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Randomised clinical trial: reducing the intake of dietary FODMAPs of breastfeeding mothers is associated with a greater improvement of the symptoms of infantile colic than for a typical diet

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Summary

Background: Therapeutic diets for infantile colic lack evidence. In breastfed infants, avoiding "windy" foods by the breastfeeding mother is common.

Aim: To examine the effects of a maternal low-FODMAP (Fermentable, Oligosaccharides, Disaccharides, Monosaccharides, And Polyols) diet compared to a typical-Australian diet on infant crying-fussing durations of infants with colic in a randomised, double-blind, crossover feeding study.

Methods: Between 2014 and 2016 exclusively breastfed infants aged ≤9 weeks meeting Wessel criteria for colic were recruited. Mothers were provided a 10-day low-FODMAP or typical-Australian diet, then alternated without washout. Infants without colic (controls) were observed prospectively and mothers remained on habitual diet. Infant crying-fussing durations were captured using a Barr Diary. Measures of maternal psychological status and samples of breast milk and infant faeces were collected.

Results: Mean crying-fussing durations were 91 min/d in seven controls compared with 269 min/d in 13 colicky infants ($P < 0.0001$), which fell by median 32% during the low-FODMAP diet compared with 20% during the typical-Australian diet ($P = 0.03$), confirmed by a two-way mixed-model analyses-of-variance ($\eta_p^2 = 0.719$; $P = 0.049$) with no order effect. In breast milk, lactose concentrations remained stable and other known dietary FODMAPs were not detected. Changes in infant faecal calprotectin were similar between diets and groups, and faecal pH did not change. Median maternal anxiety and stress fell with the typical-Australian diet ($P < 0.01$), but remained stable on the low-FODMAP diet.

Conclusions: Maternal low-FODMAP diet was associated with enhanced reduction in crying-fussing durations of infants with colic. This was not related to changes in maternal psychological status, gross changes in breast milk or infant faeces. Mechanisms require elucidation. Trial Registration Australian New Zealand Clinical Trials Registry (ANZCTR): 12616000512426 - anzctr.org.au

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1 | INTRODUCTION

Infantile colic is a common medical problem with potentially serious outcomes such as cessation of breastfeeding, post-natal depression and Shaken Baby Syndrome.[1] It is one of the most common presentations of mother-infant pairs to emergency departments.[2] Infantile colic, defined by the "Wessel Criteria" as paroxysms of crying episodes lasting for >3 h/d, for ≥3 d/week and for ≥3 weeks,[3] is reported to occur in approximately 14%-30% of infants.[4] Although infantile colic spontaneously resolves by age 3-4 months and despite the lack of evidence for dietary manipulation,[5] breastfeeding mothers often manipulate their diet in an attempt to settle their infant across multiple cultures, commonly by reducing intake of intestinal gas-producing foods, especially pulses/legumes, onion, garlic, cruciferous vegetables and breads and cereals containing wheat and rye. However, the evidence for the efficacy is limited to observational studies.[6-9] A common link between these foods is their high FODMAP (Fermentable Oligo-, Di-, Mono-saccharides And Polyols) content.[10] When a maternal diet low in FODMAPs was formally tested in an uncontrolled observational study of breastfed infants with colic, the crying-fussing durations of infants fell considerably more than that anticipated from the natural history of colic,[11] and was greater than the 25% improvement considered to be of clinical significance.[12,13] Whether this was a placebo effect and, if causal, what mechanisms by which this might occur are not known. The only other randomised controlled trials of maternal diet in breastfed infants evaluated hypoallergenic and dairy-free diets and were conducted more than 15 years ago.[13,14] Benefit over placebo was shown in one of these studies,[13] however, the FODMAP content of the mother's diet may have also been reduced by excluding wheat, soy and dairy products.

Hence, the current study aimed to address the hypothesis that a maternal diet low in FODMAPs will accelerate the reduction of symptoms of infantile colic. This was addressed by comparing, via a double-blind crossover design, changes in excessive crying-fussing durations in breastfed infants whose mothers were assigned to a diet low in FODMAPs and a typical-Australian diet of moderate FODMAPs in a random order. A cohort of mother-infant pairs without colic was studied in parallel, with the mothers remaining on their habitual diet. Effects on the psychological status of the mothers, the composition of breast milk and infant faecal profiles were also explored.

2 | METHODS

2.1 | Participants

Exclusively breastfeeding mothers with typically developing infants ≤9 weeks of age who met the Wessel criteria (crying episodes lasting for >3 h/d, for ≥3 d/week and for ≥3 weeks) were recruited by using flyers and advertising the study on websites with Monash Children's Hospital, Melbourne, maternal and child health nurse centres, the Australian Breastfeeding Association and Raising Children network and via social media and newspapers. Mothers were included if they were 18-45 years old and willing to change their diet. Mother-infant pairs were excluded if there were any underlying medical conditions or requiring medication. An additional cohort of mothers with infants who did not have colic was recruited from the community.

2.2 | Study protocol

Participants who passed a screening telephone call were sent, via email, information prior to a face-to-face consultation with the Principle Investigator (MI), at which written, informed consent was obtained. Study materials for the collection of baseline information included: a stopwatch for the timing of infant behaviour patterns, using the validated Barr Diary[15] (a Baby Day Diary), and sterilized containers for the collection of expressed breast milk (20 mL) and infant faecal sample (10 g).

The duration of crying and fussing, feeding, sleeping and periods of awake and content were recorded daily, and the data were reviewed daily for up to 7 days until criteria for colic were met. The mother was asked to complete the Depression, Anxiety and Stress Scale to assess maternal mental well-being.[16] Breast milk and infant faecal samples were immediately placed by the mother in a provided portable freezer at −20°C, picked up within 48 h and stored in the laboratory freezer at −80°C.

Eligible participants were randomised via a computer-generated randomisation application (www.randomization.com) to one of two study diets—a 10-day low-FODMAP diet or a 10-day typical-Australian diet, for which all food was provided and delivered to participant homes in a refrigerated van. Randomisation was activated when the entry criteria were reached and delivery of food was affected for the first interventional diet within 24 hours. Instructions were provided on how to store foods immediately on arrival together with a meal plan with preparation and heating instructions. On the final day of the first study diet, a second Depression, Anxiety and Stress Scale was completed by the mother and the alternate 10-day diet was delivered. No washout period was used in order to reduce the duration of the interventions, especially since the low FODMAP diet had achieved apparent efficacy after 3 days in the proof-of-concept study.[11] A third Depression, Anxiety and Stress Scale was completed on day 10 of the second diet. The mother recorded the infant's behavioural characteristics in a Baby Day Diary for each study diet and her own food intake. For each study diet, food containers or uneaten foods were collected to assess compliance.

The mothers with infants who did not have colic followed the same protocol except that mothers followed their habitual diet and were not provided any food or given dietary advice during the study.

