

Insufficient choline intake during pregnancy: cohort study

Ingestão insuficiente de colina durante a gestação: estudo de coorte

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ABSTRACT

Objective

To investigate the intake of choline during pregnancy and associated factors.

Methods

Cohort study with 353 pregnant women recruited from the primary health care network in an inland city of the State of *São Paulo*. In-house interviews were conducted in each of the gestational trimesters. In each of these points in time, a 24-hour dietary recall was collected. Subsequently, a new dietary recall collection was performed by telephone in the same trimester on a non-consecutive day, differentiating weekday versus weekend/holiday. Dietary intake data were included in the Nutrition Data System for Research software, and the habitual food intake throughout pregnancy was determined, with intra-individual variation correction in the MSM software. The influence of socioeconomic, obstetric and lifestyle factors, and of the actual diet, on choline intake during pregnancy was assessed using linear regression models, that were developed with the Stata software version 14.2, at a significance level of 95%.

Results

Choline intake (281.1±68.6 milligrams) was below the recommended adequate intake, and only energy was positively associated with this micronutrient intake.

Conclusion

Choline intake in the population studied fell far short of current recommendations, and only higher energy intake was found as a factor associated with a higher intake.

Keywords: Choline. Food Consumption. Pregnant women. Prenatal nutrition.

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RESUMO

Objetivo

Investigar a ingestão de colina durante a gestação e fatores associados.

Métodos

Estudo de coorte com 353 gestantes recrutadas na rede de assistência primária à saúde de cidade paulista. Foram realizadas entrevistas presenciais, no domicílio, em cada um dos trimestres gestacionais. Em cada momento foi coletado um recordatório alimentar de 24 horas, seguido por nova coleta via telefone no mesmo trimestre, em dia não consecutivo, diferenciando dia de semana versus final de semana/feriado. Os dados de consumo alimentar foram incluídos no software Nutrition Data System for Research, sendo obtida a ingestão habitual, durante toda a gestação, com correção da variação intraindividual, no software MSM. A influência de fatores socioeconômicos, obstétricos, de estilo de vida e da própria dieta sobre a ingestão de colina na gestação foi avaliada por modelos de regressão linear, realizados no software Stata versão 14.2, ao nível de significância de 95%.

Resultados

A ingestão diária de colina (281,1±68,6 miligramas) mostrou-se abaixo do recomendado, sendo que apenas a energia mostrou-se como positivamente associada à ingestão desse micronutriente.

Conclusão

A ingestão de colina na população estudada ficou muito aquém das recomendações atuais, sendo que apenas a maior ingestão energética foi encontrada como fator associado à maior ingestão de colina.

Palavras-chave: *Colina. Ingestão de alimentos. Gestantes. Nutrição pré-natal.*

INTRODUCTION

Choline (also known as vitamin B8) was only acknowledged as an essential nutrient in 1998, after researchers reported liver dysfunction in men consuming food with very restricted content of this micronutrient, which was reversed with supplementary administration [1]. Further studies demonstrated that the endogenous pathway of choline production (PEMT- Phosphatidylethanolamine N-methyltransferase) is not enough to meet the needs of all individuals, men in particular. Women are less vulnerable to choline deficiency, due to the effect of estrogen that works favorably by stimulating the endogenous pathway PEMT [2].

However, during pregnancy, the protection provided by estrogen is not sufficient in view of the increased choline demand. The repercussions of a low choline intake during pregnancy have shown to be important, influencing the baby's brain development and the occurrence of birth defects [2,3]. The choline Recommended Daily Intake (RDI) during pregnancy is estimated at 450 milligrams [1].

Choline is a precursor of the phospholipids necessary for cell division, growth and proper functioning of the cell membrane receptors, brain development and neurotransmission [3,4]. Literature shows unfavorable effects due to low choline intake during pregnancy such as defects in the neural tube and impairment of brain development and its functions [3,5]. Still with regard to the fetus, choline can offer protection from problems, such as those resulting from maternal alcohol use [6]. *In vitro*, it has been shown that the lack of choline in pregnancy affects the development of the placenta by reducing angiogenesis [7]. At one year of age, children of mothers affected by common infections during pregnancy but with higher levels of gestational choline showed better development of behavioral self-regulation, closer to the level of children born out of mothers who suffered no infection [8].

Eggs, milk and meat can be considered good sources of choline, while foods of plant origin contain a very low amount of this micronutrient [9,10]. Eating daily two large eggs provide 65.3% of the current choline daily recommendation [11].

