported smoking less cigarettes per day than before the pregnancy. The fact that there were more highly educated women on mainland than on islands did not come as a surprise; higher education institutions are on the mainland, and ferries that connect islands with mainland out of summer season operate rarely.

91.8% of children were born in marriage, what is in accordance with data for Split-Dalmatia County - 91.5% of marriage-born children, but both frequencies differ from country average, where 1 in 5 children (19.6%) was born outside of marriage in 2016.[30] CRIBS newborns were on average longer-at-birth, heavier-at-birth and had bigger head circumference when compared to the WHO referent population. The Generation R Study revealed significantly lower weight-at-birth in children whose mothers had low adherence with Mediterranean diet.[31] In Greece neonates of mothers from the low adherence Mediterranean Diet group had significantly higher incidence of intrauterine growth restriction (IUGR) and lower z-scores of birth weight and BMI.[32] Unlike here mentioned studies, none of the two factors, results of factor analysis of FFQ, showed association with any newborn body size parameters. But obstacle in comparing Mediterranean diet between different cohorts might be the fact that several types of Mediterranean diet were found across European Mediterranean regions, some even within the same country.

In Spanish study Mediterranean diet scores loadings differed significantly between Atlantic and the Mediterranean regions two regions. Only in the Mediterranean region women with high Mediterranean diet adherence had a significantly lower risk of delivering a fetal growth-restricted infant for weight.[33]

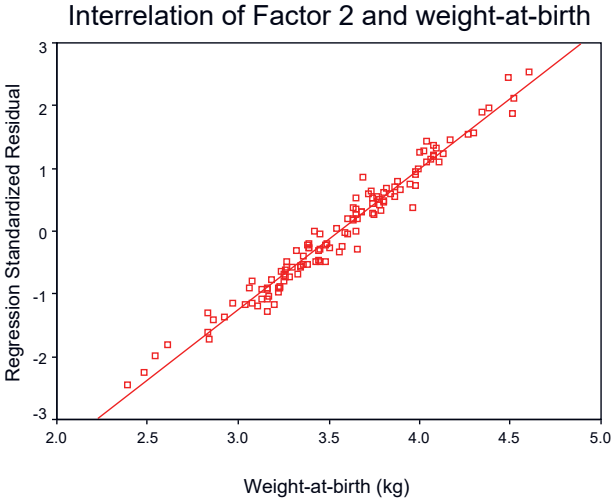

Figure 2. Interrelation of quantitative values of Factor 2 and weight-at-birth (r2=0.033 ; r=0.183; p=0.044 ; $y_{birth\text{-}weight}$= 3.557+0.039x).

Conclusions

Factor analysis of food frequency questionnaire confirmed that the pregnant women from the CRIBS study mostly follow Mediterranean diet. Even though it was not statistically confirmed in our research, this type of diet might be associated with better pregnancy outcome and optimal development of newborn. The limitation of this ongoing study is its still small sample size, so the final results might differ after completing the recruitment of participants.

Acknowledgement

This project is financed by the Croatian Science Foundation under the grant number HRZZ UIP-2014-09-6598. The CRIBS study is possible only because of the commitment of our pregnant participants, support of their husbands/partners, and their confidence in us. We thank all the participants, health professionals and researchers who have made it happen.

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MEDITERANSKA PREHRANA U TRUDNOĆI I VELIČINA NOVOROĐENČADI U DALMACIJI, HRVATSKOJ – PRELIMINARNI REZULTATI PROJEKTA KOHORTNA STUDIJA ROĐENIH NA ISTOČNOJADRANSKIM OTOCIMA

S A Ž E T A K

Kvaliteta i količina hrane koju trudnice unose utječu na veličinu novorođenčeta. Unatoč potvrđenom pozitivnom utjecaju mediteranske prehrane na zdravlje, prehrana populacije Dalmacije, obalnog područja Republike Hrvatske, slabo se podudara s preporukama mediteranske kuhinje. U ovom radu analizirani su podaci 131 para majka-novorođenče, sudionika u još uvijek aktivnom projektu „Kohortna studija rođenih na istočnojadranskim otocima (CRIBS)“, kako bi se utvrdilo: (1) konzumiraju li trudnice u studiji mediteransku prehranu, koja je tradicionalna prehrana na području na kojem žive, te (2) povezanost majčine prehrane u trudnoći s antropometrijskim mjerama novorođenčadi (težina prilikom rođenja, duljina i opseg glave). Preliminarni rezultati faktorske analize 20 tipova namirnica iz prehrambenog upitnika rezultirali su dvama značajnim faktorima, koji su oba pozitivno opterećeni komponentama mediteranske prehrane. Faktor 1 imao je najveće pozitivno punjenje plavom ribom, bijelom ribom, maslinovim uljem i masnim sirom, a Faktor 2 fermentiranim mliječnim proizvodima (jogurt), morskim plodovima, žitaricama i mahunarkama. Linearna regresija Faktora 1 i 2 i parametara veličine tijela novorođenčadi pokazala je značajnu korelaciju između težine pri rođenju i Faktora 2 ($p < 0,05$). Faktorska analiza sastavnica prehrambenog upitnika potvrdila je da trudnice iz projekta CRIBS uglavnom slijede mediteranski način prehrane, no, suprotno rezultatima nekih drugih studija, ovo istraživanje nije potvrdilo povezanost mediteranske prehrane trudnica i pokazatelja veličine novorođenčadi.