



# The Relationship Between Potential *Listeria*monocytogenes Exposure and Diet Quality and Dietary Intake During Pregnancy: A Cross-Sectional Analysis in Australian Women

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# **ABSTRACT**

**Background:** Research conducted over two decades ago indicated that more frequent consumption of foods potentially harbouring *Listeria monocytogenes* is associated with higher nutrient intakes but also higher risk of miscarriage. However, the influence of potential exposure to *Listeria monocytogenes* on pregnant women's diet quality is yet to be examined. Additionally, advancements in agricultural practices and food consumption trends in recent years may have led to changes in pregnant women's dietary intake. Therefore, the present study aimed to evaluate the associations between potential *L. monocytogenes* exposure and dietary quality, and dietary intake in two contemporary cohorts of pregnant women in Australia.

**Methods:** A secondary analysis of two combined pregnancy cohorts of women aged  $\geq$  19 years with a singleton pregnancy from the Newcastle, New South Wales (n = 441) and Perth, Western Australia (n = 1197) was conducted. Potential L. *monocytogenes* exposure was estimated by the Listeria Food Exposure Score (LFES), dietary intake was assessed using the Australian Eating Survey and diet quality using the Australian Recommended Food Score. Pearson's correlation and linear regression analyses were performed to estimate the associations between potential L. *monocytogenes* exposure and dietary quality and intake, with adjustment for potential confounders.

**Results:** Data from 1638 women (mean [SD] age 32.0 [5.0] years, 57.8% born in Australia) were included. The median (IQR) gestational age was 35 (34–36) weeks and 43.5% of women had no prior pregnancies. A higher LFES (i.e., more frequent consumption of potential food sources of *L. monocytogenes*) was significantly associated with higher diet quality score (r = 0.60, p < 0.001), higher intakes of nutrient-dense core foods (r = 0.11-0.43, p < 0.001), and higher micronutrient intakes (r = 0.24-0.52, all p < 0.001).

**Conclusion:** More frequent consumption of foods that potentially harbour *Listeria monocytogenes* is associated with higher diet quality and nutrient intakes. Further research is needed to identify how to support women to achieve optimal diet quality and nutrient intakes while simultaneously minimising risk of listeriosis.

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#### **Summary**

- In this sample of pregnant women, more frequent consumption of potential food sources of *Listeria monocytogenes* was associated with higher diet quality and nutrient intakes.
- Education should be provided to pregnant women on avoiding foods that have a high risk for harbouring *L. monocytogenes*, while focusing on lower-risk alternatives to ensure nutritional adequacy and reduce the risk of contracting listeriosis during pregnancy.
- Further training is needed for healthcare practitioners to support women in achieving optimal diet quality and nutrient intake while minimising listeriosis risk.

#### 1 | Introduction

Food safety is an important consideration in pregnancy, given that pregnant women are more likely to contract foodborne illnesses due to immune suppression associated with this physiological state [1]. Listeriosis, caused by the bacterium, Listeria monocytogenes, in its most severe form can lead to meningitis, miscarriage, stillbirth and neonatal death [2]. A systematic review and meta-analysis of 87 studies estimated that, in 2010 there were 23,150 cases of listeriosis worldwide. Of these, 20.7% were perinatal infections, and 14.9% of these resulted in neonatal death and stillbirth [3]. In Australia, a listeriosis report indicated an average of 78 cases per annum from 2010 to 2015 [4] and within the same period, a statespecific review on New South Wales (NSW) reported that, from an average of 26.3 listeriosis cases per year, seven cases (4.4%) were among pregnant women [5]. These data show that although the reported incidence of listeriosis in pregnancy is low, the infection is associated with high fatality rate, and the incidence data are likely to be under-reported [6].

In Australia, Food Standards Australia and New Zealand (FSANZ) provides dietary recommendations to minimise listeriosis risk during pregnancy [7]. Concerns have been raised that *Listeria* recommendations may overly focus on avoiding the consumption of high-risk foods rather than providing safer alternatives. Many of the food items women are recommended to avoid are rich in nutrients and are required in higher amounts during pregnancy. For example, folate in salad vegetables and iron and zinc in meats [8]. As such, while adhering to food safety recommendations may lower the risk of harbouring *L. monocytogenes* and the potential pregnancy complications, it may adversely impact the nutritional adequacy of pregnant women's dietary intake [9, 10].

