Manuscript Title: The role of fresh beef intake and Mediterranean diet adherence during pregnancy on maternal and infant health outcomes

Author List: Christifano DN, Gustafson KM, Carlson SE, Mathis NB, Brown A, Onuoha O, Taylor MK

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Study Headline: Pregnant women who eat about 1.5 ounces of beef per day as part of a Mediterranean-style dietary pattern are less likely to develop maternal anemia. Additionally, pregnant women who eat beef have higher intakes of nutrients critical to maternal and child health, including protein, iron, zinc, choline, and B vitamins.

Background: During pregnancy a woman experiences significantly increased nutrient requirements to meet the demands for optimal fetal development, while also maintaining maternal health; however, women often receive limited information or recommendations about nutrition from medical professionals during this key stage. Previous research has demonstrated that pregnant women are generally at a greater risk of nutrient inadequacy. Many nutrients of concern among pregnant women are provided by beef, such as protein, iron, vitamin B12, vitamin B6, choline and zinc,[1] indicating beef consumption may help meet nutrient needs in this population.

Dietary recommendations during pregnancy encourage choosing a variety of nutrient-dense foods including proteins, fruits, vegetables, whole grains and dairy to meet increased energy and nutrient needs. Many nutrient requirements drastically increase for women during pregnancy to meet the needs of the developing fetus. For example, iron and protein requirements increase 1.5-fold, from 18 mg/day to 27 mg/day for iron, and from 46 g/day to 71 g/day for protein. Iron deficiency is a major concern in all women of reproductive age, with 18% of pregnant women categorized as iron deficient,[2] which can lead to numerous negative health outcomes for both the mother and child. Proper iron intake –particularly heme-iron, the most bioavailable form -- is important to prevent anemia, though iron deficiency is not the only cause of anemia. Beef is a good source of iron, and predominantly heme-iron.

It is important for pregnant women to follow a healthy dietary pattern to meet nutritional needs, such as a Mediterranean dietary pattern. While dietary guidance for the Mediterranean diet generally limits red meat intake, like beef, this may not reflect a true healthful Mediterranean diet, as research shows red meat intake in many Mediterranean countries is higher than in the United States. The current study was conducted to gain insight into beef intake during pregnancy in the

[1] Agarwal S, Fulgoni VL 3rd. Contribution of Beef to Key Nutrient Intakes and Nutrient Adequacy in Pregnant and Lactating Women: NHANES 2011-2018 Analysis. Nutrients 2024;16(7):981.
[2] Mei Z, et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. Am J Clin Nutr 2011;93(6):1312-20.

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context of Mediterranean diet adherence and whether there are associations with improved nutrient intake and maternal and infant health outcomes.

Objective: The objectives are: 1) assess the relationship between fresh beef intake and macro- and micronutrient intake in a pregnant population; and 2) assess the relationship between maternal fresh beef consumption among varying degrees of Mediterranean diet adherence and maternal risk of anemia and infant health outcomes.

Study Design: This study was a secondary analysis of data from pregnant women (n=838) who participated in one of two randomized clinical trials examining the effect of docosahexaenoic acid (DHA, an omega-3 fatty acid) supplementation on rates of early preterm birth or offspring neurodevelopment outcomes. Pregnant women, enrolled between 12-20 weeks of gestation, were followed throughout their pregnancies with numerous measurements collected, including maternal characteristics (e.g. age, height, weight, hemoglobin levels), infant characteristics (e.g. birthweight, length, head circumference), maternal dietary recall (via food frequency questionnaire) and maternal supplement intake.

The researchers calculated maternal fresh beef consumption (including ground beef, steaks and roasts) from food frequency questionnaires and measured their adherence to a Mediterranean diet using a scoring system, the Mediterranean diet (MedD) score. The 18-point MedD score awards 2 points for avoidance of red meat (e.g., beef). To better understand the potential for positive outcomes from a Mediterranean diet *with* beef the researchers eliminated this criterion and modified the MedD scoring system to have a maximum 16-point. Researchers then assessed the association between fresh beef consumption, divided into tertiles, and adherence to the Mediterranean diet (low, medium and high MedD scores).