The study protocol was approved by three organisations with a strong interest on infant healthcare and development, Monash Health (HREC ref: 14033B), Department of Education and Early Childhood Development and the Australian Breastfeeding Association. The trial was registered with Australian New Zealand Clinical Trials Registry (ANZCTR): 12616000512426—anzctr.org.au after recruitment, but prior to unblinding and data analysis, as this

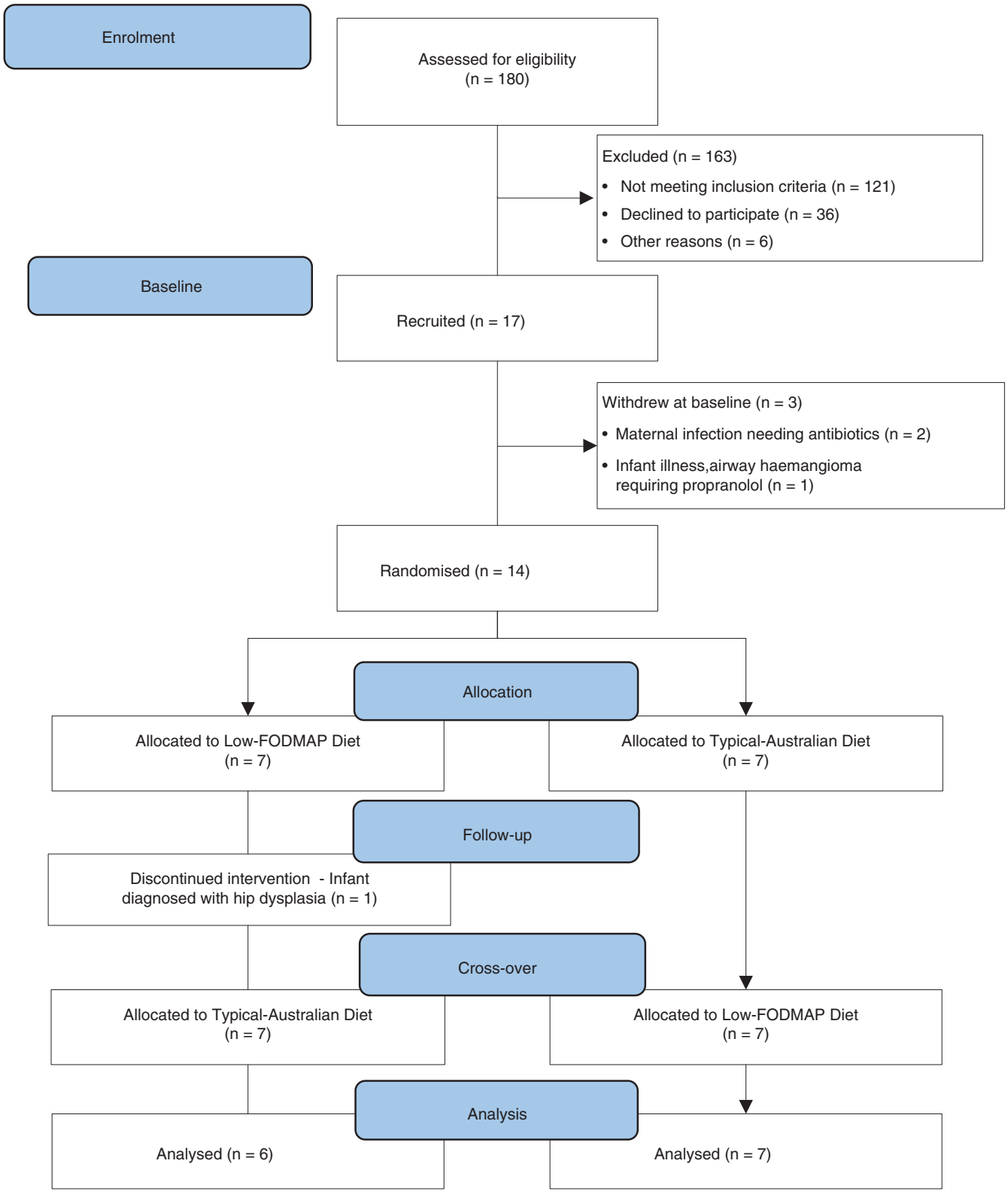

FIGURE 1 Consort flow diagram of randomised controlled trial of infants with colic. After 180 were assessed for eligibility, 17 were recruited and three withdrew at baseline. Fourteen were randomised to either the low-FODMAP diet or the typical-Australian diet. Thirteen completed the study and were analysed

obligation was overlooked. Importantly, the study protocol and primary end points, as defined prior to blinding, and conduct of the study, did not waiver from that approved and registered except for an unplanned interim analysis. This was necessary due to the difficulty in identifying eligible participants mainly due to the inclusion criterion of exclusive breastfeeding.

TABLE 1 Characteristics of mother-infant pairs who completed the study. Results are shown as number or mean (range)

	Characteristics	With colic (n = 13)	Without colic (n = 7)
Infant	Gender: Boys/Girls	6/7	2/5
	Order of birth		
	• First child	8	2
	• Second child	3	3
	• Third child	1	2
	• Fourth child	1	—
	Age at study entry (weeks)	6.3 (3.3-8.7)	7 (3.9-9)
	Weight at birth (kg)	3.5 (2.9-4.2)	3.8 (3.1-4.9)
	Gestational age (weeks)	39.6 (38-41.4)	40.1 (38.7-42)
	Mode of delivery—Caesarean	3 (23%)	3 (43%)
Mother	Age (years)	34 (28-41)	35 (32-40)
	Body mass index (kg/m^2)	24 (21-31)	25 (20-31)
	Marital status		
	• Married	11	6
	• De facto	2	1
	Irritable bowel syndrome	4 (31%)	3 (43%)
	Level of education		
	• Technical/vocational	1	1
	• Bachelor degree	6	3
	• Postgraduate or higher	6	3

2.3 | Dietary FODMAP analysis of breast milk and maternal diet

The content of known dietary FODMAPs for breast milk was determined in undiluted samples and of study diets in freeze-dried food samples that were extracted using 1 g/100 mL of deionised water in triplicate. Protocols and methods are described elsewhere in detail.[17,18] Briefly, analysis was undertaken to measure the content of fructose, glucose, lactose, xylose, sucrose, fructo-oligosaccharides (nystose, kestose), galacto-oligosaccharides (raffinose, stachyose) and polyols (sorbitol and mannitol) using high-performance liquid chromatography (Waters 515 pump, Milford, MA, USA) and ultra-high-performance liquid chromatography, both with an evaporative light scattering detector (Waters 2424 detector][17-19] and enzymatic assays for total fructans (Fructan HK Assay; Megazyme International Ireland Ltd, Wicklow, Ireland; AOAC Method 999.03 and AACC Method 32-32.01). For maternal diets, FoodWorks 8 (Xyris Software [Australia] Pty Ltd, Spring Hill, Queensland, Australia) was used to evaluate nutrient and energy composition at baseline and for each of the 10-day study diets. A modified FoodWorks database containing FODMAP data from Monash University was used to evaluate FODMAP intake of baseline diets. Resistant starch was measured using published food composition tables[20,21] and Monash University's modified FoodWorks database.

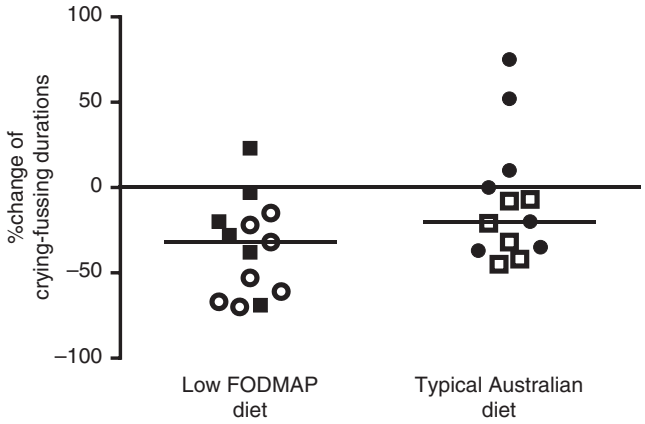

FIGURE 2 Percentage change of average time/d of crying-fussing durations over days 8-10 of a typical-Australian diet and a low-diet. Open shapes represent the first diet, and coloured shapes the second diet. The lines represent % median change regardless of order of diet. The effects of the diets were significantly different (P = 0.03; Wilcoxon signed rank test)

2.4 | Infant faecal sample analysis

Infant faecal samples were thawed and analysed for pH at room temperature using a protein-resistant pH probe (Metler Toledo LE 427, Columbus, OH, USA). Calprotectin was measured in extracts (Calpro EasyExtract™ tubes) by ELISA (CALPRO® Calprotectin ELISA Test; CALPRO AS, Lysaker, Norway) in duplicate as per manufacturer's instructions.[22]

2.5 | End points

The primary end point was the change in the duration of combined infant crying and fussing at the end of each dietary intervention (average of days 8-10) relative to that measured during 3 days prior to the commencement of the diet. This was assessed in two complementary ways—percentage change since clinical relevance has traditionally been expressed in terms of percentage change in crying and fussing durations,[5,13] and in terms of absolute time. Secondary end points included the duration of infant feeding, sleeping and periods of awake and content, maternal well-being (anxiety, depression and stress) and faecal and breastmilk indices measured.

