Eggs to improve choline intakes in pregnancy? Providing the evidence

Final Project Report | **December 2022**

A report for Australian Eggs Limited

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Australian Eggs Limited Publication No. 21NM003

ISBN 978-1-920835-75-0

Project Title: Eggs to improve choline Intakes in pregnancy? Providing the evidence

Australian Eggs Limited Project Number: 21NM003

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Published in December 2022

Foreword

Most Australians do not consume enough choline, an essential nutrient, in their diets. Choline is vital during periods of rapid growth, such as pregnancy, where it is needed in large amounts to support fetal brain development. Studies using animal models and data from a limited number of human studies suggest that choline supplementation during pregnancy improves neurodevelopmental outcomes in the offspring. There is little data on choline intakes of pregnant Australian women. This project was conducted to estimate choline intakes and food sources in a group of Australian pregnant women. We aimed to determine if pregnant women met current choline intake recommendations. We also conducted a systematic review to assess the state/quality of the evidence for maternal choline intake or status during pregnancy and associations with neurodevelopment in the offspring.

If choline intakes need improving, egg farmers are poised to capitalise on this opportunity. Just one egg provides a third of a pregnant woman's daily requirement. Increasing egg consumption would be an easy way for pregnant women to achieve choline recommendations. Egg farmers also have a competitive advantage over supplement manufacturers as the amount of choline required to meet needs cannot be incorporated into a standard prenatal supplement.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government. This report is an addition to Australian Eggs Limited's range of peer-reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

We thank Dr Yasmine Probst (Smart Foods Centre, School of Medicine, University of Wollongong, Wollongong, NSW 2522, Australia) for her help with the choline food composition database and matching the food codes to the food frequency questionnaire.

Australian Eggs Limited provided the funds that supported this project.

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Dr Jacqueline Gould is a Research Fellow in SAHMRI's Women and Kids Theme and a Senior Lecturer at the University of Adelaide. She is passionate about research to promote neurodevelopment. She is interested in the role of early nutrition in neurodevelopment, with the goal of optimising the development and functioning of the brain. Jacqueline has worked across multiple large randomised controlled trials of perinatal, neonatal and infant nutrition to improve childhood neurodevelopment. She leads a team of 15 students and research assistants that has conducted > 2500 neurodevelopment assessments, and has 30 publications in leading nutrition, medical and paediatric journals.

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Abbreviations

AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine
AI	Adequate Intake
AIHW	Australian Institute of Health and Welfare
ANNPAS	Australian National Nutrition and Physical Activity Survey
AUSNUT	Australian Food, Supplement and Nutrient Database
BMI	Body mass index
CBCL	Child behaviour checklist
DQES	Dietary Questionnaire for Epidemiological Studies
EFSA	European Food Safety Organization
FFQ	Food Frequency Questionnaire
Fagan	Fagan Test of Infant Intelligence
h	Hour(s)
HSQ	Home Screening Questionnaire
IQR	Interquartile range
K-ABC	Kaufman Assessment Battery
KBIT	Kaufman Brief Intelligence Test
MCDI	MacArthur-Bates Communicative Development Inventories
mg/d	Milligrams per day
mo	Month(s)
Mullen	Mullen Scales of Early Learning
NHMRC	National Health and Medical Research Council
NHANES	National Health and Nutrition Examination Survey
ORIP	Omega-3 fats to Reduce the Incidence of Prematurity
PPVT	The Peabody Picture Vocabulary Test
RCT	Randomised control trial
SAHMRI	South Australian Health & Medical Research Institute
SD	Standard deviation
wk	Week(s)
WPPSI	Wechsler Preschool and Primary Scale of Intelligence
WRAML	Wide Range Assessment of Memory and Learning
WRAVMA	Wide Range Assessment of Visual Motor Abilities
y	Year(s)

Executive Summary

Choline is an essential nutrient that is required for the synthesis of the neurotransmitter acetylcholine, betaine (a methyl group donor) and is part of phosphatidylcholine, a phospholipid found in high amounts in the brain. Most Australians do not receive enough choline from their diets to meet requirements. During pregnancy, choline requirements are increased as the fetus requires large amounts of choline for brain development.

We lack current data on choline intakes of pregnant women and the dietary sources of choline. Studies in animal models suggest that choline supplementation during pregnancy improves cognitive outcomes in the offspring. There are a few observational studies and randomised control trials (RCT) in humans, but these have not been evaluated using a systematic approach.

To address these gaps, we leveraged an existing RCT on folic acid to estimate choline intakes and food sources in a group of pregnant women during early and late pregnancy. We also compared current intakes during pregnancy to recommended intakes. Finally, we conducted a systematic review to address the question, "Is maternal choline during pregnancy associated with neurodevelopment in the offspring?"

Choline intakes and sources were similar in early (n = 93) and late pregnancy (n = 84). Median choline intake in early pregnancy was 362 mg/d. Only 39% and 25% of women achieved the Adequate Intake (AI) for choline intakes based on NHMRC (> 440 mg/d) and the newer EFSA (> 480 mg/d) guidelines, respectively. Eggs, red meat, nuts and legumes, and dairy accounted for 50% of choline intake, with eggs being the most significant contributor at 17%. Median egg consumption was 2.6 eggs per week, with the percentage of women consuming no eggs per week, or more than 12 eggs per week, similar at 10%. Women who consumed more than six eggs per week were seven times more likely to meet the AI for choline.

The systematic review yielded 14 eligible manuscripts. We found four eligible observational manuscripts that reported the results of three different cohorts. Of the three cohorts, two reported a positive association between choline intake or plasma choline in pregnant women, and at least one reported a measure of neurodevelopment in the offspring. Of the RCTs, seven manuscripts reported the results of four different RCTs. Three of four reported a beneficial effect of choline (or phosphatidylcholine) supplementation during pregnancy on a neurodevelopmental test. There was considerable heterogeneity in the studies, including dose and form of choline given, duration of supplementation, and population studied. Neurodevelopmental outcomes were not standardised, and in many cases, the tests used were experimental and were not well validated. RCTs were small, ranging from 26 to 140 participants. As such, a meta-analysis was not possible.