Key Findings:

- Mean total beef consumption for women in the study was 0.8 ± 0.7 oz/day. Tertiles based on beef consumption were established:
    - Tertile 1 = 0.2 ± 0.1 oz/day
    - Tertile 2 = 0.6 ± 0.1 oz/day
    - Tertile 3 = 1.5 ± 0.7 oz/day
- Women in the highest tertile of beef consumption (Tertile 3) had significantly higher intakes of energy, protein, saturated fat, monounsaturated fat, polyunsaturated fat, and carbohydrates, compared to the medium and lower tertiles (Tertiles 1 and 2).
- Furthermore, women in Tertile 3 had statistically significant higher intakes of 17 micronutrients: vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, choline, calcium, phosphorus, magnesium, iron, zinc, copper, selenium, sodium, and potassium (see

Table 1) compared to Tertiles 1 and 2, many micronutrients commonly deficient among pregnant women.

- Increased beef intake can improve nutrient intake for key nutrients of public health relevance for pregnant women including protein, iron, zinc, choline, and B vitamins.
- Women in Tertile 3 were the only group to meet the recommended daily allowance (RDA) of 71 grams of protein per day during pregnancy.
- Women in Tertile 3 consumed significantly higher amounts of all nutrients except for vitamins C and K (see Table 1) compared to Tertile 1 and 2.

Table 1. Nutrient intake by tertile of beef consumption.

	Tertile1:	Tertile2:	Tertile3:	RDA for pregnancy
Total Fresh Beef(oz/day)	0.2 ± 0.1[b]	0.6 ± 0.1[b]	1.5 ± 0.7[a]	
	(n =280)	(n =279)	(n =279)	
Energy and Macronutrients				
Energy (kcal/d)	1803.6 ± 712.5[b]	1842.3 ± 653.9[b]	2343.4 ± 767.6[a]	
Total fat(g/d)	66.9 ± 28.7[b]	76.3 ± 27.4[b]	94.2 ± 33.2[a]	
Carbohydrates(g/d)	243.9 ± 143.5[b]	222.1 ± 110.3[b]	288.9 ± 132.2[a]	175
Protein(g/d)	59.5 ± 25.3[b]	67.9 ± 23.7[b]	87.5 ± 29.0[a]	71
Saturated fat(g/day)	21.8 ± 10.2[b]	25.2 ± 9.8[b]	32.4 ± 12.7[a]	
Monounsaturated fat(g/d)	25.5 ± 11.4[b]	29.5 ± 11.0[b]	35.8 ± 12.8[a]	
Polyunsaturated fat(g/d)	13.7 ± 6.9[b]	14.9 ± 6.6[b]	17.7 ± 7.0[a]	
Dietary fiber(g/d)	16.7 ± 8.3[b]	17.3 ± 8.2[b]	19.2 ± 8.5[a]	
Micronutrients				
Retinol(mcg/d)	436.3 ± 288.0[b]	441.7 ± 235.3[b]	526.5 ± 252.0[a]	770
Vitamin E(mg/d)	8.7 ± 4.2[b]	9.1 ± 4.3[b]	9.9 ± 3.9[a]	
Vitamin K(mcg/d)	208.3 ± 224.6	194.8 ± 202.1	198.2 ± 222.9	
Vitamin C(mg/d)	152.3 ± 171.4	123.4 ± 108.0	147.9 ± 106.8	85
Thiamin(B1)(mg/d)	1.4 ± 0.6[b]	1.4 ± 0.6[b]	1.7 ± 0.6[a]	
Riboflavin(B2)(mg/d)	2.2 ± 1.1[b]	2.2 ± 0.9[b]	2.6 ± 1.2[a]	1.4
Niacin(mg/d)	19.9 ± 12.0[b]	21.0 ± 9.1[b]	27.1 ± 14.4[a]	18
VitaminB6(mg/d)	2.1 ± 1.3[b]	2.0 ± 1.0[b]	2.6 ± 1.5[a]	1.9
Folate(mcg/d)	410.6 ± 197.7[b]	407.8 ± 180.6[b]	458.6 ± 180.8[a]	600
VitaminB12(mg/d)	4.4 ± 2.9[b]	4.6 ± 2.3[b]	6.4 ± 3.4[a]	2.6
Calcium(mg/d)	1112.9 ± 613.2[b]	1079.3 ± 536.8[b]	1267.6 ± 546.9[a]	1000