2.6 | Maternal study diets

For each diet, daily meals comprised three main meals, one mid-morning and one afternoon snack and one supper meal or snack. The participant was responsible for supplying perishable items of fresh fruit and vegetables for which a detailed list of suitable items,

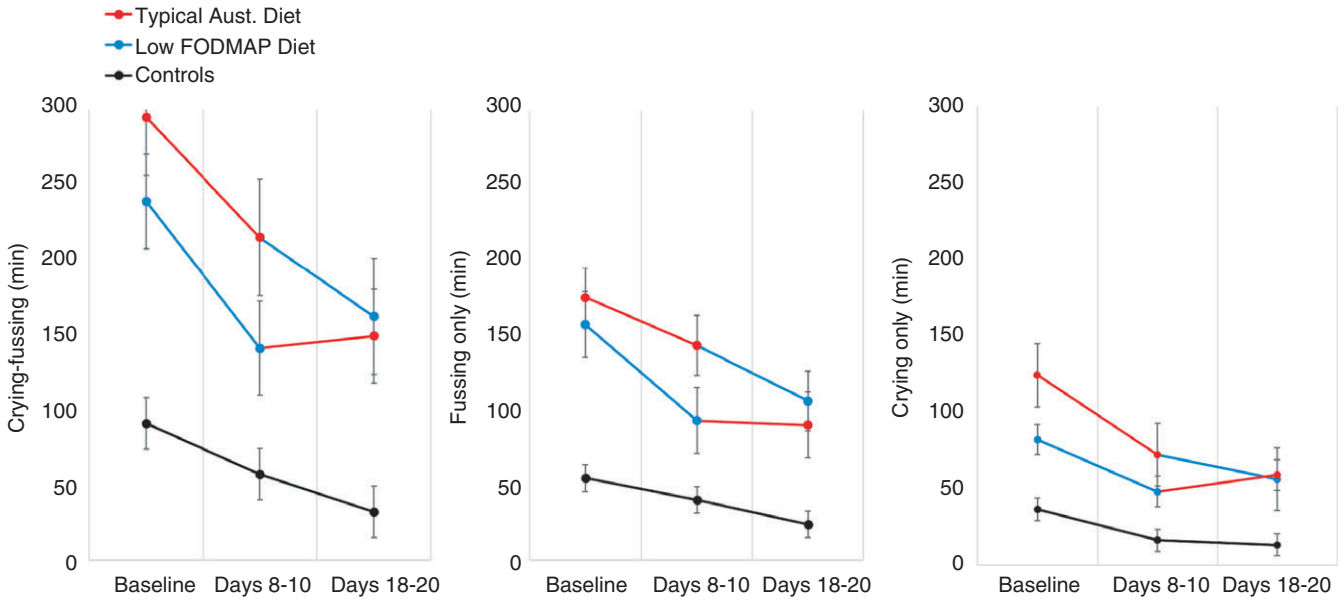

FIGURE 3 Mean (SEM) times/d for combined crying-fussing, and fussing and crying separately during the last 3 days of baseline and days 8-10 during each dietary intervention period. The blue line represents the low-FODMAP diet; red line, the typical-Australian diet; and the black line, the infants without colic where the mother followed her habitual diet. At baseline, for infants without colic crying and/or fussing were significantly less (<0.05; unpaired *t* test) compared to infant colic groups, but were not significantly different between infant colic groups (>0.05; unpaired *t* test) when comparing mothers who commenced a low-FODMAP diet first to those who commenced a typical-Australian diet first

TABLE 2 Infant behaviours during the baseline period (n = 13) and following a maternal low-FODMAP diet and a maternal typical-Australian diet. Results are shown as mean (95% confidence intervals). Analysis was performed by two-way mixed-model analyses-of-variance (order and diet) with adjustment for multiple comparisons using post hoc test least significant difference

	Diet			*P*-value		
Infant behaviour	Habitual	Typical Australian	Low FODMAP	Habitual vs typical Australian	Habitual vs low FODMAP	Typical Australian vs low FODMAP
Combined crying and fussing durations (min/d)	269 (224-314)	185 (135-234)	152 (102-203)	0.001	0.001	0.049
Crying durations (min/d)	102 (65-139)	66 (40-93)	52 (25-80)	0.012	0.009	0.136
Crying episodes (n/d)	5 (3-7)	5 (3-7)	4 (1-7)	1.0	0.7	1.0
Fussing durations (min/d)	167 (127-206)	118 (82-155)	100 (63-135)	0.002	0.001	0.089
Awake and content durations (min/d)	189 (142-236)	297 (239-355)	332 (267-398)	0.001	0.001	0.039
Feeding durations (min/d)	208 (164-253)	160 (126-194)	163 (120-206)	0.001	0.001	0.259
Feeding episodes (n/d)	9 (8-10)	8 (7-9)	8 (7-9)	0.52	0.462	0.193
Sleeping durations (min/d)	774 (730-181)	799 (755-843)	793 (747-838)	0.270	0.424	0.661

as well as drinks and alternative meals, was provided. Diets were constructed to be low or typical in FODMAP content, the latter aiming to match the dietary composition previously defined in an adult Australian population,[23] by choosing ingredients as guided by the Monash University FODMAP database. The primary ingredients (eg, protein source of fish, chicken, tofu), the names of dishes and the colour and texture of all meals were similar. Ingredients were also disguised either by hiding them in sauces and baked foods or by keeping flavours consistent. For instance, there were low-FODMAP and a moderate-high FODMAP versions of stir-fries, pasta dishes, fish meals, savoury muffins and breakfast muesli. Onion and garlic (both high FODMAP) were replaced with garlic-infused oil, chives and/or the green part of spring onion (all low-FODMAP). Herbs and spices remained consistent throughout. Vegetables such as snow peas, pumpkin, cabbage and mushrooms (all high FODMAP) were replaced with low-FODMAP vegetables of the same colour or texture such as, green beans/green capsicum, carrots and bean sprouts. Wheat- and rye-based flours/breads (high FODMAP) were replaced with spelt-sourdough breads/flour (low-FODMAP and contain gluten) and a mixture of corn-, quinoa- and potato-based

TABLE 3 Mean (range) daily energy, nutrient and FODMAP composition of the provided maternal low-FODMAP and typical-Australian diets. A paired *t* test analysis was performed on raw data

