

## Dietary Intake of Choline and Plasma Choline Concentrations in Pregnant Women in Jamaica

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### ABSTRACT

*Choline is an essential nutrient for humans and its availability during pregnancy is important for optimal fetal development. The Food and Nutrition Board of the Institute of Medicine in the United States of America has set the adequate choline intake during pregnancy at 450 mg/day. There is limited data available on normal plasma choline concentrations in pregnancy. Moreover, there are neither documented studies of choline intake among pregnant women in the Jamaican population nor of free plasma choline concentrations during pregnancy. Sixteen women presenting to the antenatal clinic of the University Hospital of the West Indies (UHWI) at 10–15 weeks of gestation were selected for this pilot study. A food frequency questionnaire was administered to estimate frequency of consumption of foods rich in choline. Fasting blood samples were collected by venepuncture and plasma assayed for choline using liquid chromatography electrospray ionization isotopic dilution mass spectrometry. Most of the women reported consumption of diets that delivered less than the recommended choline intake (mean  $\pm$  SEM, 278.5  $\pm$  28.9 mg). Mean plasma choline concentration was 8.4  $\pm$  0.4  $\mu$ mol/L. This falls below the normal concentration (10  $\mu$ mol/L) reported for individuals that are not pregnant and pregnant (14.5  $\mu$ mol/L). The results of this study may be an indication that the choline included in the diet of pregnant women in Jamaica may not be adequate to meet both the needs of the mother and fetus and that further studies are warranted to determine clinical implications.*

## La Ingestión de Colina en la Dieta y las Concentraciones de Colina en Plasma en las Mujeres Embarazadas en Jamaica

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### RESUMEN

*La colina es un nutriente esencial para los seres humanos y su disponibilidad durante el embarazo es importante para el óptimo desarrollo del feto. La Junta de Alimentos y Nutrición (Food and Nutrition Board) del Instituto de Medicina de los Estados Unidos ha establecido que la ingestión de colina durante el embarazo debe ser 450 mg/día. Los datos disponibles sobre concentraciones de colina en plasma durante el embarazo son limitados. Por otro lado, no existen estudios documentados sobre la ingestión de colina entre las mujeres embarazadas en la población de Jamaica, ni sobre las concentraciones libres de colina en plasma durante el embarazo. Dieciséis mujeres que se presentaron a la clínica de atención prenatal del Hospital Universitario de West Indies entre las 10–15 semanas de gestación, fueron seleccionadas para este estudio piloto. Se aplicó un cuestionario de frecuencia alimentaria a fin de estimar la frecuencia de consumo de alimentos ricos en colina. Se recogieron muestras de sangre en ayunas mediante venopuntura, y se sometió el plasma a análisis en busca de colina, usando la espectrometría de masa de dilución isotópica, ionización por electrospray y cromatografía líquida. La mayoría de las mujeres reportaron consumo de dietas que suministraban*

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menos de los niveles de ingestión de colina recomendados (media  $\pm$  SEM, 278.5  $\pm$  28.9 mg). La concentración media de colina en plasma fue 8.4  $\pm$  0.4  $\mu$ moles/L. Esto se halla por debajo de la concentración normal (10  $\mu$ moles/L) reportado tanto para no embarazadas como para embarazadas (14.5  $\mu$ moles/L). Concluimos que los resultados de este estudio pueden ser una indicación de que los niveles de colina incluidos en la dieta de las mujeres en estado de gestación en Jamaica no son adecuados para satisfacer las necesidades ni de la madre ni del feto, y que vale la pena la realización de estudios ulteriores al objeto de determinar las implicaciones clínicas.

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## INTRODUCTION

Choline is a nutrient that is a precursor of the phosphatidylcholine and sphingomyelin, both major components of cell membranes and important in the proper triglyceride turnover from the liver and blood. In addition, it is an important methyl-group donor needed for homocysteine metabolism and is used to make acetylcholine, a neurotransmitter (1).

This nutrient is especially important during pregnancy when mother actively transports it to the fetus (2). In rodent models, optimal development of the brain is directly influenced by the availability of choline during gestation, and can influence lifelong memory function (3–7). Choline is synthesized in the liver through the action of phosphatidylethanolamine methyltransferase (PEMT) (8).

Endogenous biosynthesis is not adequate to meet the demands for choline in humans and diets deficient in choline cause fatty liver and liver cell death (9, 10). In the United States of America (USA), the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences has set an adequate intake level for choline at 550 mg/day for males, 425 mg/day for non-pregnant females and 450 mg/day for pregnant women (9).