Previous evidence suggested that restricting potential food sources of *L. monocytogenes* may have implications on nutritional adequacy among women. A cohort study with 7486 Australian women who were either currently pregnant, trying to conceive, or who had delivered a baby in previous 12 months identified that more frequent consumption of foods potentially harbouring *L. monocytogenes* was associated with higher intakes of essential nutrients during pregnancy [11]. This study also reported a 19% higher risk of miscarriages among women

who reported more frequent consumption of foods potentially containing L. monocytogenes, compared to women with the least frequent consumption [11]. These findings supported further investigation of the relationship between risks associated with potential exposure to L. monocytogenes and nutritional adequacy of dietary intakes during pregnancy. In addition, the dietary data was not collected specifically during pregnancy and therefore may not have been representative of the relationship between dietary patterns during pregnancy and listeriosis risk [12, 13]. Moreover, the previous study focused on the analysis on single nutrient intake in relation to listeriosis risk, with no focus on overall diet quality [11]. Further, the dietary assessment in that study was conducted over 20 years ago, which may not reflect current dietary patterns due to the advancements in agricultural practices and food consumption trends [14, 15]. It is important to understand more recent dietary patterns of pregnant women in Australia and how these may be related with the consumption of foods potentially harbouring L. monocytogenes, to identify and support the nutritional needs of pregnant women. Therefore, the aim of this study was to examine the relationship between potential L. monocytogenes exposure and diet quality and dietary intake (food group and nutrient intake) in two cohorts of pregnant women in Australia.

# 2 | Materials and Methods

# 2.1 | Study Design

The current study is a secondary analysis of combined cross-sectional data from two cohorts of pregnant women recruited via two antenatal clinics in Australia. Ethics approval was received from the Hunter New England Human Research Ethics Committee (2019/ETH00954) and University of Newcastle Human Research Ethics Committee (H-2023-0239) for the John Hunter Hospital cohort in New South Wales, Australia and Human Research Ethics Committee of Joondalup Health Campus cohort (2023/ETH/0001) for the ORIGINS cohort in Western Australia. Australia.

# 2.2 | Study Population

Participants included in the analyses were women aged  $\geq 19$  years, with a singleton pregnancy, and complete dietary data, who attended the John Hunter Hospital and Joondalup Health Campus antenatal clinics for routine antenatal care. All participants provided written informed consent.

### 2.3 | Study Recruitment

# 2.3.1 | John Hunter Hospital

Participants were recruited between March and November 2018, via invitation distributed within the John Hunter Hospital antenatal clinic waiting rooms and flyers located in the pathology department. Participants were also recruited via a media release and social media posts on the Hunter Medical Research Institute and University of Newcastle Facebook pages.

The recruitment process is described in detail elsewhere [16, 17].

#### 2.3.2 | The ORIGINS Project

The ORIGINS Project is a 10-year collaboration between the Joondalup Health Campus and the Telethon Kids Institute at Western Australia which established a longitudinal cohort of 10,000 families. Participants were recruited between 2017 and 2022 via the Joondalup Health Campus by their maternity or postnatal care provider, or by a member of the research team. The recruitment process is described in detail elsewhere [18, 19].

#### 2.4 | Data Collection

#### 2.4.1 | Socio-Demographic and Health Characteristics

The current study used self-reported sociodemographic and health data including age, weeks of gestation, birth country, language spoken, education, postcode, marital status, height, and pre-pregnancy weight collected via online survey during antenatal visits. Pre-pregnancy BMI was calculated from selfreported height and weight. Parity and smoking status were obtained from hospital medical records. Postcode is linked to the Socioeconomic Indexes for Areas (SEIFA) indexes from the Australian Bureau of Statistics (ABS) as represented by Index of Relative Socioeconomic Advantage and Disadvantage (IRSAD) quintiles [20]. The IRSAD is a composite which includes economic and social conditions variables of people and household within an area. SEIFA scores were used as a proxy for socioeconomic status. A lower score indicates relatively greater disadvantage and lack of advantage whereas a higher score suggests a relative lack of disadvantage and greater advantage.

