

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

A pregnant woman's nutrition has an impact on her offspring's birth size. Although health benefits of Mediterranean diet are widely confirmed, the poor compliance with its recommendations has been detected in the population of Dalmatia, coastal region of Croatia. Data from 122 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 that is traditional in this area, and (2) to examine the association of maternal diet in pregnancy with newborns' birth weight, length and head circumference. The preliminary results of the factor analysis of 20 items from Food Frequency Questionnaire (FFQ) 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 consistent with the Mediterranean diet and Factor 2 had highest loading coefficients for fermented milk products (yogurt), sea food, cereals and legumes. Linear regression analyses resulted in model for weight at birth ($R^2=0.071$, $p<0.05$) which included as predictors living location (island or mainland) and FFQ Factor 2, which was also, in combination with moderate physical activity, predictive for newborns with z-scored weight at birth values above 15% of the lowest values in the logistic regression model. Pregnant women from the CRIBS study mostly follow Mediterranean diet but its association with weight at birth, although found in this research, has to be confirmed on a bigger sample.

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

Introduction

It is widely recognized that in industrialized countries the 1980s was a crucial period in the shift in diet toward increased intake of processed food and prepared meals, and greater consumption of cheap 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, and some types of cancer, etc.² The obesity pandemic is a problem not only in the United States and Europe, but also in low and middle income countries.³

The 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 Portu-

gal. The Mediterranean diet is characterized by the consumption of olive oil (preferably virgin oil), fruits, nuts, and whole grains daily, with moderate red wine and legumes consumption. Livestock products are consumed only occasionally, as a relish, while the priority is given to fish and white meat, over red and processed meat.⁴ The traditional diet, which includes home-prepared food made of homegrown fruits and vegetables, seafood and livestock products, is still present in smaller rural communities especially on the Dalmatian islands of Croatia.^{5,6} The population of Dalmatia has traditionally been living from agriculture and fisheries. Their diet can be seen as a variant of the Mediterranean diet, because it is mostly characterized by the consumption of brown bread, olive oil, fish, red wine, fruits and vegetables.⁷ Numerous investigations of the Mediterranean diet have confirmed its beneficial health effects on prevention of cardiovascular diseases and type 2 diabetes⁸, reversion of metabolic syndrome⁹ (MetS), and different types of cancer.¹⁰ Although traditions have historically shown strong vitality and resistance to cultural reduction and one-way development, the traditional diet of islanders is changing under the influence of modernization.^{7,11} These changes have been noted since the 1990s on the Dalmatian Islands of Hvar¹², Vis^{13,14}, and Korčula.¹⁴

Varied, balanced high quality food and adequate macro- and micronutrient intake in pregnancy are crucial for the health status of both the mother and child.¹⁵ In addition, taking into account the developmental origins of health and disease hypothesis¹⁶, nutrition in pregnancy might have long-term consequences on future generations. Recommendations for nutrients in pregnancy differ according to both the dietary tradition and nutritional status of the population.¹⁵ The traditional Mediterranean diet has shown a positive impact on maternal health during pregnancy¹⁷ and the health of children in early life^{18,19} in addition to contributing to conception in women undergoing assisted reproduction technology methods²⁰.

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. The analyses aimed: (1) to determine whether pregnant participants in the study consume a 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 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 the Croatian Dalmatian islands (Hvar and Brač) and mainland population (city of Split and its surroundings). This East-

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

ern Adriatic area has a high prevalence of the MetS, which is higher on the 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 goal of 500 mother-child pairs (expected to be achieved by October 2018). Comprehensive data on medical, anthropometric, socioeconomic, nutritional, lifestyle, psychological and other characteristics of both mothers and children, from pregnancy up to the children's second birthday (i.e. the first 1000 days), are collected. Pregnant women participated in the study voluntarily and signed informed consent for themselves at joining the research, and for their children, immediately after the birth.

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

The study sample and questionnaire measures

The sample analyzed here consists of 122 mother-newborn pairs from Split (N = 66) and islands of Brač and Hvar (N = 56). Initially, there were 131 mother-newborn pairs but nine preterm children were excluded from the

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	
alcoholic drinks that are not wine								0.896
pasta							0.747	

*Variables defining first two factors are highlighted in bold.

analyses. During pregnancy mothers filled out two specially designed questionnaires, to obtain information on maternal demographic, obstetrics, and lifestyle characteristics (diet included), well-being and mental state.