Dietary choline is supplied in the form of free choline, phosphatidylcholine (lecithin), phosphocholine, sphingomyelin and glycerolphosphocholine (11, 12). These choline derivatives can all be hydrolysed to produce choline. Some of the richest sources of choline include eggs, liver, chicken, fish, legumes, cruciferous vegetables, milk and soya products (11, 12). Also, betaine in the diet can provide methyl groups (like choline), but cannot be converted to choline to form phospholipids or acetylcholine (11) (Fig. 1). Many plant-based foods contain betaine (11, 12).

Pregnancy is a time when the dietary choline supply may not match the increased demand for choline (2) and reserves of choline are depleted. One study reported that choline concentrations in serum are higher in pregnant women than in non-pregnant women (14.5  $\mu$ mol/L vs 10.7  $\mu$ mol/L, non-fasted) (13). The reason for this is unknown, but it could be related to increased dietary intake or increased endogenous synthesis.

Considering the importance of choline to overall fetal development and the lack of information available on maternal choline levels, the aim in this pilot study was to examine the plasma choline concentrations in a group of

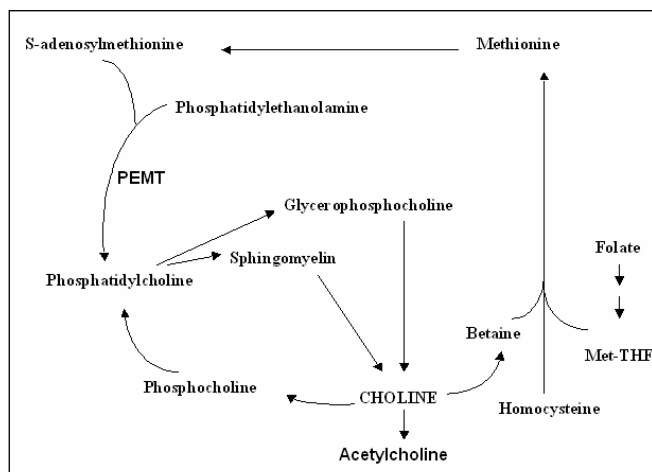


Fig. 1: Diagram showing the functions of choline in the blood and liver.

pregnant women in Jamaica and to assess their dietary choline intake.

## SUBJECTS AND METHOD

### Recruitment of subjects

Sixteen women at 10–15 weeks of gestation who attended the antenatal clinic of the University Hospital of the West Indies (UHWI), Jamaica, were recruited in this pilot study. It was decided to look only at women with normal pregnancies and therefore persons with a history of hypertension, diabetes or spontaneous abortions were excluded. The women were asked to identify their ethnicity. All participants reported compliance with standard iron and folic acid supplementation. This study was approved by the University Hospital of the West Indies/University of The West Indies Faculty of Medical Sciences Ethical Committee and the Institutional Review Board of the University of North Carolina at Chapel Hill, USA. All participants signed an informed consent form.

### Assessment of choline intake

Choline content of foods was calculated using published data (<http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html>). A food frequency questionnaire for consumption of the foods listed in Table 1 was filled out by each subject. The foods in this questionnaire were selected because they are common in Jamaica and represent high choline sources.

Table 1: Selected foods from the Jamaican diet known to be rich sources of choline. Total choline consisted of glycerolphosphocholine (GPCho), phosphocholine (Pcho) phosphatidylcholine (PtdCho) and sphingomyelin (SM). Betaine is a metabolite produced from the demethylation of choline

USDA code	Food	Free Choline	GPCho	PCho	PtdCho	SM LPC	Total Choline mg/100g	Betaine mg/100g
01129	Egg	0.7	0.5	0.5	209.9	13.6	225.2	0.6
13327	Liver	56.7	77.9	11.8	247.8	24.1	418.3	5.6
16087	Peanut	17.6	1.3	1.8	31.8	0	52.5	0.6
16098	Peanut butter	25.8	1.1	0.7	38	0	65.6	0.4
01077	Milk	3.7	7.5	1.8	0.6	0.6	141.2	0.6
43261	Yogurt	3.3	7.8	2.0	1.9	1.4	16.4	0.7
01009	Cheddar Cheese	1.6	2.3	0.6	7.4	4.6	16.5	0.7
13497	Beef	1.9	1.6	0.2	64.5	9.1	77.3	12.8
10042	Pork	2.2	22.5	1.2	70.5	6.4	102.8	1.6
05006	Chicken	6	1	3.6	40.6	8.5	59.7	7.8
98016	Fish	21.4	1.2	2.5	53.7	4.1	82.9	25.3
16028	Peas/Kidney Beans	14.2	2.2	0.6	13.5	0	30.5	0.1
16120	Soy Milk	13.1	1.3	3.4	5.7	0	23.5	0.8
11091	Broccoli	8.5	1.3	9.3	21	0	40.1	0.1
11252	Lettuce	4.8	0	1.5	0.4	0	6.7	0.1
18064	Wheat Bread	11.5	3.7	0.3	3.1	0	18.6	85.2