Despite the importance of this micronutrient intake during pregnancy, studies that have assessed choline intake during this period are rare. Regarding the choline dietary intake, American pregnant women evaluated in 2004-2005 ingested an average of 319 milligrams/day and only 8.51% of pregnant women had an intake higher than the RDI [12]. Among South African pregnant women, the average choline intake values were between 250 to 340mg/day [13]. To date, no investigation with Brazilian pregnant women has been published. This article presents the results of the evaluation of choline dietary intake in a cohort of Brazilian pregnant women and the investigation of the factors associated with such intake.

METHODS

Cohort study that used the data of an investigation called “Impact of intervention to promote leisure walking and healthy eating in pregnant women attended by the Family Health Strategy: a controlled cohort study”, carried out in 2013/14. The objective of this parent study was to evaluate the impact of walking practice during leisure and on the eating practices of pregnant women after a continuing education action implemented with those responsible for prenatal care in the primary health care network in the municipality of *Botucatu*, in the inland region of the State of *São Paulo*. This is a municipality located in the south-central region of the State of *São Paulo*, 235km from the capital, with a territorial area of 1,482,642km², which had, at the beginning of the data collection, approximately 130,201 inhabitants; the infant mortality rate was 4,512.20 deaths/100 thousand live births, HDI 0.800, with a population predominantly urban (93%).

Details of this parent study have been reported in a previous publication [14]. In summary, two groups of pregnant women were monitored: cohort A, composed of pregnant women attended at the nine Family Health Units (USFs) in the city of *Botucatu* and accompanied by doctors and nurses who participated in educational activities and cohort B, composed of pregnant women attended at the eight Basic Health Units (UBSs), monitored by doctors who did not participate in educational activities. The dietary practices addressed in the educational action consisted in promoting the consumption of three fruits daily, two servings of vegetables and beans in the two main meals, at least five days a week, and the incorporation of advice to avoid consumption of soft drinks or industrialized biscuits or consume them only sporadically. The promotion of physical activity included walking at leisure at least five days a week.

The present study followed the same inclusion criteria of the parent study (pregnant women enrolled in public prenatal care programs for primary health care in the municipality, between November 2012 and June 2013, aged over 18 and in the 1st gestational trimester) and the same exclusion criteria (twinning, presence of factors that would cause high risk in prenatal care), conducting only one *a posteriori* data analysis. The sample size designed for the original study was 280 pregnant women, 140 in each group/cohort, a sample size necessary to detect differences in the proportions of pregnant women who performed the recommended leisure walk in the third trimester of 5% to 15%, with a 5% significance level and 80% power. In addition, 15% were added for multiple statistical analyses and 10% for possible follow-up losses. The minimum sample size also allowed detecting changes in the diet from 40 to 50%.

Interviews were conducted, in each gestational trimester, by a previously trained staff, in each pregnant woman's home; in the 1st gestational trimester, 353 pregnant women were interviewed. Only 3.0% of pregnant women refused to continue participating in the study which, added to some follow-up losses (changes in the prenatal location, change to private health services or change to a reference hospital for high-risk pregnancies, moved to a different location and abortions), 281 pregnant women were assessed in the second trimester and 267 in the 3rd trimester (Figure 1).

In each face-to-face interview, data were collected about the course of pregnancy, highlighting the first interview that also included the collection of sociodemographic, lifestyle and obstetric data. The variables from this interview used in this study were: age (21 |- 34 years, 18 to 20 years, 35 |- years),