Energy/nutrients/FODMAPs	Provided typical Australian diet	Provided low FODMAP diet	*P*-value
Energy (kJ)	9929 (9457-10 364)	10 242 (9747-10 550)	NS
Protein (g)	82 (74-87)	85 (72-92)	NS
Total fat (g)	79 (60-91)	94 (78-126)	NS
Carbohydrate (g)	316 (277-356)	299 (246-322)	NS
Sugars (g)	125 (97-148)	129 (101-156)	NS
Fibre (g)	33 (25-42)	33 (24-43)	NS
Resistant starch (g)	3.41 (2.71-4.59)	3.34 (2.55-4.31)	NS
Total FODMAPs (g)	20.32 (13.69-29.97)	3.34 (2.30-4.70)	<0.0001
Total Oligosaccharides (g)	8.68 (4.70-12.42)	2.39 (1.66-3.65)	0.0137
Galacto-oligosaccharides (g)	1.36 (0.62-2.92)	0.20 (0-0.84)	0.0129
Fructans (g)	7.32 (4.08-9.50)	2.19 (1.40-3.58)	0.0152
Fructose in excess of glucose (g)	7.74 (5.87-12.30)	0.55 (0.07-1.40)	0.002
Total polyols (g)	3.44 (2.83-4.28)	0.02 (0-0.04)	<0.0001
Sorbitol	3.09 (2.89-3.34)	0	<0.0001
Mannitol	0.35 (0.06-0.94)	0.02 (0-0.04)	NS
Lactose (g)	0.46 (0.18-0.97)	0.50 (0-0.87)	NS
Gluten, dairy, fish, nuts, soy and egg	Present	Present	—
Food chemicals: amines, salicylates and glutamates	Present	Present	—

NS, not statistically significant.

flours. For vegetarians and pescatarians, suitable alternative meals in the menu plan were provided. The diets were designed to ensure that breastfeeding mothers met their estimated energy requirements including an additional 2 MJ due to the energy expenditure associated with lactation.[24] Matching of the diets for total fibre was achieved by adding unprocessed oat-bran to meals. Both diets were low in lactose.

2.7 | Blinding of study diets

Signed participant information consent forms did not name or describe the two study diets—only that two diets would be provided to examine if any of them had benefit in improving infantile colic. There was no mention of FODMAPS or a typical-Australian diet in the participant information and consent form or during the study. Mothers remained blinded to the nature of the two study diets using the food preparation techniques and design as outlined above. For each participant, study meals for each study diet were coded and placed in cooler bags in a commercial kitchen freezer, located remotely from the Principal Investigator at Notting Hill, Monash University. A second code was applied to the freezer bags. Study codes were kept in a secured computer file. Only one person, not involved in the study, knew the study codes and was responsible for passing on the meals to the courier. Codes, to which the researcher, laboratory staff, statisticians, courier and supervisors were blinded, were also applied to sample collection containers and study materials. Unblinding occurred only after laboratory analyses were completed and the database locked. At study completion, participants were asked if they could identify any differences between the two study diets. Responses were documented by the researcher.

2.8 | Dietary compliance and tolerance

Dietary compliance was assessed according to the types rather than quantity of foods consumed on each diet, as reported by participants in their daily food diary entries and by the return of food containers. Participants were compliant when 90% of intake, regardless of quantity, was appropriate to that dietary intervention arm. The mothers were encouraged to report their tolerance of the diets directly or to enter responses in their diary records.

2.9 | Data analysis

In order to achieve a medium effect size ($\eta p^2 = 0.09$) with a power of 0.8 and α of 0.05, a sample size of 18 mother-infant pairs were required. Results showed the power calculations to be conservative with a large effect size ($\eta p^2 > 0.25$). Due to difficulties in recruitment, an interim analysis of the primary end point only was performed after 13 mother-infant pairs had completed the interventions by the statistician without unblinding the identity of the diets. It was found that one of the study diets had a significant effect. Recruitment was subsequently stopped.

Statistical calculations were performed with SPSS statistical software version 23 using the intention-to-treat principle and included participants who had evaluable data from both dietary arms. The primary end point was examined in two ways. First, the

TABLE 4 Actual dietary intake of mothers with and without colicky infants during the baseline and dietary intervention periods. Results are shown as mean (range) in g/day unless otherwise stated. Sub-analysis (paired *t* test) of two-way analyses-of-variance measures are shown

		Mothers of infants with colic (n = 13)			*P*-values			
Food content	Control (n = 7) Baseline diet	Baseline diet	Typical-Australian diet (TAD)	Low-FODMAP diet (LFD)	Baseline control vs colic	Baseline vs TAD	Baseline vs LFD	TAD vs LFD
Energy (kJ/d)	9894 (8169-13003)	9290 (6184-10983)	8957 (5978-10518)	9089 (6685-10556)	NS	NS	NS	NS
Protein	103 (63-141)	97 (60-137)	102 (68-144)	100 (80-115)	NS	NS	NS	NS
Total fat	96 (76-142)	89 (35-117)	90 (55-116)	90 (57-111)	NS	NS	NS	NS
Carbohydrate	253 (211-304)	236 (163-298)	213 (151-290)	225 (161-286)	NS	NS	NS	NS
Sugars	111 (67-148)	94 (63-146)	91 (54-181)	103 (65-163)	NS	NS	NS	NS
Fibre	29 (22-44)	30 (10-49)	30 (22-41)	27 (23-33)	NS	NS	NS	NS
Resistant starch	1.94 (0.88-3.89)	2.51 (0.87-4.81)	3.82 (2.24-6.64)	2.74 (1.07-3.64)	NS	0.005	0.421	0.01
Total FODMAPs	15.69 (5.67-25.12)	20.59 (9.09-36.48)	17.68 (10.90-22.21)	3.95 (3.14-6.97)	NS	NS	0.001	0.001
Total oligosaccharides	3.58 (3.01-4.03)	8.22 (4.04-18.75)	11.57 (7.96-16.11)	2.43 (1.89-3.40)	0.025	0.011	0.001	0.001
Galacto-oligosaccharides	0.61 (0.29-0.92)	1.69 (0.29-3.68)	1.36 (0.58-3.52)	0.22 (0.04-0.47)	0.044	NS	0.002	0.001
Fructans	2.97 (2.30-3.48)	6.53 (3.21-15.14)	10.21 (7.02-14.91)	2.21 (1.69-3.00)	0.032	0.001	0.002	0.001
Fructose in excess	1.06 (0.37-2.12)	3.08 (0.70-8.81)	4.11 (2.18-6.08)	0.88 (0.29-2.60)	0.063	NS	0.003	0.001
Total polyols	0.81 (0.54-1.51)	2.34 (0.35-9.43)	1.4 (0.68-4.08)	0.13 (0.01-0.64)	0.099	0.06	0.007	0.001
Sorbitol	0.39 (0.29-0.58)	1.87 (0.21-8.72)	1.16 (0.53-3.57)	0.05 (0-0.28)	0.099	NS	0.015	0.001
Mannitol	0.42 (0.12-1.09)	0.47 (0.01-1.10)	0.24 (0.15-0.51)	0.08 (0.01-0.60)	NS	0.028	0.005	0.018
Lactose	10.24 (1.20-19.01)	6.95 (0-26.23)	0.59 (0-1.30)	0.51 (0.33-0.93)	NS	0.004	0.004	0.408
Gluten/dairy[a]/ fish/nuts/soy/egg		Present	Present	Present		—	—	—
Food chemicals: amines, salicylates and glutamates		Present	Present	Present		—	—	—

TAD, typical-Australian diet; LFD, low-FODMAP diet.
[a]Dairy was not consumed by one participant at baseline.

percentage change in crying and fussing durations were compared using a Wilcoxon signed rank test. Secondly, a two-way mixed-model analyses-of-variance (ANOVA) (order and diet) was performed for infant behaviour variables. Similar analyses were performed for actual maternal daily dietary intakes at baseline and during the typical-Australian and low-FODMAP diets, maternal psychological status, lactose content of breast milk and pH of infant faeces. Adjustments were made for multiple comparisons using post hoc test least significant difference. Shapiro-Wilk and Levene's tests were used to evaluate the assumptions of normality and homogeneity of variance, respectively. For normally distributed data, analyses of covariance were used to determine if infant age, a major confounding factor of infant colic studies, had any effect. A Friedman's test was performed where the sample data was not normally distributed and for the noncontinuous variables. Psychological variables were compared between mothers of colicky and noncolic infants using the Wilcoxon rank sum test. A *P*-value of 0.05 or less was regarded statistically significant.