### Plasma collection

Subjects were required to be fasted overnight. Blood samples were collected by venepuncture at 10–15 weeks gestation. By this gestational period none of the subjects reported vomiting. The blood samples were centrifuged immediately for the collection of plasma and stored at  $-80^{\circ}\text{C}$  until time for analysis, which was carried out at the University of North Carolina at Chapel Hill, USA.

### Analysis of plasma

Choline and choline metabolites were extracted and analyzed using standardized methods described by Koc *et al* (14). Briefly, lipids were extracted from plasma (200  $\mu\text{l}$ ) with methanol:chloroform (2:1, by vol), vortexed vigorously and left at  $-20^{\circ}\text{C}$  overnight. Samples were then centrifuged and the supernatant collected. The residue was re-extracted with methanol: chloroform: water (2:1:0.8, by vol). The supernatant from both extractions were combined. Chloroform (200  $\mu\text{l}$ ) and water (200  $\mu\text{l}$ ) were added to the supernatant to separate an aqueous and an organic phase. Quantification was done using liquid chromatography electrospray ionization isotopic dilution mass spectrometry. All samples were done in duplicates and final values reflect the average of these two readings.

## RESULTS

### Subject profile

All the women in this study were Afro-Caribbean (based on self-assessment) in ethnicity and had parity in the range of one to four (Table 2). Five subjects reported a history of

chronic illness (asthma or manic depression) with and without pharmacological management.

### Dietary choline consumption and plasma choline concentration

Most of the participants reported low consumption of the choline-rich foods selected from the Jamaican diet (Fig. 2). Two of the subjects showed adequate or close to adequate intake of choline (over 400 mg) from the consumption of known choline-rich foods. The mean ( $\pm$  SEM) dietary choline consumed was 278.5 mg  $\pm$  28.9 mg.

The fasting plasma choline ranged between 6.8 to 11.2  $\mu\text{mol/L}$  (Fig. 3) with a mean ( $\pm$  SEM) plasma choline concentration of 8.4  $\pm$  0.4  $\mu\text{mol/L}$ . In further analysis of the plasma choline concentrations, it was observed that subjects with a history of chronic illness, with or without being on medication showed significantly (student's *t* test,  $p < 0.01$ ) higher plasma choline concentrations (9.7  $\pm$  1.7  $\mu\text{mol/L}$ ,  $n = 5$ ) compared to those not having chronic illnesses (7.7  $\pm$  0.9  $\mu\text{mol/L}$ ,  $n = 11$ ).

## DISCUSSION

During pregnancy, large amounts of choline are delivered across the placenta for fetal development and maternal stores are depleted in rodent models (2). This suggests that dietary intake of choline is likely to be very important during pregnancy to ensure that normal plasma choline concentrations are maintained. In this study, an assessment of dietary choline intake was made using a food frequency questionnaire consisting of foods that are rich sources of choline.

Table 2: Profile of subjects (n = 16)

Subject Code	Age Yr	weight/ 12 wks gestation Kg	height cm	parity	BP(12–16 wks) mmHg	Hb g/dL	Comments
PL001	25	66.2	173.5	2	100/60	11.4	bi-polar disorder, medication included lithium
PL002	24	97.6	164	1	100/80	11.3	
PL003	29	66.5	159.5	2	90/60	11.2	asthmatic/ventolin
PL004	28	60.7	175.3	1	100/70	11.4	
PL005	29	72.4	159	2	120/80	11.9	Asthmatic
PL006	28	72.8	170	2	120/70	12.2	
PL007	18	55.6	166.5	1	100/60	12.3	Asthmatic
PL008	20	48.3	162	1	100/60	18.4	Asthmatic/spina bifida
PL009	34	67.6	160.5	2	100/70	11.4	
PL010	38	91.7	173	4	120/70	13.1	
PL011	29	92.1	178	1	100/60	11.8	
PL012	20	62.2	164.7	1	100/90	12.2	childhood chickenpox
PL013	21	97.6	154	2	120/70	10.6	shellfish allergy
PL014	32	66.9	171	1	114/70	9.8	
PL016	24	76.34	155.2	1	120/80	11.8	
PL017	18	70.9	165.2	1	120/70	12	