Phosphorous(mg/d)	1127.3 ± 477.3 [b]	1205.5 ± 471.3 [b]	1482.0 ± 513.1 [a]	
Magnesium(mg/d)	311.3 ± 120.3 [b]	321.2 ± 128.5 [b]	356.8 ± 125.2 [a]	350
Iron(mg/d)	13.0 ± 5.9 [b]	13.4 ± 5.6 [b]	16.4 ± 5.8 [a]	27
Zinc(mg/d)	9.7 ± 4.2 [b]	10.8 ± 4.1 [b]	13.9 ± 4.4 [a]	11
Copper(mg/d)	1.4 ± 0.6 [b]	1.5 ± 0.6 [b]	1.7 ± 0.6 [a]	
Selenium(mcg/d)	78.9 ± 36.4 [b]	89.9 ± 33.1 [b]	112.3 ± 39.2 [a]	60
Sodium(mg/d)	2569.4 ± 1061.7 [b]	2841.7 ± 966.4 [b]	3621.5 ± 1172.6 [a]	1500
Potassium(mg/d)	2740.3 ± 1162.6 [b]	2768.8 ± 1095.4 [b]	3291.9 ± 1231.2 [a]	4700
Choline(mg/d)	274.8 ± 125.0 [b]	299.4 ± 106.8 [b]	377.7 ± 132.7 [a]	550

Note: statistically significant differences between [a] and [b]

- Overall, adherence to the Mediterranean diet was low. Mediterranean diet adherence was measured out of a maximum possible score of 16. The MedD scores were as follows:
  - Low adherence: 2.1 ± 0.8
  - Medium adherence: 4.0 ± 0.7
  - High adherence: 6.4 ± 1.2 ($p < 0.001$).
- Mean intakes of fresh beef across MedD adherence groups were not significantly different, with daily intake for low MedD adherence: 0.8 ± 0.7 oz., medium MedD adherence: 0.7 ± 0.6 oz., and high MedD adherence: 0.7 ± 0.7 oz ($p = 0.37$).
- Neither beef intake nor adherence to the Mediterranean diet independently impacted maternal or infant health outcomes; **however, a synergistic effect was seen in women with the highest beef intake and highest MedD adherence,** where statistically significant higher hemoglobin levels (beta = 0.19; SE = 0.08; $p = 0.01$) were observed, with a similar trend among women in the medium tertile of beef consumption.
- Clinically, 36% of the total study participants met the criteria for anemia. The women with medium to high MedD adherence had a reduced risk of anemia with higher intake of fresh beef.
  - Women in the medium MedD group had 31% lower odds of anemia (OR: 0.69; 95% CI: 0.46–0.99; $p = 0.05$), and women in the high MedD group had 38% lower odds of anemia with every one-ounce increase in fresh beef intake (OR: 0.62; 95% CI: 0.41–0.89; $p = 0.01$).

Study Implications:

- Independent from one another, neither fresh beef intake nor Mediterranean diet adherence impacted maternal and infant health outcomes; however, **the combination of greater beef consumption within a Mediterranean dietary pattern synergistically decreased the odds of maternal anemia.**

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- RDAs were established as the average daily level of intake sufficient to meet the nutrient needs of most of the healthy population and there are specific nutrient requirements established for pregnant women.
    - Women with the highest beef consumption (Tertile 3), consumed on average 1.5 ounces per day, and met the RDA for protein, zinc and iron, while the women in the middle and lower tertiles did not meet the RDA for those respective nutrients.
    - However, women in all tertiles, did not meet certain micronutrients essential for healthy pregnancy and fetal development such as iron, choline, potassium, folate, and retinol (vitamin A), indicating an increase in beef intake and improvement in Mediterranean diet adherence could potentially result in a greater percent of the pregnant women population meeting nutrient needs.
- Bioavailable heme iron, inherently found in beef, may be a key element for preventing anemia when red meat is included in a Mediterranean diet, indicating current Mediterranean diet indices that negatively score red meat may be unnecessarily doing so.
- Pregnant women consumed fresh beef consistently across the varying adherence levels of the Mediterranean diet, indicating beef is consumed in a variety of approaches to this dietary pattern.
- Diet quality indices and scoring systems may be misrepresenting the role of beef within healthy eating patterns, as demonstrated in this study, had the avoidance of red meat been included in the overall MedD adherence score, the improved nutrient intake would have been inversely related to MedD adherence.

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