3 | RESULTS

3.1 | Participants

As shown in Figure 1, between September 2014 and March 2016, 180 pairs of mothers and colicky infants were assessed for eligibility. Inclusion criteria were not met in 121 pairs mainly due to infant age (>9 weeks, n = 59/121) and introduction of infant formula (complete formula-feeding n = 20/121 and combined breast-feeding and formula-feeding, n = 24/121). Of the 17 mother-infant pairs recruited, three withdrew from the study during baseline, with one infant having an airway hemangioma causing respiratory obstruction thereby requiring propranolol and two mothers with infections requiring antibiotic treatment. Of the 14 mother-infant pairs randomised, one withdrew during the first arm when the infant was diagnosed with hip dysplasia requiring a hip brace. Hence, 13 pairs had evaluable data from each dietary arm. Seven of the 13 pairs were randomised to a typical-Australian diet first.

Participant characteristics of mother-infant pairs are outlined in Table 1.

3.2 | Effect of maternal diet on infant behaviour

At baseline, crying-fussing times of infants without colic had a mean (95% CI) 91 (68, 114) minutes compared to 269 (224, 314) minutes in infants with colic ($P < 0.0001$, t test). In addition, at baseline, crying-fussing times in infants whose mothers commenced a low-FODMAP diet first had a mean of 239 (173, 305) minutes, which was not different to 295 (221, 368) minutes in infants whose mothers commenced a typical-Australian diet first ($P = 0.19$; unpaired t test). The changes that occurred from the average crying-fussing times during the 3 days prior to that over the last 3 days of each diet are shown in Figure 2. The median change of 32% with the low FODMAP diet was significantly greater than 20% during the typical Australian diet ($P = 0.03$; Wilcoxon signed rank test). As is also shown in Figure 2, no order effect was evident ($P = 0.19$). Details of the changes of crying-fussing, crying and fussing times over time are shown in Figure 3.

The effects of maternal diet on infant behaviour was further analysed by analyses of covariance, as shown in Table 2. The two interventional diets were also found to be significantly different with respect to crying-fussing times ($P = 0.049$) with a large effect size ($\eta_p^2 = 0.719$). There were as expected significant main effects of maternal low-FODMAP and typical-Australian diet compared with the baseline diet for crying-fussing durations. However, there was no effect of maternal diet ($P > 0.05$) on infant behavioural variables, crying episodes, fussing episodes and sleeping durations. Importantly, there were no significant effects of the order of maternal diet, and no interaction between diet and order, and infant age had no significant influence on any of the infant behaviour variables.

3.3 | Dietary analysis of maternal intake

The provided diets were well matched on all indices except the average daily intake of FODMAPs, this being 20.32 g/d for the typical-Australian and 3.34 g/d for the low-FODMAP diet (Table 3). However, actual intake (Table 4) differed not only for FODMAP content, but also for resistant starch, which was higher during the typical-Australian diet ($P = 0.01$; $\eta_p^2 = 0.432$). While there were no significant differences in the total FODMAP intake on the typical-Australian diet when compared to baseline, the types of FODMAPs varied. Baseline dietary intake of the control mothers differed in that the FODMAP intake was considerably less (Table 4). The dietary intake did not change over the ensuing 20 days of observation (data not shown).

3.4 | Dietary compliance and tolerance

Participants were compliant ≥98% of the time except one who had 70% compliance during the low-FODMAP arm. Participants reported that the meals were plentiful, enjoyable and provided variety. On direct questioning, all 13 participants reported that the diets were similar, consisted of the same meals and were not able to identify what "type" of diet they were following both during and at the end of each study diet. Adverse events were uncommon; one participant

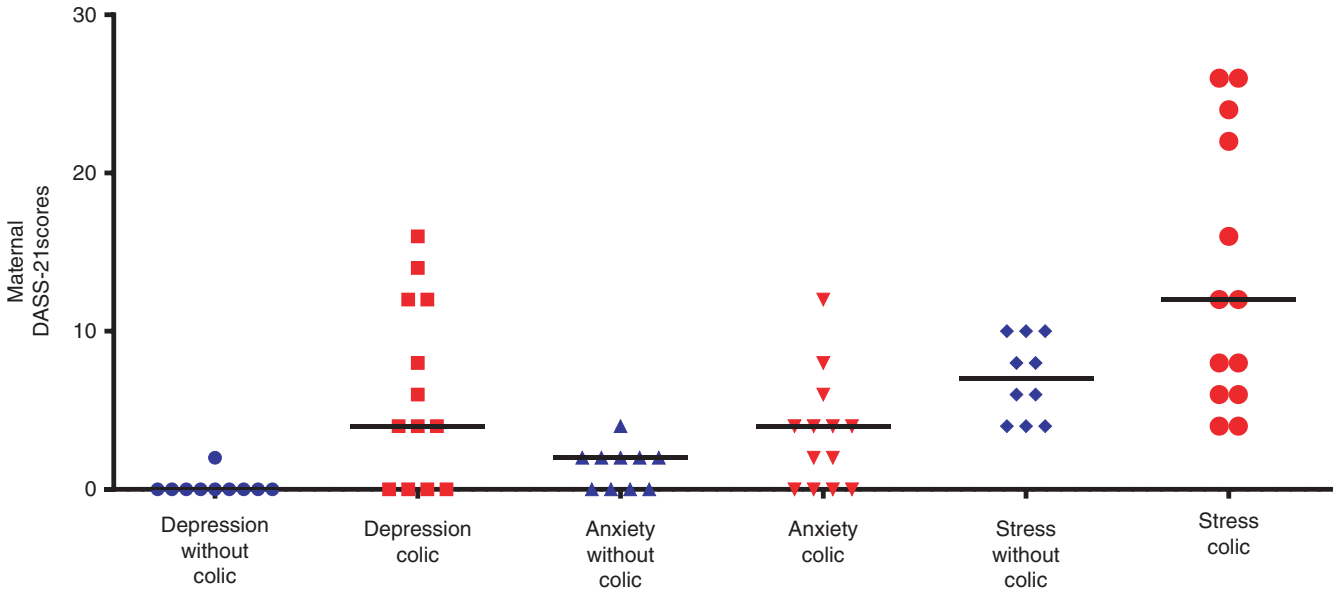

FIGURE 4 Baseline comparisons of depression, anxiety and stress scores between breastfeeding mothers whose infants had (n = 13) and were without colic (n = 10). Noncolic group includes seven mothers who completed the study and three who completed baseline and met noncolic criteria, but withdrew from the prospective study. The bars represent median values. Only depression scores were statistically different between infant with and without colic ($P = 0.002$); Wilcoxon rank sum test)

had bloating on the low-FODMAP diet, and gas and abdominal pain on the typical-Australian diet. Another had gas and more bowel actions on the typical-Australian diet.