# 2.4.2 | Potential L. monocytogenes Exposure

Potential *L. monocytogenes* exposure was assessed using the Listeria Food Exposure Score (LFES). This scoring was developed based on 11 foods from six categories (vegetables, fruit, dairy foods, meat, seafood, deli meats) within the Australian Eating Survey (AES) food frequency questionnaire (Supporting Information S1: Table S1), which are identified as potential food sources of *L. monocytogenes* based on the FSANZ public health recommendations [7]. The LFES was derived based on the reported frequency of consumption of each food. Responses ranged from 'never' (scored as zero) to 'two or more times per day' (scored up to seven points). The maximum LFES was 57 points, with higher scores indicative of more frequent consumption of foods that potentially harbour *L. monocytogenes*.

# 2.4.3 | Diet Quality

Diet quality was calculated using the Australian Recommended Food Score (ARFS) to generate a food-based diet quality score as a single continuous variable (Supporting Information S1: Table S2) [21]. The ARFS assesses dietary variety within food groups recommended in the Australian Guide to Healthy Eating (AGHE) [22] and was derived from a sub-set of 70 AES questions within eight sub-scales, of which 20 questions relate to vegetables, 12 to fruit, 12 to breads and cereals, 10 to dairy foods, seven to meat, six to meat alternatives, two to spreads/ sauces and one to water. Most food items are awarded one point for a consumption frequency of once per week or more, with exception of red meat and some dairy foods (e.g., ice-cream, frozen yoghurt), where a limit is applied for higher intakes as they are associated with higher saturated fat intake or disease risk. For these food items, zero points are awarded for a higher frequency of consumption (e.g., more than four times per week for red meat). Additional points are awarded for greater consumption of vegetables, healthier choices of breads (e.g., brown, multigrain), and milk. If participants indicate they follow a vegetarian eating pattern, they are assigned a zero score for the meat subscale, with double points awarded for each of the meat alternatives questions. An additional point is also awarded if soybeans, tofu, other beans and lentils (e.g., chickpeas, split peas) were reported as consumed once per week or more. The total score, ranging from zero to 73, is calculated by summing the points for each food item, with higher scores indicating a higher diet quality [23]. The ARFS is categorised as: 'needs work' (< 33 points), 'getting there' [24-29], 'excellent' [30-37], and 'outstanding' (> 47) [23]. The ARFS has been validated and shown high level of reliability and consistency in evaluating diet quality and estimating fruit and vegetable intakes among Australian adults [23, 38].

# 2.4.4 | Dietary Intake

Usual dietary intake of participants was assessed using the AES, a validated semi-quantitative food frequency questionnaire (FFQ) consisting of 120 questions including beverages, milk and dairy foods, breads and cereals, sweet and savoury snacks, main meals, vegetables, fruit, and other foods [39]. In addition, the AES contains 15 supplementary questions including age, use of nutritional supplements and intake-related behaviours (e.g., frequency of having takeaway meals, consumption of breakfast, eating meals in front of the television, etc.) [39]. Participants were asked to report usual frequency of consumption over the previous three to 6 months. Responses were assessed using a Likert Scale with response options ranging from 'never' to 'four or more times per day', and for some beverages up to 'seven times per day'. Standard portion sizes for each food item in the AES were derived from the most recent National Nutrition Survey data [40]. Nutrient intakes from the AES FFQ were computed using data in the AUSNUT 2011-13 database [41]. The AES has been validated in the adult population and demonstrated high level of accuracy in estimating nutrient intake and fruit and vegetable intakes [42, 43]. It has also been used to harmonise pregnancy cohort studies across Australia including ORIGINS Project [18], Baby1000 [44], Queensland Family Cohort [45], and NEW1000 [46]. Participants' usual daily servings of vegetables and legumes/beans, fruits, grains (cereals), meat and alternatives, and dairy and alternatives (nutrientdense core foods) were derived from the AES to compare with the Australian Guide to Health Eating (AGHE). Macro- and micronutrient intakes captured from the AES for each participant were compared to the respective Nutrient Reference Values (NRVs) to determine adequacy of intake [8]. The estimated average requirement (EAR) describes the daily nutrient level targeted to meet the requirements of half of the healthy individuals in any particular life stage [8]. When EAR is unavailable, adequate intake (AI) is used to estimate the average daily nutrient level that is assumed to be adequate by a group (or groups) of apparently healthy individuals [8]. Total contribution of energy intake from core and noncore foods were derived from the nutrient-dense core foods and foods that are high in saturated fat, added sugars, added salt and/or alcohol (energy-dense, nutrient-poor noncore foods) from the AES.