Overall Conclusions

The choline intakes of Australian pregnant women are less than that recommended by the NHMRC and EFSA. Eggs are the most important source of choline for pregnant women. If women consumed the equivalent of one extra egg a day, the percentage of women with adequate choline intakes would increase from 39% to 80%. Current evidence is insufficient to support or refute the hypothesis that increasing choline intake in pregnancy improves neurodevelopmental outcomes. A high-quality trial of choline or egg supplementation during pregnancy is needed.

1 Introduction

Choline is an essential nutrient that is required for the synthesis of the neurotransmitter acetylcholine and betaine, a vital methyl donor and osmoregulator. Choline is also a component of phosphatidylcholine, a phospholipid found in high amounts in neural tissue[1]. Therefore, choline is involved in a broad range of critical physiological functions across all life cycle stages.[2] Choline is synthesised endogenously from phosphatidylcholine, but endogenous choline synthesis is insufficient to meet needs, especially during periods of rapid growth[1]. Most Australians do not have adequate choline intakes based on recommendations established by the National Health and Medical Research Institute (NHMRC).[3] In the Australian National Nutrition and Physical Activity Survey (ANNPAS) 2011–12, less than 10% of the population met the Adequate Intake (AI) for choline[4,5]. Animal source foods are the primary source of choline: are the single largest contributor to the choline intake of Australians.[5]

During pregnancy, choline requirements are increased as the fetus requires large amounts of choline for brain development.[6] In the ANNPAS 2011-2012, less than 1% of pregnant women met their choline AI[4]. However, this survey is over ten years old and did not look at food sources of choline in pregnant women. Although most Australian women take prenatal supplements, they do not usually contain choline[7]. The amount of choline required cannot easily be incorporated into a single prenatal supplement. Thus, improving choline intake through diet would be the best approach, with eggs playing a pivotal role as they are a rich source of choline[5]. No studies have looked at blood choline concentrations and related metabolites in pregnant Australian women.

There is presently a lot of interest in whether increasing maternal choline intake through diet or supplements improves neurodevelopmental outcomes in the child. Several animal studies suggest that choline supplementation during pregnancy improves cognitive outcomes in the offspring[8]. There are a few pregnancy cohorts and RCTs in humans, but these have not been evaluated using a systematic approach. Here we aim to address these gaps.

1.1 Aims

1. To determine the choline intakes of a group of Australian pregnant women.
2. To determine the percentage of Australian pregnant women with choline intakes meeting AIs set by the NHMRC[3] and the European Food Safety Authority (EFSA)[9].
3. To determine food sources of choline in a group of Australian pregnant women.
4. If choline intakes are low, to determine the best means of increasing choline intake through diet.
5. To measure plasma choline and related metabolites in a group of Australian pregnant women.
6. To conduct a systematic review addressing the question, "Is maternal choline intake or status during pregnancy associated with neurodevelopment in the offspring and does supplementing maternal diets with choline improve neurodevelopmental outcomes in the offspring?"

2 Methodology

2.1 Choline intakes and biomarkers

This study leveraged an existing RCT that aimed to determine if removing folic acid from supplements after 12 weeks of gestation results in a lower concentration of unmetabolised folic acid (UMFA), a marker of excess folic acid intake. This study was a double-blind, placebo-controlled RCT comparing serum unmetabolised folic acid concentrations at 36 weeks' gestation following supplementation with a prenatal supplement with or without 800 µg folic acid. Full details of the study design have been described in the published trial protocol[10]. Here, we highlight the parts of the study procedures relevant to the choline sub-study.

2.1.1 Study participants and procedures

Pregnant women living in Adelaide, South Australia, were recruited to the trial between December 2019 and June 2020[10]. Women with a singleton pregnancy between 12 and 16 weeks' gestation, taking a folic acid supplement and planning to continue throughout pregnancy were eligible to participate. Women were excluded if they: were carrying a fetus with a confirmed or suspected fetal abnormality; had a prior history of an NTD-affected pregnancy; or were taking medications shown to interfere with folate metabolism. Women were recruited in person at their first antenatal appointment or through a Trial Recruitment Company (TrialFacts Australia, Melbourne), utilising an online digital marketing campaign and a pre-screening survey. Due to covid restrictions, after March 2020, screening, consent, and collection of participant information was completed online.

All women provided informed consent, and the study was approved by the Women's and Children's Health Network Research Ethics Committee – HREC/19/WCHN/018 and Flinders Medical Centre – SSA/20/SAC/61. The trial was prospectively registered with the Australia New Zealand Clinical Trials Registry (ACTRN12619001511123). An online food frequency questionnaire was completed close to enrolment (12–16 weeks) and at late pregnancy (36 weeks). A blood sample was collected from women at 36 weeks' gestation.

2.1.2 Choline intakes

Choline intakes were determined using the online version of the Dietary Questionnaire for Epidemiological Studies (DQES v3.2) developed by the Cancer Council of Victoria. The DQES v3.2 consists of 142 items (food and food groups) and has undergone validation in several populations[11]. DQES v3.2 does not include choline. Thus, we used choline food composition values recently added to the AUSNUT 2011-2013 database by Probst et al.[5]. With the assistance of Yasmine Probst, choline values were assigned to each of the foods in the Food Frequency Questionnaire (FFQ). DQES v3.2 provides the daily quantity of each item on the FFQ consumed. Daily choline intakes from each food were calculated and then summed to get the total choline intake for each group. The choline values for the 142 food items were collapsed into food categories.

2.1.3 Serum choline and related biomarkers

Serum concentrations of free choline, betaine and dimethylglycine were quantified by isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Waters I-class ACQUITY Ultra-Pure Liquid Chromatographic (LC) system connected to a Quattro Micro tandem MS-MS configured with an electrospray source (Waters Corporation, Milford, MA, USA). TLC LC was equipped with a pre-

column (2.1 x 12.1 mm) and a column (2.1 x 150 mm), both Zorbax Rx-SIL with a particle size of 5 µm (Agilent Technologies, Santa Clara, CA, USA)[12,13].