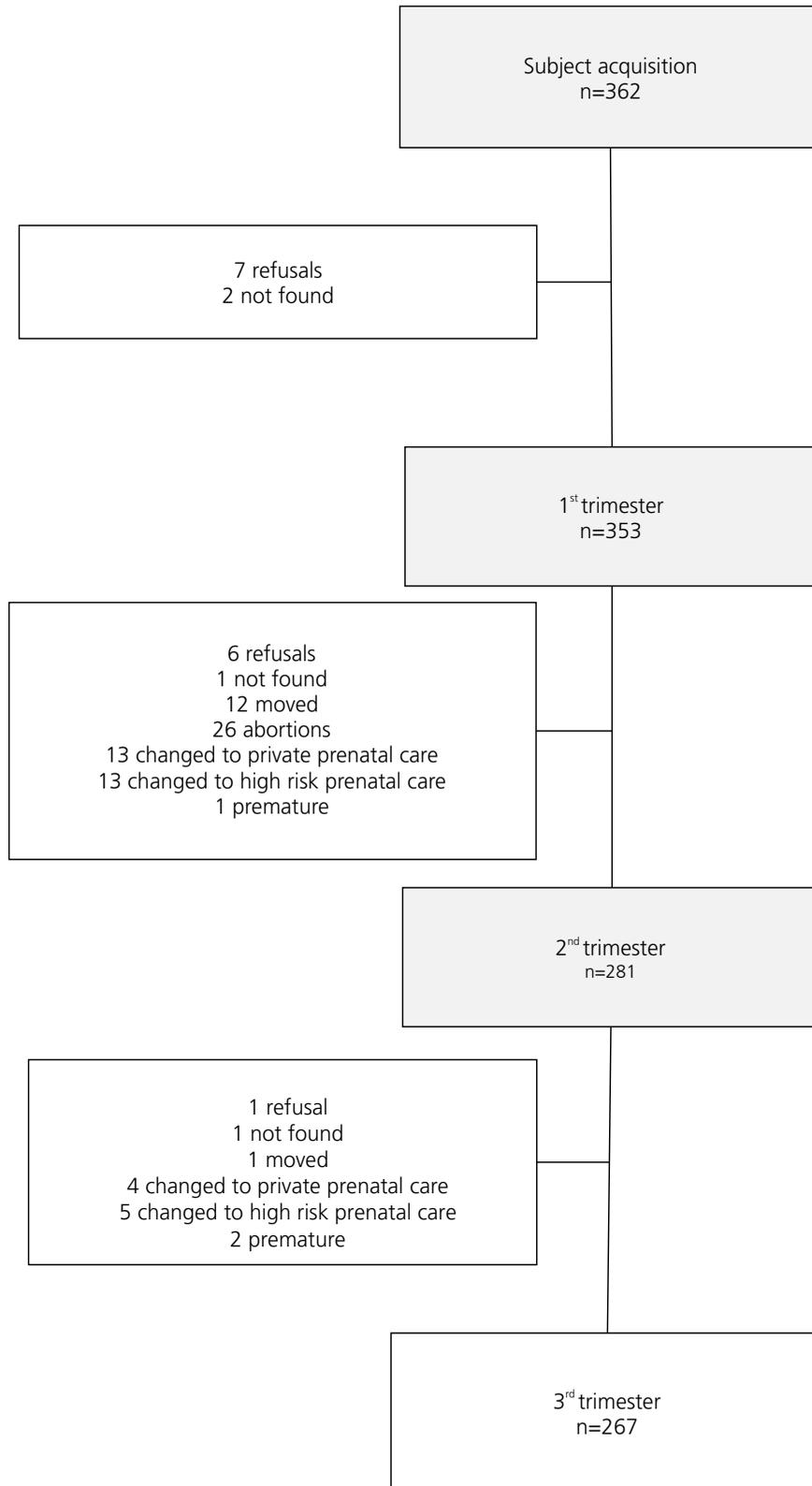


Figure 1 – Flowchart of pregnant women participating in the study. *Botucatu (SP), Brazil, 2012-2014.*

education (≥ 11 years of study, 8 | 11 years of study, < 8 years of study), socioeconomic classification (class B, class C, class D/E), working outside home (yes, no), living with a partner (yes no), parity (0, 1, 2 or more deliveries), self-reported skin color (white, not white), pre-gestational tobacco use (yes, no), pre-gestational alcohol use (yes, no) and walking during leisure (yes, no). The pre-gestational BMI variable was also used, which characterizes the nutritional status of women before pregnancy ;women were classified as low weight, normal weight (eutrophy), overweight or obesity [15]. For the composition of this variable, weight and height were obtained from the pregnant women's medical records in each health unit where each pregnant woman performed her prenatal care. Pre-gestational weight was considered the weight recorded before the woman became pregnant, or the first weight measured in the 1st gestational trimester, if ± 2.0 kg different from the pre-gestational weight.

The outcome variable of this study is the intake of dietary choline throughout pregnancy. To this end, the food consumption data originated from six 24-hour food recall (R24h), two of them applied in each trimester of pregnancy, the first one in the interview conducted at home and the second one, on a day not consecutive with the first one, by telephone. The R24h were applied using the Multiple Pass Method, which contributes to obtain a greater accuracy of the information collected [16]. All R24h foods and preparations were then standardized and quantified in grams or milliliters according to the Brazilian homemade measurement tables and, later, included in the Nutrition Data System for Research (NDSR) software, version 2010 [17]. Periodic consistency analyses were performed when entering data. R24h with energy intake higher than 6000kcal or lower than 600kcal were excluded and the choline intake was obtained using the actual NDSR software [18]. The usual choline intake during pregnancy for each pregnant woman, with the correction of intra-individual variability, was obtained with the MSM software.