#### 2.5 | Statistical Analysis

Analyses were performed using STATA 18.0 [24]. Sociodemographic, health, and dietary characteristics were reported for participants as mean (standard deviation) or median (interquartile range) for continuous variables (age, weeks of gestation), and count (percentages) for categorical variables (parity, country of birth, language, education level, marital status, pre-pregnancy BMI and smoking status). The LFES was described by quartiles to examine the trends and association with diet quality (ARFS total and subscale scores) and dietary intakes (AGHE core food group serves, macro- and micronutrient intakes) of participants. The associations between LFES with diet quality and dietary intakes were assessed using Pearson's correlation. Linear regression analyses were used to model the relationship between diet quality (ARFS total and subscale scores) and LFES, adjusting for potential confounders including smoking status, parity, maternal age, prepregnancy BMI, and SEIFA IRSAD decile [25]. As a descriptive and exploratory study, a formal power calculation was not conducted. With 1,638 participants, this study was able to estimate the association between LFES and diet quality scores with a margin of error of ±0.048 for the standardised regression coefficient (95% confidence level). Both adjusted and unadjusted models were evaluated to compare their explanatory power. Before conducting Pearson's correlation and linear regression analyses, we performed checks to ensure the data met the required assumptions. For Pearson's correlation, we assessed linearity and normality of all included variables by visual inspection. For linear regression, we examined linearity using scatter plots and residual versus fitted value plots, checked normality of residuals using Q-Q plots, and evaluated homoscedasticity using residual versus fitted value plots and the Breusch-Pagan test. Multicollinearity was assessed using correlation matrices.

Overall, 5.12% of data points were missing, ranging from 0% (listeria score, ARFS) to 20.35% (BMI), with 75.8% of cases having complete data for all variables. Multiple imputation was employed to address missing values. The multiple imputation process was conducted using the multivariate imputation by chained equations (MICE) approach. We included all variables from the planned regression models in the imputation process. We generated five imputed datasets with ten iterations each to ensure convergence of the imputation algorithm. This method helps to maintain the complex associations between variables and reduces bias that could arise from complete case analysis or single imputation methods. This method helps to maintain the complex associations

between variables and reduces bias that could arise from complete case analysis or single imputation methods. Estimates within each data set were combined following Rubin's rules to provide a single set of results using the mibeta command in Stata. All tests were two-tailed, and a *p*-value of less than 0.05 was considered statistically significant.

#### 3 | Results

# 3.1 | Participant Characteristics

Table 1 summarises the sociodemographic and dietary characteristics of the participants. Of 1671 women assessed for eligibility, 33 were excluded due to age and missing dietary data, with 1,638 women (JHH: n = 441; ORIGINS Project: n = 1,197) included in the analysis. The mean (SD) age of participants was 32.0 (5.0) years, and the median (IQR) gestational age at the time of the survey was 35 [25-27] weeks. Close to half of the women (43.5%) had no prior pregnancies, and more than half (57.8%) were born in Australia and spoke English at home (76.7%). Almost half (42.4%) of the women reported holding a University Degree. In terms of socioeconomic status, the greatest proportion of participants (31.0%) resided in SEIFA IRSAD quintile 7-8. Most participants (82.6%) were married or in a de facto relationship. Pre-pregnancy BMI data were available for 1300 participants, and 35.6% had a healthy BMI classification, while 41.4% had a classification of overweight or obese. In total, 4.7% women reported smoking during pregnancy.

# 3.2 | Potential L. monocytogenes Exposure, Diet Quality and Dietary Intake of Pregnant Women

The potential L. monocytogenes exposure, diet quality and dietary intake of participants (n = 1638) are summarised in Table 2. The mean (SD) LFES score was 16.3 (5.2) out of 57 points. Based on frequency of consumption, the food items contributing most to the total LFES score (mean [SD]) were lettuce (3.2 [1.4]) with consumption frequency reported as once per week, followed by spinach (2.5 [1.4]) and melon (2.5 [1.4]) with consumption frequencies of one to three times per month (Figure 1). For overall diet quality, the mean (SD) ARFS score was 33.6 (9.7) points, categorised as 'getting there' [26]. Mean (SD) scores for ARFS subscales were the following: vegetables 12.2 (4.8) points, fruit: 5.5 (2.8), grains: 5.5 (2.1), dairy: 4.1 (1.9), and meat: 2.5 (1.3). The mean (SD) serves for nutrient-dense core food groups were vegetables: 3.5 (1.7) points, fruits: 1.9 (1.2), grains (cereals): 3.0 (1.5), dairy and alternatives: 1.6 (1.1), meat and alternatives: 2.2 (1.3). The mean (SD) percentage energy (kJ per day) from carbohydrate, protein, fat, and saturated fat were 44.7 (6.4)%, 17.9 (3.0)%, 37.4 (4.6)%, and 14.4 (2.5)% respectively. In terms of key pregnancy micronutrients, the mean (SD) intakes were, iron: 10.5 (3.5) mg, iodine 132.6 (57.5) µg, zinc 11.3 (3.8) mg, and folate 540.4 (199.4) µg.