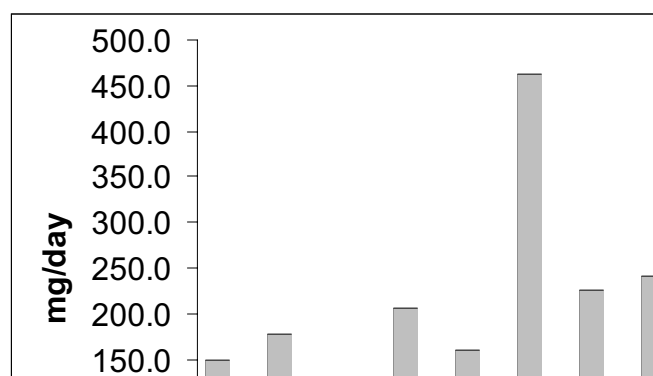


Fig. 2: Graph showing daily consumption of choline and betaine for each subject. Choline bar represents free choline, phosphatidylcholine, phosphocholine, sphingomyelin and glycerolphosphocholine content. Most of the women reported a diet that provided less than 450 mg of choline (adequate allowance of choline in pregnancy).

Although food frequency questionnaires are of limited accuracy, they have been validated as adequate for assessment of single micronutrient intake (15). In this study, there were however further limitations associated with an incomplete database for the choline content of many foods, as well as whether the values of choline in foods listed on the United States Department of Agriculture database are accurate for Jamaican foods.

The pilot study suggests that pregnant women in Jamaica are eating foods that may not be delivering adequate amounts of choline. Although the implications of inadequate choline in the diet have not been fully examined in humans, several animal studies show that in terms of memory development, the role of dietary choline during pregnancy is significant (3–7).

Normal plasma choline concentrations in fasted non-pregnant humans vary from 7–20  $\mu\text{mol/L}$  (with most

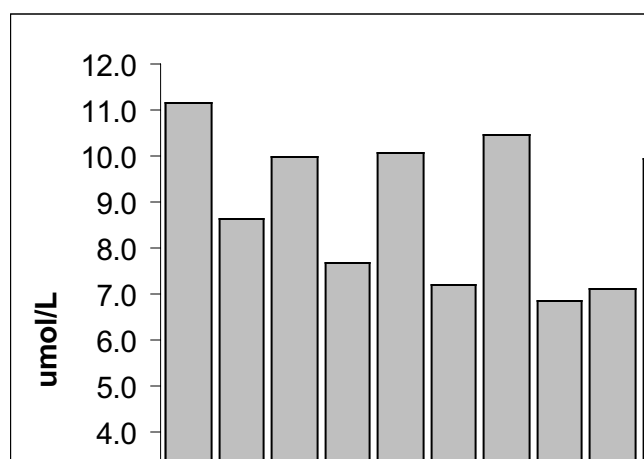


Fig 3: Graph showing the plasma choline concentration measured at 10–15 weeks of gestation for each participant. Plasma choline concentrations tended towards low to normal values (normal fasted plasma choline range from 7–20  $\mu\text{mol/L}$  (9,15) with most subjects having low concentrations.

individuals having 10  $\mu\text{mol/L}$ ) (9, 16) and are controlled not only by the supply (endogenous and dietary), but also by the ability of all tissues to accumulate choline (17). The uptake and storage of choline by the liver is integral in this control. Choline is also significantly taken across the blood-brain barrier through a carrier mediated mechanism, the rate of which is positively dependent on plasma choline concentrations (17). Additionally, hormonal influences, namely cortisol, insulin and prolactin participate in maintaining plasma choline concentrations (18). Previous reports suggest that pregnant women have increased plasma choline concentration (14.5  $\mu\text{mol/L}$ ) when compared with non-pregnant women (13). Reasons for this elevation have not been fully determined, but occur, possibly to meet the increasing de-

mands of the fetus including the increased accumulation in breast milk (19).

The authors found that pregnant women in Jamaica have low to normal plasma choline concentrations during the first trimester. The trend of more normal plasma choline concentrations in subjects with chronic illness was an interesting finding and although the observation was limited by numbers (and type of illness), the power obtained (0.78 at  $p = 0.05$ ) from this pilot study suggest that future work should examine this association.

In conclusion, this is the first documented study evaluating plasma choline concentrations in the Jamaican population and suggests a need to assess further whether diets of this population ensure adequate plasma choline concentration during pregnancy. An extension of this study should also examine the implications of low plasma choline concentration including the significance it may have in ensuring healthy fetal brain development in humans.

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