Initially, descriptive analyses were performed with the exposure and outcome variables. The difference between the intake means according to the characteristics of the pregnant women was tested by ANOVA. The analyses of difference between the consumption averages in the different trimesters were tested by the paired t-test. The normality of the outcome variable was tested by the Kolmogorov-Sminorv test and by analyzing the intake distribution histogram. None of the practices addressed in the educational action was aimed at promoting choline sources, so that the pregnant women were grouped and considered as a single cohort in the analyses of this study. However, considering the hypothesis that eventually the pregnant women could differ in terms of choline intake due to the fact that they were assisted in prenatal care by professionals with different training in the promotion of healthy eating practices, the variable belonging to cohort A or B was inserted in the analyses, so that its potential role on choline intake could be controlled.

Linear regression analysis between choline intake and exposure variables were performed, with the initial adjustment by cohort, and those with $p < 0.25$ were included together and maintained in a multiple model. All analyses were performed using the Stata software version 14.2, considering $p < 0.05$ as the level of statistical significance.

At the time of the first home interview, all pregnant women were informed about the study and signed the free and informed consent form for their participation. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the *Botucatu* Medical School (CAAE: 32407314.0.0000.5411– Opinion number: 1.526.510).

RESULTS

The pregnant women assessed belonged mostly to the socioeconomic class C (66.6%), had 11 years or more (49.3%) education and were caucasian (64.3%). Almost half worked outside their home (48.2%) and about 74% lived with a partner. More than half of them were in their second pregnancy or further pregnancies (57.8%) and 47% had pre-pregnancy overweight. Choline intake differed only in relation to the parity of pregnant women (Table 1).

Table 1 – Choline intake (milligrams), according to socioeconomic, demographic, obstetric characteristics and nutritional status of pregnant women (n=353). Botucatu (SP), Brazil, 2012-2014.

Characteristics	Frequency		Intake (mg)		<i>p</i> ^d
	N	%	Mean	SD	
Age (years)					0.987
18 to 20	85	24.1	282.0	74.2	
21 - 34	238	67.4	280.9	65.0	
35 - and more	30	8.5	280.0	81.3	
Education (years of passing grade)					0.132
≥11 years	174	49.3	288.3	62.5	
8 - 11 years	102	28.9	271.7	73.1	
<8 years	77	21.8	277.3	74.5	
Socioeconomic classification ^a					0.352
B	34	9.9	278.6	61.8	
C	235	68.1	286.0	69.0	
D/E	76	22.0	273.3	69.0	
Works outside home					0.300
Yes	170	48.2	285.0	67.1	
No	183	51.8	277.4	69.9	
Lives with partner					0.375
Yes	261	73.9	279.8	67.4	
No	92	26.1	284.9	71.9	
Number of deliveries					0.032
0	149	42.2	286.0	69.0	
1	98	27.8	289.4	73.0	
2 or more deliveries	106	30.0	266.7	61.9	
White skin color ^b					0.814
Yes	225	64.3	282.8	69.3	
No	125	35.7	281.0	65.4	
Pre-gestational nutritional status ^c					0.491
Low weight	17	5.0	301.5	66.0	
Eutrophy	162	47.9	282.9	64.5	
Overweight	97	28.7	285.9	73.5	
Obesity	62	18.4	274.5	66.5	
Pre-gestational tobacco use					0.500
No	263	74.5	282.6	66.1	
Yes	90	25.5	276.9	75.6	
Pre-gestational alcohol use					0.945
No	217	61.5	280.9	70.1	
Yes	136	38.5	281.4	66.4	
Walks regularly during pregnancy					0.371
Yes	85	24.1	287.0	69.2	
No	268	75.9	279.3	68.4	

Note: ^aMissing information about lost purchasing power n=8; ^bAbsent information about skin color n=3; ^cMissing information about Body Mass Index n=15; ^dOne-Way ANOVA.

The average energy intake during pregnancy was 1876.5 (SD=416.1) kcal. The average choline intake was 281.1 (SD=68.6) mg, with a minimum intake of 84.0mg and a maximum intake of 533.0mg. Only five pregnant women showed consumption above the RDI (1.4%). Analyzing this consumption separately, pregnant women consumed, on average, 266.5 (SD=78.9) mg, 297.0 (SD=57.9) mg and 288.2 (SD=60.4) mg in the first, second and third gestational trimesters, respectively, and consumption was statistically different ($p<0.05$) between the first and second trimester and between the first and third trimester (data not shown in tables).