For analysis, aliquots of 50 µL of serum were transferred to Eppendorf tubes (1.5 mL) containing 10 µL of internal choline d-9, betaine d-9, and dimethylglycine d-6 standards (CDN Isotopes Inc., Pointe-Clair, QC, Canada). Plasma protein was precipitated, and the supernatant was mixed with the mobile phase consisting of 19% 15 mM ammonium formate containing 0.1% formic acid and 81% acetonitrile. A standard curve including choline (1.0 to 20.0 µmol/L), betaine (5.0 to 100.0 µmol/L), and dimethylglycine (0.5 to 10.0 µmol/L), and an in-house pooled plasma sample analysed in every run to ensure quality assurance.

2.2 Systematic review

Overarching question: Is maternal choline during pregnancy associated with neurodevelopment in the offspring?

- Is dietary choline intake during pregnancy associated with neurodevelopment in the offspring?
- Is circulating choline during pregnancy associated with neurodevelopment in the offspring?
- Is choline supplementation during pregnancy associated with neurodevelopment in the offspring?

2.2.1 Search strategy and selection of studies

We searched EMBASE, Pubmed, PsychInfo, The Cochrane Library until 31 December 2021. The study is registered with Prospero. Studies were eligible for inclusion in the review: 1. if they were observational studies that evaluated the effects of choline intake from diets or supplements or biomarkers of choline status during pregnancy and neurocognitive outcomes in the offspring; or 2. if they were an RCT that evaluated the effects of choline supplementation or dietary approaches during pregnancy on neurocognitive outcomes in the offspring. Two review authors independently assessed the titles, abstracts and, when necessary, the full text of the article for study eligibility, whereas a third author was available to review disagreements.

3 Results

3.1 Choline intakes and biomarkers

Between December 2019 and November 2020, 103 women entered the study and were randomised to treatment. Of the 103 women, 93 completed the FFQ in early pregnancy (11–16 weeks), and 84 women completed the FFQ in late gestation (34 weeks). Ninety women provided a blood sample at 36 weeks' gestation.

3.1.1 Participant characteristics

The mean maternal age was 31.4 years, and more than 85% of the participants were of European ethnicity. More than 87% of the women had completed secondary education, and 60% had an annual household income higher than AUD$105,000 (Table 3-1). Of the women, 55% had previously given birth to one or more children. Most women had a BMI in the healthy range.

Table 3-1 Characteristics of participants at study entry

Characteristic	Mean ± SD or N (%) (n=103)
Age (y)	31.1 ± 4.8
Gestational age at study entry	
12 – <14 weeks	64 (62)
≥14 – 16 weeks	39 (38)
Pre-pregnancy BMI (kg/m^2)[1]	24.1 ± 4.7
Healthy (18.5–24.9)	68 (72)
Overweight (25.0–29.9)	12 (13)
Obese (30.0 and above)	14 (15)
European ethnicity	85 (83)
Completed secondary education	90 (87)
Annual household income	
$70,000 or less	18 (17)
$70,001 – $105,000	19 (18)
$105,001 – $205,000	49 (48)
>$205,000	12 (12)
Prefer not to disclose	5 (5)
Parity	
0	47 (46)
1	45 (44)
>1	11 (11)

[1] Body Mass Index (n=94).

3.1.2 Choline intakes

Mean choline intakes were similar at early (11–16 weeks) and late (34 weeks) pregnancy, 394 and 418 mg/d, respectively (Table 3-2). In early pregnancy, only 39% of women met the NHMRC's AI for choline during pregnancy (>450 mg/d) which rose to 51% by late pregnancy. Using EFSA's higher AI (> 480 mg/d) for choline, only 25% and 33% of women met the AI in early and late pregnancy, respectively.

Table 3-2 Choline intakes in early and late pregnancy and the percentage (95% CI) of women not meeting the Adequate Intake for choline

	Early pregnancy (n=93)	Late pregnancy (n=84)
Mean ± SD (mg/day)	394 ± 125	418 ± 143
Median (IQR) (mg/day)	362 (298, 484)	414 (303, 509)
< NHMRC AI (440 mg/day)[1]	61 (51, 70)	49 (39, 59)
< EFSA AI (480 mg/d)[2]	75 (65, 84)	67 (56, 77)

IQR Interquartile range.

[1] National Health and Medical Research Council Adequate Intake.

[2] European Food Safety Association Adequate Intake.

3.1.3 Sources of choline

Food or food groups ranked by their contribution to daily choline intake during pregnancy are given in Table 3-3. Eggs were the most significant contributor to choline intake at both timepoints providing around 75 mg/day and 17% of total choline intake. Eggs and red meat, nuts and legumes, dairy, vegetables, and chicken accounted for around 70 % of choline intake. All other food groups account for 5% or less of choline intake.

Table 3-3 Choline intake by food or food group in early and late pregnancy[1]

Early pregnancy (n=93)			Late pregnancy (n=84)		
	mg/day	*% intake*[2]		*mg/day*	*% intake*[2]
Eggs	72 ± 55	17 ± 11	Eggs	76 ± 59	17 ± 10
Red meat	49 ± 39	12 ± 11	Red meat	58 ± 42	14 ± 9
Nuts and legumes	46 ± 58	11 ± 13	Nuts and legumes	50 ± 61	11 ± 12
Dairy	40 ± 29	10 ± 7	Dairy	43 ± 27	11 ± 6
Vegetables	35 ± 22	9 ± 5	Vegetables	30 ± 20	8 ± 5
Chicken	30 ± 19	8 ± 6	Chicken	29 ± 19	8 ± 5
Fruit	20 ± 13	5 ± 4	Fruit	19 ± 14	5 ± 4
Pasta	18 ± 11	5 ± 3	Pasta	19 ± 12	5 ± 3
Fish	16 ± 16	4 ± 4	Fish	18 ± 16	4 ± 4
Bread	12 ± 8	3 ± 3	Breakfast cereals	13 ± 15	3 ± 3
Breakfast cereals	12 ± 10	3 ± 3	Beverage	13 ± 12	3 ± 3
Other savoury foods	10 ± 6	3 ± 2	Bread	12 ± 7	3 ± 3
Beverages	10 ± 14	2 ± 3	Processed meat	9 ± 8	2 ± 2
Processed meat	7 ± 7	2 ± 2	Sweets	8 ± 5	2 ± 1
Sweets	7 ± 6	2 ± 1	Other savoury foods	8 ± 5	2 ± 1
Plant-based milk	4 ± 20	1 ± 4	Plant-based milk	4 ± 15	1 ± 3
Yeast spreads	2 ± 2	1 ± 1	Other	2 ± 6	1 ± 1
Plant oils	2 ± 1	0 ± 0	Plant oil	2 ± 2	1 ± 1
Other	1 ± 4	0 ± 1	Yeast spreads	2 ± 2	0 ± 1
Rice	1 ± 1	0 ± 0	Rice	1 ± 2	0 ± 0