# 3.3 | The Relationship Between Potential L. monocytogenes Exposure and Diet Quality

Table 3 reports participants' mean (SD) diet quality and intake by quartiles of LFES, and correlations between LFES and dietary variables.

**TABLE 1** | Sociodemographic characteristics of the participants (n = 1638).

Variable	Value
Age, Mean (SD)	32.0 (5.0)
Weeks of gestation, Median (IQR)	35 (34–36)
<b>Parity</b> , <i>n</i> (%)	
0	712 (43.5)
1	457 (27.9)
2	183 (11.2)
≥3	65 (4.0)
Not reported	221 (13.5)
Born in Australia, n (%)	
Yes	946 (57.8)
No	459 (28.0)
Not reported	232 (14.2)
Language spoken at home, $n$ (%)	
English	1,257 (76.7)
Other	150 (9.2)
Not reported	231 (14.1)
Highest education level, n (%)	
University Degree	695 (42.4)
Trade Certificate or Diploma	319 (19.5)
High School Certificate (Year 10–12)	326 (20.0)
Other/no formal qualifications	67 (4.1)
Not reported	231 (14.1)
SEIFA IRSAD quintile, $n$ (%)	
1–2	98 (6.0)
3–4	135 (8.2)
5–6	413 (25.2)
7–8	508 (31.0)
9–10	261 (15.9)
Not reported	223 (13.6)
Marital status, $n$ (%)	
Married/defacto	1,353 (82.6)
Never married	38 (2.3)
Divorced/separated	22 (1.3)
Not answered/reported	225 (13.7)
Pre-pregnancy BMI (kg/m²), n (%)	
Underweight (< 18.5)	39 (2.4)
Healthy weight ( $\geq 18.5$ to $\leq 24.9$ )	583 (35.6)
Overweight ( $\geq 25$ to $\leq 29.9$ )	324 (19.8)
Obese (Class I–III) ( $\geq 30$ to $\geq 40$ )	354 (21.6)
Not reported	338 (20.6)
Smoking during pregnancy, $n$ (%)	
No	1343 (82.0)
	(Continues)

TABLE 1 | (Continued)

Variable	Value
Yes	77 (4.7)
Not reported	218 (13.3)

Abbreviations: BMI, body mass index; IRSAD, the index of relative socioeconomic advantage and disadvantage; SEIFA, socioeconomic indexes of areas.

Assumption checks for correlation and linear regression revealed that the variables of interest were approximately normally distributed, and the relationships between the dependent variables of interest and the listeria score were approximately linear. Residuals were approximately normally distributed. Some heteroscedasticity was observed for the fitted values of overall diet quality (ARFS), and diet quality meat and vegetables subscales. To address the violation of the homoscedasticity assumption identified in our diagnostic tests, we employed robust standard errors in the affected models, ensuring more reliable standard error estimates and inference. Additionally, mibeta provides a pooled R-squared value across the imputed datasets. We note that the interpretation of R-squared in the context of multiple imputation is not fully settled in the statistical literature, and thus the pooled R-squared should be interpreted cautiously as an approximate measure of model fit, rather than a definitive proportion of explained variance as in single-data set regression. Pearson's correlation matrices indicated no problematic multicollinearity among the independent variables.

A moderate positive correlation was observed between potential  $L.\ monocytogenes$  exposure (LFES) and total diet quality (ARFS) ( $r=0.60,\ p<0.001$ ), indicating that as the consumption frequency of foods potentially containing  $L.\ monocytogenes$  increased, participants' diet quality also increased (Table 3). A moderate positive correlation was observed between LFES and all five ARFS subscales ( $r=0.29-0.52,\ p<0.001$ ). The vegetable subscale demonstrated the strongest positive correlation with LFES ( $r=0.52,\ p<0.001$ ) and the dairy subscale demonstrated the weakest positive correlation ( $r=0.29,\ p<0.001$ ).