The main source of choline was beef, which provided 20.4% of the total choline present in all food recalls made with pregnant women throughout the gestational trimesters, followed by milk, chicken, legumes, eggs, pork, liver, fish, broccoli and kale (Table 2).

Crude regression analysis (with adjustment by the cohort) showed differences in choline intake according to total energy consumption ($p < 0.001$), with education and number of deliveries being potentially confounding factors ($0.25 > p > 0.05$). The results of the multiple analysis showed that only energy intake was associated with choline intake, and with each 1 kcal increment, choline intake increased 0.13mg (Table 3).

Table 2 – Main food sources of choline consumed by pregnant women. Botucatu, SP, Brasil, 2012-14.

Food	% ¹
Beef	20.4
Milk	10.3
Chicken meat	8.6
Legumes	7.3
Eggs	4.1
Pork meat	2.8
Liver	2.2
Fish	1.0
Broccoli and kale	0.5

Note: ¹In relation to the total choline consumed in all R24h.

Table 3 – Results of the crude and multiple linear regression analysis, between maternal characteristics and choline intake, during pregnancy.

Characteristics	Crude analysis ¹			Multiple analysis ¹		
	β	95% CI	p	β	95% CI	p
Total energy	0.13	0.12; 0.14	<0.001	0.13	0.12; 0.14	<0.001
Age (years)	0.17	-1.04; 1.39	0.773			
Education (years of passing grade)	1.60	-1.00; 4.19	0.227	0.66	-1.07; 2.40	0.453
Socioeconomic classification	-5.36	-18.54; 7.82	0.424			
Works outside home	-7.59	-21.97; 6.79	0.300			
Lives with partner	5.10	-11.28; 21.48	0.541			
Number of deliveries	-4.99	10.53; 0.55	0.077	1.07	-2.56; 4.69	0.564
White skin color	1.79	-16.70; 13.12	0.814			
Pre-gestational BMI	0.09	-1.20; 1.38	0.887			
Pre-gestational tobacco use	-5.66	-22.15; 10.83	0.500			
Pre-gestational alcohol use	0.52	-14.27; 15.30	0.945			
Walks regularly during pregnancy	-7.69	-24.56; 9.18	0.371			

Note: ¹Cohort adjustment.

DISCUSSION

According to the literature searches that we carried out, this is the first Brazilian study to report data on the intake of choline in pregnant women. The intake in the cohort studied in *Botucatu*, SP was lower (281.1mg) than the recommendation of 450mg/day and the percentage of pregnant women who met the RDI was extremely low (1.4%).

Among Canadian pregnant women, monitored during pregnancy and lactation, the average daily intake of choline during pregnancy was 337.8 mg, with 18% meeting the RDI [19]. Lower intake values, but still higher than those of Brazilian pregnant women, were found in American pregnant women, as already mentioned, with an average intake of 319mg and with 8.51% of pregnant women with an intake above the recommendation [12]. The choline intake range of pregnant women in South Africa, also previously

mentioned, is close to that of Brazilian pregnant women: between 250 and 340mg/day [13]. These results deserve the attention of professionals who assist pregnant women and of those who develop food and nutrition policies, since a high percentage of pregnant women do not meet the recommended intake, exposing themselves and their conceptions to many adverse events, such as those previously mentioned.

The situation of the pregnant women studied does not seem to be a particular feature of the place where the present study was conducted. Considering that studies in developed countries had already detected an inadequate situation for most of their pregnant women, a high prevalence of insufficient intake could be expected. The local and international picture may be even more serious, as the recommendation for choline intake during pregnancy is being challenged, with some investigators indicating that those recommendations are likely to be below the actual requirements, since doses well above the RDI have been associated with several beneficial effects [20].

The results of this study indicate that choline deficiency may be another relevant nutritional deficiency to affect the maternal and child populations in Brazil, although further studies are needed in other frameworks to provide evidence whether the conditions found in the location of this study are also equivalent in other country's locations. Among the nutritional deficiencies that are already acknowledged as being prevalent in the Brazilian maternal and child population are iron and folate deficiency, both of which are considered public health problems in Brazil and are included in individual and collective programs and interventions [21-23]. However, the low intake of choline during pregnancy does not appear as a public health problem or as a concern and there are no specific actions by the Brazilian health authorities to deal with it, a fact that is expected to be modified with the advent of new evidence.