[1] Values are Mean ± SD.

[2] Mean daily percentage of choline from food or food group.

3.1.4 Eggs as a source of choline

In early pregnancy, the median (IQR) intake of eggs was 2.6 (2.2, 7.0) per week. There was a significant variation in egg consumption, with 10% of women consuming no eggs and a similar percentage consuming more than 12 eggs per week (Figure 3-1). Egg consumption was similar in early and late pregnancy. Women who consumed more than six eggs per week were seven times [OR (95%CI)] more likely to have a choline intake that met the EFSA [7.2 (2.4,20.1); $P < 0.01$] and NHMRC AI [7.1 (2.7,18.9); $P < 0.001$].

If women increased their consumption by one egg per day, the proportion of women above the EFSA AI for choline would increase from 25% to 60%. Using the NHMRC AI for choline proportion would increase from 39% to 80%.

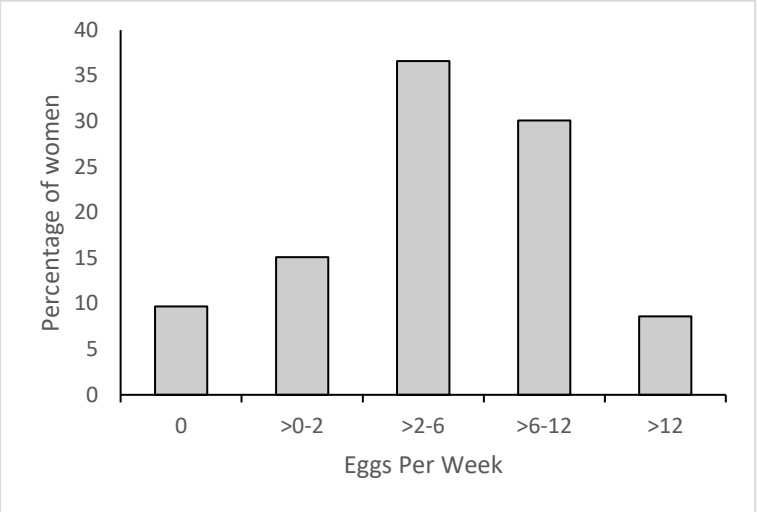

Figure 3-1 Weekly egg consumption in early pregnancy

3.1.5 Choline and related biomarkers

Plasma choline and related metabolites are given in Table 3-4. There are no cut-offs for choline during pregnancy, and the normal range in non-pregnant adults is from 10–20 µmol/L. Only one woman had a serum choline < 10 µmol/L. There was no association between total serum choline and total choline intake or any of the food sources of choline.

Table 3-4 Choline and related biomarkers

Metabolite	Mean ± SD	Median [IQR]
Choline (µmol/L)	15 ± 4	13 [13, 17]
Betaine (µmol/L)	17 ± 4	14 [16, 20]
Dimethylglycine (µmol/L)	15 ± 4	2 [2, 3]
AdoMet (nmol/L)	73 ± 17	65 [74, 82]
AdoHcy (nmol/L)	25 ± 9	19 [22, 28]

3.2 Systematic review

The search returned 1425 articles, 959 of which remained after duplicates were removed (Figure 3-2). Of these, 927 were excluded after the abstracts were reviewed because they did not meet the inclusion criteria. The full text of 32 citations was examined in detail, of which 21 were excluded. A total of 5 articles based on 4 observational cohorts and 6 reports based on 4 RCTs were included.

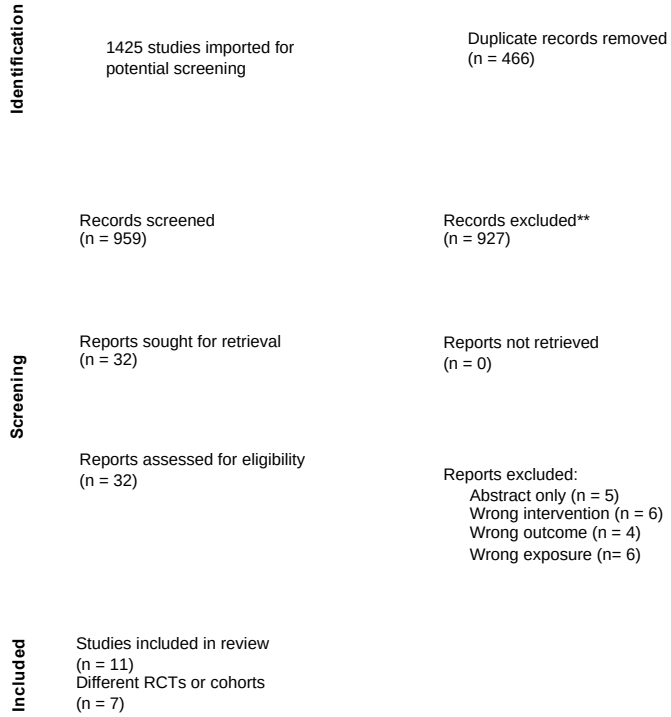

Figure 3-2 Progress of RCTs and cohorts identified and included in the systematic review

3.2.1 Participants

The three observational studies included a total of 2682 participants but ranged in size from 154[14] to 2128[15] participants. The manuscript by Boeke et al.[14] was a follow-up of participants in Villamor et al.[15] and included 895 participants. All three studies were conducted in high-income countries. Two studies were in healthy women. The other was described as a population in which 95% of women had identifiable risk factors for growth restriction[16]. All studies excluded non-singleton pregnancies or restricted their analysis to singleton births. Other exclusion criteria included inability to answer questions in English and women > 22 weeks' gestation[15], increased risk of pre-term birth[16], or non-full-term infants[16].