3.5 | Maternal mental well-being

During the baseline period, mothers of infants with colic had higher Depression Anxiety and Stress Scale scores than those of infants without colic for depression ($P = 0.002$) and tended to be higher for stress and anxiety (Figure 4). During the typical-Australian diet, the changes in mean scores for anxiety and stress were significantly lower compared to baseline scores and stress was lower when compared to the low-FODMAP dietary arm, where no changes were observed (Figure 5). Maternal anxiety fell from a median of 4 to 0 ($P = 0.009$), depression 4 to 2 ($P = 0.03$) and stress 12 to 8 ($P = 0.004$) in the typical-Australian diet, but not the low-FODMAP diet where anxiety changed from 4 to 2, with no change for depression, and for stress from 12 to 10. There were no significant changes for maternal depression in the dietary intervention groups throughout and the control group showed no overall change in any index across time points (Figure 5).

3.6 | Infant faecal analysis

There were no consistent changes in infant stool frequency with either diet and the frequency was similar to that of the noncolicky infants (data not shown). Only complete sets of three technically suitable samples of faeces were analysed. Faecal pH at baseline was 5.8 (range 4.8-7.2) in six infants with colic and 6.2 (5.5-7.5) in five controls. No change was observed over the 20 days of dietary intervention for either diet or in the controls (data not shown). Mean faecal calprotectin at baseline was 343 (65-1158) μg/g in nine infants with colic and 522 (138-794) μg/g in five controls ($p = 0.24$; t test), and tended to fall over the study period in infants with and without colic. The rate of fall was similar for both dietary interventions and in the control group (Figure 6).

3.7 | Breast milk FODMAP analysis

Trace amounts only of glucose, fructans, fructose, sorbitol and mannitol were detected but known dietary galacto-oligosaccharides were not. Lactose was present at 4.9 (3.1-5.6) g/100 mL during baseline in the control and 5.1 (3.1-5.8) g/100 mL in treated cohorts, and these did not change with time or diet (data not shown).

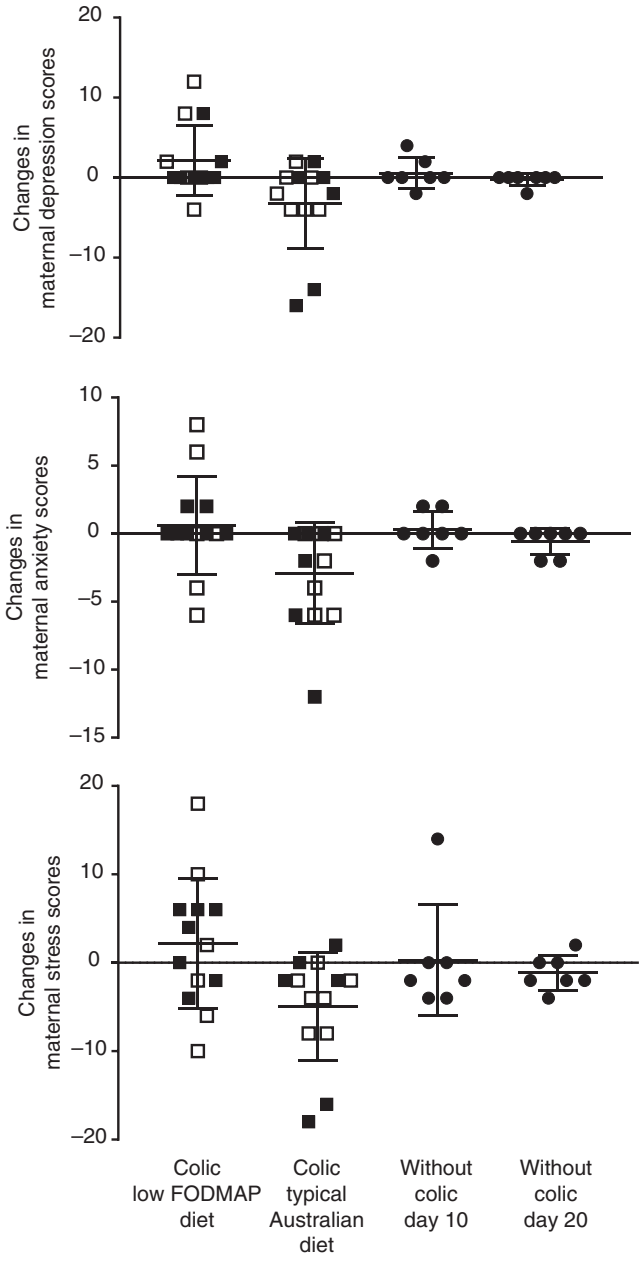

FIGURE 5 Changes in maternal depression, anxiety and stress scores in mothers of infants with colic (n = 13) during the low-FODMAP diet and the typical-Australian diet compared to the changes in scores over the similar time periods in mothers of infants without colic (n = 7). For colic group, open shapes represent first dietary intervention and closed shapes the second. Day 10 and 20 for noncolic group represent the days after completion of the baseline observations. On a two-way mixed-model analyses-of-variance (order and diet) for the colicky infants, significant fall in the maternal anxiety ($P = 0.009$), depression ($P = 0.03$) and stress ($P = 0.004$) were observed with the typical-Australian diet, but not the low-FODMAP diet. There were no other statistically significant differences

4 | DISCUSSION

The change in crying-fussing duration associated with infantile colic was significantly greater while a low-FODMAP diet was being consumed by the exclusively breastfeeding mother than compared to that with a typical-Australian diet. The reduction in crying-fussing duration associated with the typical-Australian diet was only

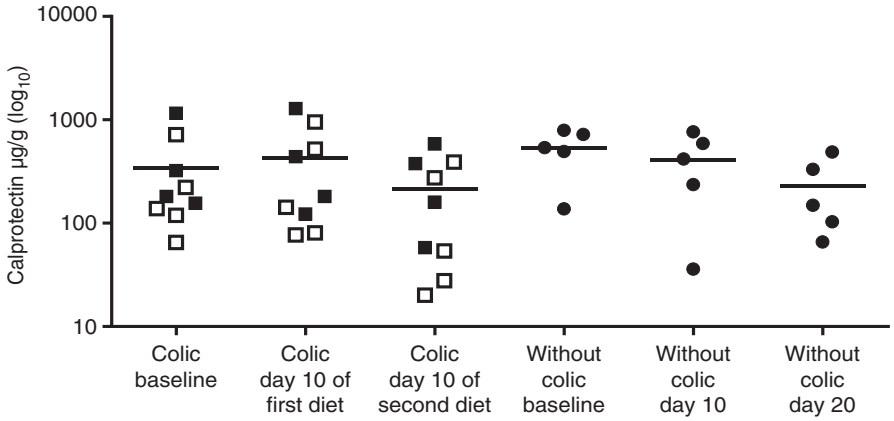

FIGURE 6 For colic, infant faecal calprotectin measures at baseline, day 10 of the first diet and day 10 of the second diet. Open shapes represent low-FODMAP and closed shapes typical-Australian diets. Only nine complete sets (three samples) from 13 participants could be analysed for infant faecal calprotectin. There was a significant difference over time (P = 0.04; one-way analyses-of-variance), but a sub-analysis paired t test found no difference between time points. For infants without colic (n = 5) there was a significant decrease from mean baseline 538 µg/g to the end of the study 228 µg/g (P = 0.024; one-way analyses-of-variance)

apparent in infants of mothers who were allocated that diet first, consistent with the natural resolution of colic symptoms over time. As the second diet, there was either no further improvement or increases in crying-fussing duration, consistent with the efficacy exerted by the preceding low-FODMAP diet. The magnitude of improvement with the low-FODMAP diet (median 32%) was remarkably similar to that observed in the proof-of-concept study of 18 mother-infant pairs (mean 30%).[11] The benefit was not related to an order effect and was independent of the infant's age. Whether the often-quoted >25% reduction in crying-fussing times as an index of clinical significance of an intervention is valid,[5,13] this was considerably surpassed in most of the infants in association with the low-FODMAP diet.