Assessment of dietary intake in pregnancy was determined using the Dietary adequacy assessment questionnaire for adults²¹ (DAAQA), the food frequency questionnaire adapted from the Harvard Semiquantitative Food Frequency Questionnaire²². 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 in PCA/FA should be no lower than five²³, 20 food variables (foods or food groups) characteristic for the Mediterranean diet were selected for the factor analysis (listed in Table 2). The first part of this questionnaire included questions about number of meals per day (1, 2, 3, 4 and 5 or more), number of times in a week when person eats breakfast,

brunch, lunch, snack, supper and late-night meal, and food preparation habits.

Only a subset of the data is analyzed here.

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. Anthropometric parameters of newborns were Z-score standardized according to the WHO referent values²⁴ and compared between sexes using a t-test. A multiple linear regression analyses were conducted for each of the significant FFQ factors as dependent and mothers' characteristics (age, employment status, level of education, living place and smoking) as independent variables. All these variables were further used as independent variables in the linear and the logistic regression analyses with the z-standardized weight at birth, height at birth and head circumference at birth as dependent variables. In logistic regression analyses, dependent variables were defined by grouping the 15% of newborns with the lowest body size z-scores vs. all the remaining newborns, and later by

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								
alcoholic drinks that are not 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 Chi square 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.

grouping the 15% of newborns with the highest z-scores and comparing them with the remaining 85% of newborns. Following the recommendations of commonly accepted guideline²⁵, only two independent variables were included in each of the regression models, one of which was always the significant FFQ factor. Size for gestational age could not be used as a dependent variable in the logistic regression analyses because of a low proportion of children small for gestational age (8.19%) and large for gestational age (8.19%) which thus did not meet criteria for minimal sample size.

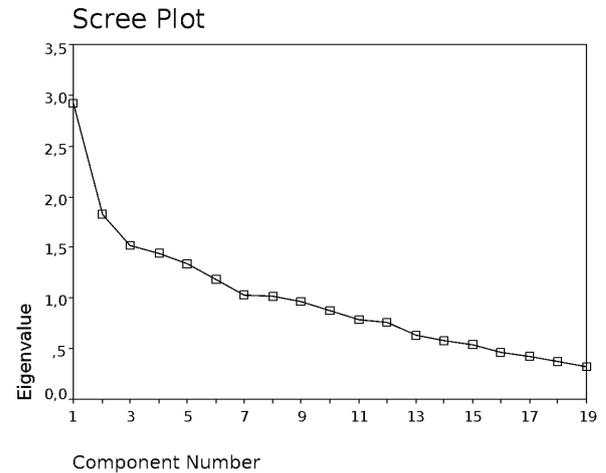


Fig. 1. Scree plot from the principal component analysis of the Food Frequency Questionnaire data with factor loading coefficient >0.20.

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 explained 15.354% of variance. Factor 2, which explained additional 9.630% of variance, had an eigenvalue of 1.830, and 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 in maternal age 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 as 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

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	0.5190±0.9153	0.5475±0.8504	ns
Length	0.7133±0.9503	0.7392±0.9245	ns
Head circumference	0.9228±0.8832	0.7477±0.7051	ns
BMI	0.2209±0.8928	0.2521±0.8513	ns
Weight-for-length	0.0229±0.8644	0.0138±0.8907	ns

*The differences between groups were tested by Student's t-test.

number of cigarettes per day since they found out they were pregnant. Most of the women reported being moderately active in pregnancy.

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 for length, head circumference and birth weight 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 at least 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.