The four RCTs included 349 participants and ranged in size from 29 to 140 participants[17-22]. Three studies were conducted in the US, all on healthy women. The other study was conducted on pregnant women in South Africa who were described as heavy drinkers. For three of the studies[17-19], analyses of later neurocognitive outcome assessments when the children were older are published[20-22].

3.2.2 Exposure or intervention

Two of the three observational studies used plasma/serum choline to measure status in pregnancy Signore et al.[23], measured serum total and free choline multiple times during pregnancy, and cord blood. Wu et al.[16], measured total plasma choline at 16- and 36-weeks' gestation. Villamor et al.[15] assessed choline intake during the 1st and 2nd trimester using a 166-item semi-quantitative FFQ, widely used in large US epidemiological studies and calibrated for use in pregnancy[24].

Of the RCTs, two studies gave choline as phosphatidylcholine [25,26] and the other two as choline salts, either chloride[18] or bitartrate[19]. Three studies included a placebo[17,19,25], two used corn oil as the

placebo[17,25], and the other did not specify[19]. Caudill et al. did not have a placebo[18] but used two doses of choline 480 or 930 mg/d choline. The supplementation period varied greatly: two studies continued supplementation until delivery; one started in the third week of pregnancy[18]; and the other midway in the second trimester[19]. Two studies began in the 2nd trimester and continued to 90 days postpartum, one supplementing the mother[25] postpartum, and the other the infant directly[17]. Maternal doses of choline ranged from 480[18] to 2000 mg/d[19].

Table 3-5 Summary of observational studies included in the review[1]

Author	Participants	Exposure	Neurocognitive assessments	Adjustments	Results
Signore et al. 2008[23] AB, USA Infant Growth Project cohort	N:400 Healthy women receiving care from the public health department	*Period:* 16–18, 24–26, 0–32, and 36–38 wk pregnancy and cord. *Measure:* Serum total and free choline.	*Age*: 5 y *Outcomes:* WPPSI-R	Maternal PPVT-R raw score, HSQ score, poverty status, maternal race, education level, smoking, alcohol intake GA at delivery, and child sex.	There was no association between maternal or cord serum total and free choline at any timepoint for any neurodevelopmental outcome.
Villamor et al. 2012[15] MA, USA Project Viva cohort	N:2128 Healthy women receiving care at a large practice	*Period:* 1st and 2nd trimester *Measure:* Choline intake measured by FFQ	*Age*: 3 y *Outcomes:* PPVT-III, WRAVMA	Maternal ethnicity, age, parity, smoking, BMI, PPVT-III, education, energy intake, fish and iron intake, paternal education, household income, the sex of the child and primary language.	There was no association between choline intake and neurodevelopmental outcomes.
Boeke et al. 2013[14]	N:895		*Age*: 7 y *Outcomes:* WRAML-2, KBIT-2		The top quartile choline intake in the 2nd trimester was associated with modestly better WRAML (child visual memory) at 7 y. No other associations.
Wu et al. 2012[16] Vancouver, Canada	N:154 Healthy	*Period:* 16, 36 wk. of pregnancy. *Measure:* Plasma choline	*Age:* 18 mo. *Outcome:* Bayley-III	MateIQal IQ, ethnicity, age phosphatidylethanolamine docosahexaenoic acid, infant sex, and breastfeeding duration.	A positive association was found between infant cognitive test scores and maternal plasma choline at 16 wks.

[1] FFQ Food Frequency Questionnaire.
WPPSI Wechsler Preschool and Primary Scale of Intelligence.
R Revised.
PPVT The Peabody Picture Vocabulary Test.
HSQ Home Screening Questionnaire.
WRAVMA Wide Range Assessment of Visual Motor Abilities.
KBIT Kaufman Brief Intelligence Test.
WRAML Wide Range Assessment of Memory and Learning.
Bayley Bayley Scales of Infant Development.
K-ABC Kaufman Assessment Battery.
MCDI MacArthur-Bates Communicative Development Inventories
Mullen Mullen Scales of Early Learning.
CBCL Child Behaviour Checklist.
Fagan Fagan Test of Infant Intelligence.

Table 3-6 Summary of randomised controlled trials included in the review[1]