Additional indirect observations supported the efficacy of the low-FODMAP diet and debunked some behavioural hypotheses of underlying reasons for infantile colic. First, awake and content times significantly increased following the low-FODMAP diet. This provides strong evidence to reject the notion that infants who are awake for long periods of time are "over-stimulated", resulting in excessive crying-fussing.[25] Secondly, infant sleeping times remained stable throughout the study, dismissing the notion that excessive crying-fussing in infants is a result of an 'over-tired' infant due to insufficient sleep.[25] Finally, feeding durations significantly decreased, indicating that crying-fussing times were not related to a hungry infant, another factor previously associated with infant crying.[26]

Interestingly, significant reductions in maternal anxiety and stress occurred while following the typical-Australian, while no change was observed with the low-FODMAP diet. The scores in most mothers were within the normal range and the magnitude of diet-associated changes was relatively small, questioning their clinical significance. Reasons for these specific effects are unclear. Maternal psychological indices were higher than in mothers of infants without colic, a finding that was anticipated. However, whether such effects have a pathogenic role in infantile colic cannot be addressed by this study, except to imply that greater improvement of colic with the low-FODMAP diet was not occurring via such a mechanism.

The use of parallel protocols for infants with and without colic enabled a valid comparison between the measures examined in order to improve understanding of mechanisms underlying infant colic. They confirmed that crying and fussing do reduce over time whether severe (as in the colicky infants regardless of diet) or mild (as in the controls). The main differences between groups were crying-fussing durations, and these were threefold greater in infants with colic. Interestingly, six of the seven mothers of infants without colic reported changing their habitual diet early post-partum because they believed certain foods would trigger colic. Of particular interest was a significantly lower intake of oligosaccharides by mothers of infants without colic. Whether a maternal low-FODMAP diet prevented the occurrence of infantile colic in the control group cannot be determined in the present study, but is an interesting concept that warrants further research.

A potential problem in demonstrating efficacy of dietary change is colinearity. This issue was exemplified by apparent efficacy of hypoallergenic maternal diet,[13] which may be inadvertently low in FODMAPs via removing wheat. For this reason, care was taken in the design of both diets of the present study to ensure inclusion of potentially allergenic foods, as well as keeping other putative factors such as food chemicals similar in each arm. Beneficial effects of the low-FODMAP diet were, therefore, unlikely to be related to the exclusion of food allergens and chemicals.

Manifestations of infantile colic appear to be consistent with abdominal pain of intestinal origin, poor gastrointestinal tolerance to dietary factors, gastrointestinal dysbiosis and gastrointestinal inflammation,[27] but without strong evidence. In the current study, gross changes in intestinal fermentation were unlikely as faecal pH, which is largely dependent upon the degree of fermentation by colonic microbiota,[28] was similar with each dietary intervention. Likewise,

changes in intestinal inflammation were unlikely due to similar patterns of faecal calprotectin in all infants, although the use of this marker in infants is less established than it is in adults.[29] Indeed, the levels fell over time in the present study, probably reflecting increasing maturity of the infant's gut.[30] Thus, the conclusion that improvement in colic in association with a maternal low-FODMAP diet is not associated with altered gastrointestinal inflammation must be guarded.

A strong candidate in the link between maternal diet and abdominal symptoms in the infant is the breast milk. The composition of the milk can change in terms of, for example, its soluble molecular content or microbiota. Of importance to infant gut microbiota and health are human milk oligosaccharides, which are unique to breast milk.[31,32] What is known, is that fucosyl-oligosaccharides are common and protect against infection by enteric pathogens. In particular, 2′-fucosyllactose accounts for more than 30% of total human milk oligosaccharides.[33] Low levels of 2′-fucosyllactose have also been linked to higher rates of diarrhoea in breastfed infants.[34] Measuring these oligosaccharides is a highly specialised area,[35] and although outside of the scope of the present study, exploring the effects of diet on them is an attractive concept and is worthy of further exploration.

Research into the microbiota of breast milk is in its infancy, but it is presumed that its composition will influence gastrointestinal microbiota in the infant. Maternal factors that influence such composition are poorly understood, but the migration of bacteria from the gut of the mother via dendritic cells to the breast milk has been documented in experimental animals.[31] Altering the content of FODMAPs in the diet will change the composition of the host faecal microbiota, but whether that has any influence on breast milk bacteria composition remains to be determined.

The concept that foods such as garlic, onion and cabbage (high FODMAP-containing foods) alter human milk is not new. In an earlier study, after ingestion of garlic by breastfeeding mothers, the sensory qualities of the milk were altered to produce a garlic-like odour.[36] This change was observed to take as little as 2-3 hours. Early animal studies have shown that major sulphur-containing volatiles (natural food chemicals) found in garlic are transmitted to the milk.[37] Since then, it has been identified that the odour change in milk is not actually related to a direct transfer of garlic odorants, but rather garlic-derived metabolites. One of these is allyl-methyl-sulphide, which has also been shown to appear in breast milk in as little as 2.5 hours after maternal ingestion.[38] Other sulphur-containing foods include onion and cruciferous vegetables such as cauliflower, cabbage and Brussels sprouts —all of which are high in FODMAPs. Whether food chemicals or metabolites such as allyl-methyl-sulphide have an effect on gut function is unknown. However, recent studies have shown that a low-FODMAP diet in patients with IBS reduced urinary histamine levels and increased in p-hydroxybenzoic acid and azelaic acid.[39] Although further studies are needed to understand the variability of these metabolites and their relationship with IBS symptoms, they may provide another clue to the efficacy of a maternal low-FODMAP diet for the treatment of infantile colic in breastfed infants.

The current study has limitations. The small sample of participants somewhat limits the strength of the observations made and may impact on generalisability of the results. Recruitment of exclusively breastfeeding participants is very challenging as it is uncommon in Australia, with only 39% of infants exclusively breastfed for 3 months and ~40% are introduced to other fluids by 1 month of age.[40] Furthermore, a common response to colic is to change the feeding method, especially by introduction of supplements. The time constraints of studying therapeutic strategies in infants with colic are also challenging. Contacting eligible mothers and decisions regarding involvement in the study needs to be made rapidly. Despite the small number, however, the effect size was large and the study was deemed sufficiently powered following an interim analysis. The provision of all meals was a major strength in that it enabled the hypothesis to be addressed due to high dietary adherence and reduced likelihood of other potentially confounding dietary changes. Conversely, provision of all meals does not represent a realistic setting. Transmission of a placebo effect to an infant is difficult to conceive.

In conclusion, a low-FODMAP diet was associated with a clinically significant improvement in crying-fussing durations of infants with colic compared with a typical-Australian diet. Potentially confounding factors such as maternal stress and anxiety, insufficient infant sleep and feeding and infant over-stimulation were not associated with improvement. As a short-term intervention, the results might encourage the use of a low-FODMAP diet in breastfeeding mothers who have an infant with colic. While the mechanism remains unclear, future work will need to define the mechanisms of action by which changes in maternal dietary change can influence infant behaviour. Not only will this inform our understanding of the pathophysiology of infantile colic, but may also have wider implications regarding influences over infant behaviour.