Table 4 reports the adjusted (and unadjusted) regression results of LFES as a predictor of diet quality indicators. There was a significant association between LFES and total ARFS (Adjusted model:  $\beta=1.06$ ; 95% CI 0.99, 1.13, p<0.001), indicating that for every one-point increase in LFES, there was a 1.1-point increase in total ARFS. The adjusted  $R^2$  value (Adj.  $R^2=0.39$ ) suggests that 39% of variance in total ARFS can be explained by LFES. The adjusted linear regression estimates indicate a significant association between LFES and each ARFS subscale (all p<0.001). Based on the adjusted model, the one-point increase in the ARFS subscales was highest for the vegetables subscale ( $\beta=0.47$ ; 95% CI 0.43, 0.50, p<0.001, Adj.  $R^2=0.27$ ), and lowest for the dairy subscale ( $\beta=0.10$ , 95% CI 0.08, 0.12, p<0.001, Adj.  $R^2=0.10$ ).

# 3.4 | The Relationship Between Potential L. monocytogenes Exposure and Dietary Intake

(Continues)

Significant weak to moderate positive correlations were observed between potential  $L.\ monocytogenes$  exposure and

**TABLE 2** | Dietary characteristics of the participants (n = 1638).

Variable		Mean (SD)
Potential Listeria monocytogenes exposure		
LFES*	Points	
Total score	0-57	16.3 (5.2)
Lettuce	0–7	3.2 (1.4)
Spinach	0–5	2.5 (1.4)
Cabbage, brussel sprouts	0–5	1.4 (1.3)
Melon	0–5	2.5 (1.4)
Cottage cheese or ricotta	0–3	0.5 (0.9)
Ice cream	0–5	1.6 (1.6)
Liver-beef, calf, chicken (including pate)	0–5	0.1 (0.5)
Other Seafood (e.g., prawns/shrimp, lobster)	0–4	0.7 (0.9)
Devon, salami	0–5	0.5 (0.9)
Bacon, ham	0–5	2.1 (1.2)
Sausages, frankfurts, pluto pup/battered	0–4	1.2 (1.0)
Diet quality		
$\mathbf{ARFS}^\dagger$	Points	
Total	0–73	33.6 (9.7)
Vegetables	0–21	12.2 (4.8)
Fruit	0–12	5.5 (2.8)
Grains	0–13	5.5 (2.1)
Dairy	0–11	4.1 (1.8)
Dietary intakes		
Food group servings	AGHE (serves/day)	
Vegetables and legumes/beans	5	3.5 (1.7)
Fruit	2	1.9 (1.2)
Grain (cereal) foods	8.5	3.0 (1.5)
Dairy and alternatives	2.5	1.6 (1.1)
Meat and alternatives	3.5	2.2 (1.3)
Macro- and micronutrients intake	NRVs (unit/day)	
Carbohydrate (%E)	AMDR 45%-65%	44.7 (6.4)
Protein (%E)	AMDR 20%-30%	17.9 (3.0)
Total fat (%E)	AMDR 20%-35%	37.4 (4.6)
Saturated fat (%E)	< 10%	14.4 (2.5)
Fibre (g)	AI 28	26.2 (9.0)
Calcium (mg)	EAR 840	841.1 (358.0)
Iron (mg)	EAR 22	10.5 (3.5)
Sodium (mg)	AI 460-920	1859.2 (707.7)
Potassium (mg)	AI 2800	3222.5 (991.0)
Zinc (mg)	AI 9.0	11.3 (3.8)
Iodine (μg)	EAR 160	132.6 (57.5)
Magnesium (mg)	EAR 290-300	379.2 (103.5)
Phosphorus (mg)	EAR 580	1430.4 (472.5)
Thiamin (mg)	EAR 1.2	1.5 (0.7)