Author /Setting	Participants	Interventions	Follow-up	Results
Cheatham et al. 2012[25] Chapel Hill NC, USA	N:140 Healthy	*Period*:18 wk. of gestation to 90 d postpartum *Treatment:* 750 mg/d choline as phosphatidylcholine or placebo (corn oil)	*Age*: 10 and 12 mo. *Outcome*: Visuospatial Memory Delayed Response Task, Long-term episodic memory, MCDI, MSEL	No effect of treatment on any outcome.
Ross et al. 2013[17] Denver CO, USA	N:100 Healthy	*Period*: 2nd trimester 70–90 d postpartum *Treatment:* 900 mg/d choline as phosphatidylcholine or placebo (corn oil). Infants 100 mg/d phosphatidylcholine or placebo in an oral suspension.	*Age*: 5 and 13 wk. *Primary Outcome*: electrophysiological recordings of cerebral inhibition *Age:* 6 mo. Outcome: Mullen Scales of Early Learning	Infants whose mothers received choline versus placebo were more likely to have normal inhibition at 5 wks. No difference at 13 wks. No effect on Mullen Scales of Early Learning.
Ross et al. 2016[20]	N:49		*Age:* 40 mo. *Outcome:* CBCL-parent report	Fewer attention problems were reported and less social withdrawal in choline versus the placebo group. Aggression, emotionality, anxiety/depression, sleep, somatic, internalising, externalising, and total scores did not differ.
Caudill et al. 2018[18] Ithaca NY, US	N:29 Healthy	*Period:* 3rd wk. of gestation until delivery *Treatment:* 480 or 930 mg/d choline chloride	*Age:* 4, 7, 10, and 13 mo. *Outcome*: Memory-guided, anticipatory saccade reaction.	Reaction time averaged across the four ages was significantly faster for infants born to mothers in the 930 vs. 480 mg/d choline group.
Bahnfleth et al. 2022[21]	N:20		*Age*: 7 y *Outcome*: signal detection task	930 versus 480 mg/d choline group showed better performance on the signal detection task.
Jacobson et al. 2018[19] Cape Town, South Africa	N:69 Heavy drinkers	*Period*: by 23 wk. of gestation until delivery *Treatment:* 2000 mg/d of choline bitartrate or placebo (not defined)	*Age*: 6.5 and 12 mo. *Outcomes*: eyeblink conditioning, Fagan (recognition memory, information processing speed)	At 6.5 mo., the choline group was more likely to be classified as meeting the criterion for eyeblink conditioning. At 12 but not 6.5 mo., the choline group had faster recognition memory processing speed. No difference in recognition processing speed.
Warton et al. 2021[22]	N:67		*Age*: 1-7 wk. *Outcomes:* Regional brain volumes were measured using structural magnetic resonance imaging. Subcortical regions were manually segmented. All volumes were adjusted for age and total intracranial volume.	Six of the 12 regions in the brain were larger in the choline than in the placebo group. Larger right putamen and corpus callosum were related to higher Fagan scores at 12 mo. An NS trend toward partial mediation of the choline effect on recognition memory.

[1] MCDI MacArthur-Bates Short Form Vocabulary Checklist: Level I.
MSEL Mullen Scales of Early Learning.
CBCL Child Behaviour Checklist.

3.2.3 Neurodevelopmental outcomes

A wide variety of neurocognitive tests were used in both the observational and RCTs. The (Revised) Wechsler Preschool and Primary Scale of Intelligence was used at age 5 years in the largest of the observational studies[23]. Villamor et al.[15] used the Peabody Picture Vocabulary Test and the Wide Range Assessment of Visual Motor Abilities in children at age 3y. The former test measures English-speaking children's receptive (hearing) vocabulary. In contrast, the latter test provides a reliable, accurate evaluation of the visual-motor skills of children[15]. A subset of these children was tested again at age 7[14], using the Wide Range Assessment of Memory and Learning (WRAML2) and the Kaufman Brief Intelligence Test (KBIT2). The WRAML2 includes a measure of design memory and picture memory. The KBITs is a brief intelligence test. Wu et al.[16] used the Bayley Scales of Infant Development-III, the most established test to measure neuro-development in children less than 42 months of age.

In contrast to the observational studies, most of the neurocognitive tests in the RCTs are experimental and have not been well validated. Of the tests used, the MacArthur-Bates Short Form Vocabulary Checklist (MCDI) Level I and the Mullen Scales of Early Learning (MSEL) used by Cheatham et al.[25], and the Child Behaviour Checklist (CBCL) used by Ross et al. in the follow-up of their earlier study, are the best established and validated[17,20]. The remainder are tests of physiological responses or imaging of brain scans that have not been validated and may not be predictive of intelligence or future abilities. Tests measure different neurological domains and are done at different ages, making it impossible to compare studies.

3.2.4 Risk of bias

In the observational studies, the authors adjusted for potential confounders in the observational studies, such as maternal ethnicity, educational attainment, gestational age at birth, and maternal scores on intelligence tests.

In the RCTs, the method used to generate the randomisation sequence was given in three[18,25,27] of the four studies; allocation and blinding of participants was reported in all studies. Blinding of outcome assessors was not mentioned explicitly in any of the studies. Only two[18,19] of four RCTs had a greater than 80% follow-up for the primary assessment. Follow up rates for these studies when children are assessed at older ages ranged from 50% to 75%.

Table 3-7 Summary of risk of bias in randomised control trials

Author	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Follow-up > 80%
Cheatham et al. 2012[25]	+	+	+	?	-
Ross et al. 2013[17]	-	?	?	?	-
Caudill et al. 2018[18]	+	+	+	?	-
Jacobson et al. 2018[19]	+	+	+	?	+

+ Adequate (low risk of bias).
? unclear (unknown risk of bias).
– inadequate (high risk of bias).

4 Discussion

4.1 Choline intakes and biomarkers

4.1.1 Choline intakes and Adequate Intakes

As expected, choline intakes were low and below NHMRC and EFSA recommendations in our sample of pregnant Australian women. The choline intake was similar in early and late pregnancy, averaging 401 mg/d over both periods. More women exceeded the NHMRC AI in 51% versus 39% in late than early pregnancy. Table 4-1 summarises the studies that have estimated choline intakes in pregnant women, and our intakes are generally higher than those reported in other studies. Indeed, the lowest intakes published were in pregnant women in the ANNPAS 2011–2012, where the mean choline intake was only 251 mg/d, and < 1% of women met the NHMRC AI.[5]

There are several reasons why the choline intakes are higher in our study than in ANNPAS 2011–2012. We used an FFQ, whereas 24-h recalls on two different days per individual were used in ANNPAS 2011–2012. Compared to FFQs, 24-h recalls tend to underestimate intakes of infrequently consumed foods such as eggs that may be eaten once or twice a week but contain high amounts of choline. Conversely, the FFQ, due to its long lists of foods, tends to overestimate consumption of food items because there is a tendency to indicate a specific food has been eaten more than it has[28]. In the ANNPAS 2011–2012, the 24-h recall energy intakes were under-reported by 22% (ANNPAS underestimation), which leads to all nutrients being underestimated. Finally, a 24-h recall only recalls two days, whereas our FFQ was designed to assess habitual dietary intake over the previous month[28].