Multiple linear regression analyses (backward method), conducted separately for each of the two significant factors and mothers' characteristics, showed that employment status (1=employed, 2=unemployed) and level of education (1=secondary school, 2=College or University degree) of pregnant women were predictive for the FFQ Factor 2 ($y = -0.122 + (0.298 \times \text{Education}) - (0.213 \times \text{Employment status})$; $F(2, 118) = 3.353$, $p<0.05$), with an R^2 of 0.054. The linear regression models for newborns' body size parameters (z-scores of weight, length and head circumference at birth) and two FFQ factors, which were tested separately in each model, resulted in regression model predictive for z-scored weight-at-birth which included Factor 2 and living place ($p<0.05$, Figure 2). The same FFQ Factor 2 and physical activity were predictive for weight at birth in the logistic regression analysis; newborns born to women with lower Factor 2 values and heavy-to-competitive physical activity status in pregnancy, were more likely to be in the lowest 15% of the CRIBS

sample according to z-standardized birth weight, in comparison with women with higher Factor 2 values (OR=1.834) and who were moderately physically active in pregnancy (OR=4.213) ($N=121$, $\chi^2=9.855$, $df=3$, $p=0.0198$; -2 Log Likelihood 91.919, Goodness of fit 141.715, Cox & Snell - R^2 0.078, Nagelkerke - R^2 0.138).

Discussion

Early-life factors influence fetal programming, birth and child health outcomes later in life.²⁶

Diet and lifestyle of mothers are important determinants of health of both mother and offspring, starting from the preconceptional period.²⁷ 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 diseases in adulthood.²⁸

Mediterranean diet in pregnancy has been found to affect favorably not only the risk of maternal hypertension/preeclampsia, but also the intrauterine growth and certain acute and chronic complications of prematurity.²⁹ 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 1990ies¹² and confirmed on Korčula¹⁴ 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.³⁰ 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

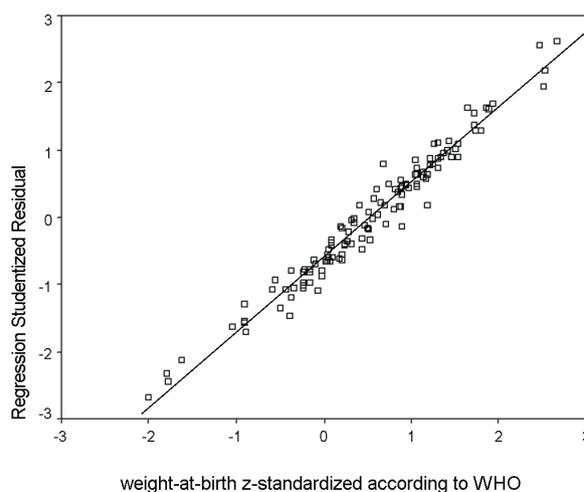


Fig. 2. Interrelation of quantitative values of FFQ Factor 2 and weight at birth z-standardized according to WHO recommendations. The linear regression model predictive for z-standardized weight at birth included Factor 2 and living place ($R^2=0.071$; $R=0.267$; $F=4.576$, $df1=2$, $df2=119$, $p=0.012$; $y_{\text{birth-weight}} = 1.003 + (0.161 \times \text{FFQ Factor 2}) - (0.323 \times \text{living place})$). Living place was binary variable in which 1=mainland and 2=island.

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

Prehrana tijekom trudnoće utječe 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 ovome radu analizirani su podaci 122 para majka-novorodenče, sudionika u projektu „Kohortna studija rođenih na istočnojadranskim otocima (CRIBS)“, kako bi se utvrdilo: (1) konzumiraju li trudnice u studiji mediteransku prehranu, koja je tradicionalna prehrana područja na kojemu žive, te (2) je li majčina prehrana tijekom trudnoće povezana 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 regresijska analiza rezultirala je modelom za težinu pri rođenju ($R^2 = 0,071$, $p < 0,05$) koji je kao prediktore uključivao mjesto stanovanja ispitanice (otok ili kopno) i FFQ Faktor 2, koji je također bio, u kombinaciji s umjerenom fizičkom aktivnošću, prediktivan i za novorođenčad sa z-vrijednostima iznad 15 % najnižih vrijednosti težine pri porodu u logističkom regresijskom modelu. Trudnice koje sudjeluju u projektu CRIBS uglavnom slijede mediteranski način prehrane, ali povezanost ovog tipa prehrane s porođajnom težinom, unatoč tome što je pronađena u ovom istraživanju, treba se dodatno potvrditi na većem uzorku.