In developed countries, where poor choline intake is already a concern of the health authorities, clinical trials have been conducted to assess the effectiveness of choline supplementation during pregnancy on obstetric, neonatal and childhood outcomes [20,24,25]. However, the way of coping with low choline intake using medication supplementation for pregnant women does not seem to be the best option for Brazil. Considering that the food route is viable and of relatively low cost, recommendations for the daily intake of food choline would be an action more in line with the National Food and Nutrition Policy and the Dietary Guidelines for the Brazilian Population [26].

On the other hand, the results found, revealed only a positive association between energy intake and choline intake. However, encouraging energy intake increase as a way to meet choline recommendations does not seem to be an appropriate strategy. Energy intake is positively associated with gestational weight gain [15] and the prevalence of excessive weight gain is increasing in Brazil and worldwide, being associated with adverse maternal and child health outcomes, such as increased risk for diabetes and gestational hypertension, big babies for their gestational age, macrosomia, cesarean delivery and higher postpartum weight retention rates [15,27].

Thus, it is necessary to think about stimulating consumption of foods that are sources of choline, especially those with less energy input. As already pointed out, the consumption of two eggs already exceeds half of the choline RDI; it is a food that does not have a high energy density if prepared without oils/fats [11,28]. An American study found that adults over 19 who consumed eggs were more likely to reach adequate choline RDI compared to non-consumers. Egg consumers ingested almost twice the usual choline amounts compared to non-consumers [12]. Another significant food source is liver; however, this food contains a high amount of vitamin A, which makes its daily intake inadequate due to the risk of exceeding the maximum limit of vitamin A tolerance [28].

Stimulating consumption of eggs as a way to improve the nutrition of children and mothers in low and middle income countries has been advocated by researchers in recent articles [11,29]. For these authors, eggs advantage over drug supplementation derives from the widespread recognition and acceptance of eggs as a human food and its high nutritional value, capable of providing, in addition to choline, other

important nutrients such as high-quality proteins, essential fatty acids, cholesterol, vitamin A and carotenoids, among other ingredients. For pregnant women, in addition to providing more than half of the daily choline requirements, two eggs provide 1.7% of protein and between 20 to 35% of vitamins A, riboflavin, vitamin B12, pantothenic acid and phosphorus [28]. However, in the cohort assessed in this study, the eggs ranked third as source of choline, behind meat (1st) and milk and milk products (2nd). It is worth pointing out that this same population already had the dietary patterns investigated during pregnancy and in none of the three identified patterns eggs were present [30].

From a cultural point of view, the widespread concern with eggs' high cholesterol content and their potential cause of food poisoning, when undercooked, may cause pregnant women and health professionals suppose that it is not a healthy and safe food in this life stage. However, such claims are at odds with current Brazilian recommendations and that of many scholars of maternal and child nutrition [11,26]. In addition, the consumption of eggs was inversely associated with the consumption of ultra-processed foods [31]. There is also an association with the egg being a simpler, normal and day-to-day food, such as rice-beans-egg-steak, a fact that also has to be considered when implementing the guidelines [32].

The validity of the results of this study is supported by the fact that six 24-hour food recall surveys were carried out during pregnancy and the adoption of the Multiple pass method technique, accepted as the best way to minimize the survey's respondents' memory's failures [16]. However, it is important to consider that the cohort was made up of pregnant women, users of the public network of the primary health care units and that, although they correspond to about 70% of the population in the locality, care should be taken in extrapolating the results to the population as a whole, even though a large portion of Brazilian pregnant women undergo prenatal care in the public health system [33]. Studies in other geographic, cultural and socioeconomic contexts are necessary so that the national scenario regarding the intake of choline in food by pregnant women be better known and, that actions to promote choline intake be outlined.

One can also consider as a limitation of this study the use of international tables to assess the consumption of Brazilian pregnant women. However, the software involved is one of the most used in the world in food consumption analyses; in addition, there is a lack of choline quantification in national tables.

CONCLUSION

Choline intake among Brazilian pregnant women who are assisted by the public primary health care network was well below the recommendations. Given the important role of choline during pregnancy, stimulating the consumption of food sources of this micronutrient is a relevant strategy in prenatal care.

CONTRIBUTORS

CB Gomes and MABL Carvalhaes participated in the conception and design, analysis and interpretation of data, writing, revision and approval of the final version of the article.

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