Although choline intakes of pregnant women in our study are higher than those reported in most other studies, there is considerable variation in intake. In the US NHANES, mean choline intake was ~320 mg/d despite using 24-h recalls. In contrast, studies in Vancouver Canada[16] and Eastern Massachusetts[15] using an FFQ report a mean intake of 383 and 344, respectively, using an FFQ in pregnant women. All studies report considerable variation in choline intake within a population; in many cases, the SD is as large as the mean. This variation is not surprising given that choline is found in high quantities in select foods a woman may or may not eat, such as eggs, fish and seafood.

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Table 4-1 Studies reporting choline intake in pregnant women

Author/Setting	Age/years	Study design	Sample size	Assessment method	NCD	Choline intake (mg/d)
Fawcett et al. 2020[29] USA	NG	Prospective observational study	*n*, 251	3d-FR	Nutrient Data System for Research	Mean 281
Probst et al. 2019[5] Australia	19–50 y	Cross-sectional study Australian Survey 2011–12	*n*, 116	24-h recall	Database derived from local & international sources	Mean (95% CI) 252 (232-273)
Bailey et al. 2019[30] USA	20–40 y	From NHANES 2001–2014	*n*, 533	24-h recall ×2	USDA FCD	Mean ± SD 321 ± 231
Pauwels et al. 2017[31] Belgium	25–41 y	Prospective observational study	Trimester: 1st *n*, 94; 2nd *n*, 85; 3rd *n*, 82	FFQ	Not reported	Trimester; Mean ± SD 1st 274 ± 72; 2nd 268 ± 68; 3rd 280 ± 78
Wallace et al. 2016[32] United States	13–44 y	2009–2014 & 2005–2014 NHANES	*n*, 593	24-h recalls ×2	Various USDA FCD	Mean ± SD 319 ± 241
Groth et al. 2017[33] United States	18–36 y	Prospective observational study	Trimester: 1st *n*, 90; 2nd *n*, 68; 3rd *n*, 67	24-h recalls x 3	Nutrition Data System for Research software 2009	Trimester: Mean ± SD: 1st 318 ± 68; 2nd 289 ± 28; 3rd 306 ± 28
Masih et al. 2015[34] Canada	Mean ± SD 32 ± 5 y	Prospective observational study	*n*, 290	FFQ; 0–16 & 23–27 wks.	Primarily USDA FCD	Trimester; Mean ± SD 1st 306 ± 127; 3rd 302 ± 122
Lewis et al. 2014[35] Canada	17–45 y	Prospective observational study	Trimester: 1st *n*, 123; 2nd *n*, 562; 3rd, *n*, 493	24-h recall	USDA FCD	Trimester; Mean ± SD 1st 340 ± 148; 2nd 349 ± 154; 3rd 353 ± 144
Wu et al. 2012[16] Vancouver, BC Canada	Not reported	Prospective observational study	*n*, 154	FFQ; from 16 wks. gestation	USDA FCD	Mean ± SD 383 ± 99
Villamor et al. 2012[15] MA; USA	Mean ± SD 33 ± 5 y	Prospective observational study	Trimester; 1st *n*, 1148; 2nd *n*, 1083	FFQ	Harvard FCD	Trimester; Mean ± SD 1st 332 ± 63; 2nd 325 ± 64
EFSA 2016[36], 2011[37] Latvia	15–45 y	EFSA European Food Consumption Database	*n*, 990	24 h recall	Not reported	Pregnant Adolescents Mean, 336; Pregnant women Mean, 356

NG Not given.
NHANES National Health and Nutrition Examination Survey.
FR Food record.
IQR Interquartile range.
USDA United States Department of Agriculture.
FCD Food composition database.
FFQ Food frequency questionnaire.
EFSA European Food Safety Authority.

4.1.2 The contribution of eggs to choline intake

Eggs were the number one contributor to choline intake in our study in both early and late pregnancy, providing around 17% of total intake or 75 mg/d. Overall, in the ANNPAS 2011–2012, eggs were the highest contributor (9%) to Australian choline intakes for all population groups[5]. The contribution of eggs to the choline intake of Australian pregnant women is not provided. Still, it would appear that the combination of their 'Eggs' category and 'Dishes where egg is the major ingredient' would make eggs the number one contributor to choline intake[5]. Most of the other studies in Table 4-1 do not provide the breakdown of choline intake by dietary source. One exception is the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort, where the authors report that eggs were the second leading contributor to choline in pregnant women at 12%, only exceeded by 'Dairy' at 21%[35].

In our study, eggs were the number one contributor to choline intake in pregnant women. Similar results were reported by Wallace et al.[38] in non-pregnant women of reproductive age in US NHANES, where the usual intakes of choline were nearly twice as high in 'Consumers' of eggs than 'Non-Consumers'. For example, the mean $\pm$ SE choline intakes for women 19–30 years in 'Consumers' versus 'Non-Consumers' was 427 $\pm$ 11.4 and 225 $\pm$ 3.8 mg/d, respectively. To our knowledge, we are the first group to model increased consumption of eggs to help meet choline requirements during pregnancy. By consuming seven eggs per week or one egg a day, over 80% of pregnant women would meet their AI for choline during pregnancy.

4.1.3 Serum choline and related biomarkers

Serum choline values were in the normal range for non-pregnant individuals (10–20 µmol/L) in all but one woman, who had serum choline of 9.8 µmol/L. There are no established choline reference ranges in pregnancy. Plasma choline is higher in pregnant women compared to non-pregnant women despite haemodilution that occurs in pregnancy[16,18].

Serum choline is not reflective of choline intake. Circulating choline levels only begin to drop after several weeks of consuming a diet low in choline[39]. Even after fasting for one-week, plasma choline did not drop beyond 50% of initial concentrations, likely due to the release of choline from plasma membrane phospholipids[40].

4.1.4 Strength and limitations of pregnancy study

We acknowledge that our sample size is small, which would affect our confidence in the estimates of choline intake. However, our participants were similar to the broader population of Australian pregnant women. For example, the age of first-time mothers in Australia was 29.4 y in 2019[41], which is slightly lower than our mean age of 31.1 y. However, over 50% of women in our study had given birth to at least one previous child. Of participants, 83% described themselves as of European ethnicity, which is similar to AIHW data[41]. Sixty per cent of participants had an annual family income over 105,000 AUD, which is similar to the average Australian family income (2020) of 120,000 AUD[42].