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AUTHORSHIP

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manuscript, conducted the literature search, data interpretation and preparation of tables and figures. SSC provided support in the recruitment of participants and contributed to the manuscript. GWY provided assistance in statistical analysis and data interpretation and contributed to the manuscript. JSB and JGM assisted with the study design and contributed to the manuscript. PRG assisted with the study design, interpretation of data and contributed to the manuscript.

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REFERENCES

1. Barr RG, Trent RB, Cross J. Age-related incidence curve of hospitalized Shaken Baby Syndrome cases: convergent evidence for crying as a trigger to shaking. *Child Abuse Negl*. 2006;30:7-16.
2. Morris S, St. James-Roberts I, Sleep J, Gillham P. Economic evaluation of strategies for managing crying and sleeping problems. *Arch Dis Child*. 2001;84:15-19.
3. Wessel MA, Cobb JC, Jackson EB, Harris GS Jr, Detwiler AC. Paroxysmal fussing in infancy, sometimes called colic. *Pediatrics*. 1954;14:421-435.
4. Halpern R, Coelho R. Excessive crying in infants. *J Pediatr*. 2016;92:S40-S45.
5. Iacovou M, Ralston RA, Muir J, Walker KZ, Truby H. Dietary management of infantile colic: a systematic review. *Matern Child Health J*. 2012;16:1319-1331.
6. Abdallah E, Elneim A. Dietary habits during the postpartum period among a sample of lactating women in Sudan. *J Nurs Health Sci*. 2014;3:1-6.
7. Maldonado M, Chomat AM, Solomons N. Food avoidance: potential impact on dietary variety during pregnancy and early lactation in the Mam-Mayan western highlands of Guatemala. *Ann Nutr Metab*. 2013;63:690.
8. Lust KD, Brown JE, Thomas W. Maternal intake of cruciterous vegetables and other foods and colic symptoms in exclusively breast-fed infants. *J Am Diet Assoc*. 1996;96:46-48.
9. Subbiah N, Jeganathan A. Socio-cultural beliefs influencing breastfeeding practices among primi postnatal mothers residing in urban slum area of Delhi. *Health Popul Perspect Issues*. 2012;35:61-73.
10. Gibson PR, Shepherd SJ. Personal view: food for thought - Western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Aliment Pharmacol Ther*. 2005;21:1399-1409.
11. Iacovou M, Mulcahy EC, Truby H, Barrett JS, Gibson PR, Muir JG. Reducing the maternal dietary intake of indigestible and slowly absorbed short-chain carbohydrates is associated with improved infantile colic: a proof-of-concept study. *J Hum Nutr Diet*. 2018;31:256-265.
12. Hill DJ, Hudson IL, Sheffield LJ, Shelton MJ, Menahem S, Hosking CS. A low allergen diet is a significant intervention in infantile colic: results of a community-based study. *J Allergy Clin Immunol*. 1995;96:886-892.
13. Hill DJ, Roy N, Heine RG, et al. Effect of a low-allergen maternal diet on colic among breastfed infants: a randomized, controlled trial. *Pediatrics*. 2005;116:e709-e715.
14. Evans RW, Allardyce RA, Fergusson DM, Taylor B. Maternal diet and infantile colic in breast-fed infants. *Lancet*. 1981;317:1340-1342.
15. Barr RG, Kramer MS, Boisjoly C, McVey-White L, Pless IB. Parental diary of infant cry and fuss behaviour. *Arch Dis Child*. 1988;63:380-387.
16. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behav Res Ther*. 1995;33:335-343.
17. Muir JG, Rose R, Rosella O, et al. Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J Agric Food Chem*. 2009;57:554-565.
18. Muir JG, Shepherd SJ, Rosella O, Rose M, Barrett JS, Gibson PR. Fructan and free fructose content of common Australian vegetables and fruit. *J Agric Food Chem*. 2007;55:6619-6627.
19. Halmos EP, Bogatyrev A, Ly E, Liels KL, Muir JG, Gibson PR. Challenges of quantifying FODMAPs in enteral nutrition formulas: evaluation of artifacts and solutions. *J Parenter Enteral Nutr* 2017;41:1262-1271.
20. Englyst KN, Liu S, Englyst HN. Nutritional characterization and measurement of dietary carbohydrates. *Eur J Clin Nutr*. 2007;61:S19-S39.
21. Sambucetti ME, Zuleta A. Resistant starch in dietary fiber values measured by the AOAC method in different cereals. *Cereal Chem*. 1996;73:759-761.
22. Johne B, Kronborg O, Tøn HI, Kristinsson J, Fuglerud P. A new fecal calprotectin test for colorectal neoplasia: clinical results and comparison with previous method. *Scand J Gastroenterol*. 2001;36:291-296.
23. Barrett JS, Gibson PR. Development and validation of a comprehensive semi-quantitative food frequency questionnaire that includes FODMAP intake and glycemic index. *J Am Diet Assoc*. 2010;110:1469-1476.
24. National Health and Medical Research Council. *Nutrient Reference Values for Australia and New Zealand*. Canberra, ACT: Australian Government. https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n35.pdf. Accessed 9 February 2015.
25. Lawrence RA, Lawrence RM. *Breastfeeding: A Guide for the Medical Profession*. 7th ed. Missouri: Elsevier; 2011.
26. Wood C, Kempson C, Beech C, Brooke OG. An investigation of crying in the newborn in relation to maternal and infant factors. *J Obstet Gynaecol*. 1987;7:201-205.
27. Kunze WA, Mao YK, Wang B, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J Cell Mol Med*. 2009;13:2261-2270.
28. Fleming I, Defazio J, Zaborina O, Alverdy JC. *Critical Illness and Intestinal Microflora: Ph as a Surrogate Marker. Diet and Nutrition in Critical Care*. New York: Springer; 2015:397-404.
29. Selimoglu MA, Temel I, Yildirim C, Ozyalin F, Aktas M, Karabiber H. The role of fecal calprotectin and lactoferrin in the diagnosis of necrotizing enterocolitis. *Pediatr Crit Care Med*. 2012;13:452-454.
30. Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. *J Pediatr Gastroenterol Nutr*. 2002;34:323-324.
31. Fernández L, Langa S, Martín V, et al. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res*. 2013;69:1-10.
32. Gnoth MJ, Kunz C, Kinne-Saffran E, Rudloff S. Human milk oligosaccharides are minimally digested in vitro. *J Nutr*. 2000;130:3014-3020.
33. Lee WH, Pathanibul P, Quarterman J, et al. Whole cell biosynthesis of a functional oligosaccharide, 2' -fucosyllactose, using engineered *Escherichia coli*. *Microb Cell Fact*. 2012;11:48.
34. Morrow AL, Ruiz-Palacios GM, Altaye M, et al. Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. *J Pediatr*. 2004;145:297-303.

35. Nijman RM, Liu Y, Bunyatratchata A, Smilowitz JT, Stahl B, Barile D. Characterization and quantification of oligosaccharides in human milk and infant formula. *J Agric Food Chem*. 2018;66:6851-6859.
36. Mennella JA, Beauchamp GK. Maternal diet alters the sensory qualities of human milk and the nursling's behavior. *Pediatrics*. 1991;88:737-744.
37. Bassette R, Fung DYC, Mantha VR. Off-flavors in milk. *Crit Rev Food Sci Nutr*. 1986;24:1-52.
38. Scheffler L, Sauermann Y, Zeh G, et al. Detection of volatile metabolites of garlic in human breast milk. *Metabolites*. 2016;6:18.
39. McIntosh K, Reed DE, Schneider T, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut*. 2017;66:1241-1251.
40. Australian Institute of Health and Welfare. *2010 Australian National Infant Feeding Survey: Indicator results*. Canberra: AIHW; 2011.

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