We measured choline at two timepoints, early (12–16 weeks) and late pregnancy (36 weeks). We updated a validated Australian FFQ for choline, with the choline values derived from a recent update of AUSNUT 2011–2013. FFQs tend to overestimate dietary intake, which may be the reason for the higher choline estimates than the ANNPAS 2011–2012. On the other hand, the FFQ may be better at capturing usual intake, especially of foods eaten less frequently but that are rich sources of choline, such as eggs. In contrast, 24h-recalls, the method used in the ANNPAS 2011–2012, lead to the under-reporting of energy and nutrients. We also identified food sources of choline and determined the contribution made to choline intake by eggs.

4.2 Strengths and limitations of the systematic review

It was impossible to conduct a meta-analysis of either the observational studies or the RCT[43]. The observational studies use different exposures at different timepoints in pregnancy. Although most outcomes were well validated neurocognitive assessments, they were administered at different timepoints. A meta-analysis of the RCTs is even more problematic. The doses and the forms of choline varied, the duration of choline supplementation varied, and not all studies were placebo-controlled. The study populations were heterogeneous (i.e. health versus heavy alcohol users), and the cognitive tests were administered at different ages and assessed the different cognitive domains.

The two largest observational studies reported no association between choline and any neuro-developmental outcome assessed[15,23]. Villamor et al.[15] reported in 1210 mother-child pairs that there was no association between 1st and 2nd-trimester choline intake assessed by FFQ and PPVT-III or WRAVMA at 3 years of age. Boeke et al.[14] followed up a subset of these children (n = 895) and administered WRAML2, Design and Picture Memory subtests, and KBIT tests when the children turned 7y. The WRAML2 was chosen because, unlike the tests administered at 3 years, it measures visuospatial memory, the cognitive domains most affected by choline in animal models and a study of older people[44]. Children of mothers with a choline intake in the highest quartile in the second trimester scored 1.4 points higher on the WRAML. This difference was moderately large, approximately 1/3 of a standard deviation in this population (4.4 points). However, no association was observed between choline intakes in the first trimester and WRAML2 or with KBIT-2 at either timepoint.

The other two observational studies[16,23] both used circulating choline as the exposure. The use of circulating choline as an exposure indicator is a limitation as it does not correlate with choline intake. The larger of the two studies that had 404 maternal-child pairs found no association between serum choline measured multiple times during pregnancy, including cord blood found no difference in child IQ at 5y of age. In contrast, Wu et al.[16] reported that plasma choline at 16 weeks' gestation was associated with improved Bayley Scales of Infant Development–III (B = 6.0, SE = 2.33, p = 0.009) administered at 18 months. However, the study was small (n = 154), and no association was reported with choline intake at 36 weeks. Moreover, the authors assessed choline intake by FFQ at two timepoints but did not indicate whether there was an association with the Bayley-III, suggesting no association.

All studies were conducted in North America, limiting generalisability. The neurocognitive tests used are well validated and were age appropriate. Of the three studies, two used circulating choline, which is considered a poor biomarker of choline intake. The observational studies adjusted for several confounders.

The systematic review of RCTs included three separate studies. Children whose mothers received choline supplements performed better on at least one of the tests administered than those whose mothers received a placebo, or a lower dose of choline, in three[17-19] out of four[25] studies. Aside from the different forms of choline used, gestational age at the start of the study, different doses and duration of choline, and age of assessment, several points should be noted:

1. All studies were small, ranging from 20 to 140 mother-child pairs and were underpowered to the modest effects that might be expected from a nutritional intervention. The only study with over 100 participants did not find an effect of choline on any neurocognitive test[25]. Caudill et al.[18] only had 29 participants and included no placebo, just an arm providing 480 mg/d choline (similar to the NHMRC AI) and twice that amount[18].

2. One of the studies and its follow-up was conducted on women who were heavy drinkers, which aimed to mitigate the adverse effects of prenatal alcohol exposure[19,22]. Choline has been shown to reduce the impact of alcohol in animal models.[45]

3. Several studies used quasi-neurocognitive tests that have not been validated for the age groups assessed.

5 Conclusions

In our study, the median choline intakes of Australian pregnant women were less than that of NHMRC and EFSA recommendations. Eggs are the most important source of choline for pregnant women. If women consumed the equivalent of one extra egg a day, the percentage of women with adequate choline intakes would increase from 39% to 80%.

Current evidence is insufficient to support or refute the hypothesis that increasing choline intake in pregnancy improves neurodevelopmental outcomes. The RCTs are too small, use varying amounts of choline in different forms, commence at various times in pregnancy and continue for a variable duration. Many do not use validated assessment techniques that measure outcomes other than neurodevelopment. It is also not clear if the tests predict long-term outcomes. The cognitive assessments are not standardised in either timing of the test or the type of assessment used. A high-quality pregnancy trial of choline or egg supplementation with sufficient power is needed. The trial should use a neurocognitive assessment tool that is well established and at an older age.

6 Future directions

We suggest the following:

1. Obtain data on the choline intake of children under two years. Our recent Australian first study of dietary and nutrient intakes of Australian children under 2 years could be used to generate this data.
2. Develop an online choline calculator for pregnant women. One could then advise on how to improve choline intake. Ten foods or food groups would be sufficient to estimate choline intakes with some confidence.
3. Evaluate choline intakes in lactation in a group of lactating women. The AI requirements increases from 440 mg/d during pregnancy to 550 mg/d during lactation.
4. Make a choline 'content claim' on eggs under FSANZ regulations.
5. Increase awareness of choline in pregnancy. Unlike other micronutrients, a prenatal supplement cannot accommodate enough choline to meet a pregnant woman's needs. This is because the amount of choline needed as a supplement including the anion, excipients and fillers would need to be over two grams, and would require two large capsules.
6. Finally, a high-quality trial is needed to show if choline supplementation (or advice to increase eggs) improves neurodevelopmental outcomes.

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