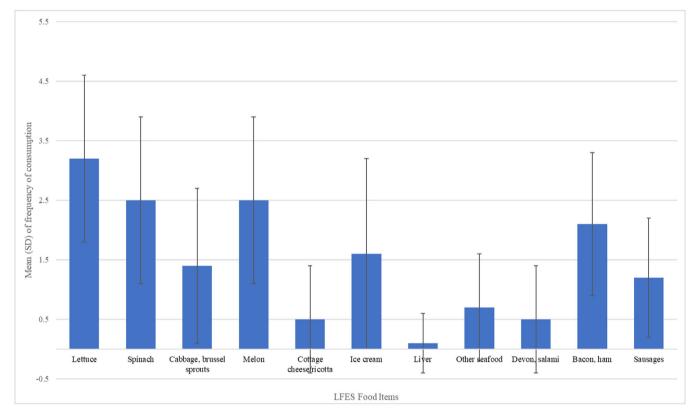
(Continues)

TABLE 2 | (Continued)

Variable		Mean (SD)
Riboflavin (mg)	EAR 1.2	2.0 (0.8)
Niacin (mg)	EAR 14	38.2 (13.5)
Vitamin C (mg)	EAR 40	165.6 (80.0)
Dietary folate equivalents (ug)	EAR 520	540.4 (199.4)
Retinol equivalents (μg)	EAR 550	924.7 (664.7)
Vitamin E (mg)	AI 7	10.1 (3.3)
Energy intake		
Total energy intake (kJ/day)	_	8568 (2645)
Energy from nutrient-dense core foods (%)	_	66.0 (12.0)
Energy from energy-dense nutrient-poor noncore foods (%)	_	34.0 (12.0)

Abbreviations: AGHE, Australian Guide to Healthy Eating; AI, Adequate Intake; AMDR, Acceptable Macronutrient Distribution Range; ARFS, Australian Recommended Food Score; EAR, Estimated Average Requirement; kJ, kilojoule; LFES, Listeria Food Exposure Score; NRVs, nutrient reference values; SFA, saturated fat.

<sup>&</sup>lt;sup>†</sup>This is described based on food groups.



**FIGURE 1** | Mean (SD) of LFES food group subscale scores of participants (*n* = 1638). LFES, Listeria Food Exposure Score; SD, standard deviation.

intake of all core food groups (r = 0.11-0.43, p < 0.001) (Table 3), with the strongest correlation observed between LFES and vegetable/legumes serves (r = 0.43, p < 0.001) while dairy and alternatives demonstrated the weakest positive correlation (r = 0.11, p < 0.001).

For macronutrient intake, there were significant weak positive correlations observed between LFES and percentage of total energy from total fat (r = 0.18, p < 0.001), saturated fat (r = 0.12, p < 0.001) and protein (r = 0.11, p < 0.001), and a significant

weak negative correlation between LFES and percentage of total energy from carbohydrate (r = -0.21, p < 0.001).

In terms of micronutrient intake, there were significant weak to moderate positive correlations observed between LFES and all 15 micronutrients (r = 0.24–0.52, p < 0.001). The strongest correlation was observed between LFES and Vitamin E (r = 0.52, p < 0.001) and the weakest correlation was observed with iodine (r = 0.24, p < 0.001). Significant moderate positive correlations were observed between LFES and intake of key pregnancy

<sup>\*</sup>This is described based on food items.

**TABLE 3** | Diet quality (ARFS) and dietary intake (foods group serves, nutrient intake) of participants by LFES quartiles (n = 1638).

		Onartile 1*	ام <u>ا</u>	Onartile 2	ile 2	Onartile 3	الم ع	Onartile 4 <sup>†</sup>	ile 4 <sup>†</sup>	
		(n = 491,	30.0%)	(n = 388, 23.7%)	23.7%)	(n = 418, 25.5%)	25.5%)	(n = 341, 20.8%)	, 20.8%)	
LFES		Mean 10.4	SD 2.4	Mean 15.0	SD 0.8	Mean 18.5	SD 1.1	Mean 23.6	SD 2.6	Correlation, $p^{\ddagger}$
Diet quality										
ARFS	ARFS maximum									
	score									
Total	73	26.7	8.9	32.5	7.8	36.9	7.4	41.0	7.8	0.60, < 0.001
Vegetables	21	9.1	4.6	11.8	4.3	13.8	4.0	15.1	3.6	0.52, < 0.001
Fruit	12	4.2	2.7	5.3	2.6	6.1	2.4	6.9	2.7	0.41, < 0.001
Grains	13	4.7	2.1	5.3	1.9	5.8	2.1	6.2	2.2	0.30, < 0.001
Dairy and alternatives	11	3.5	1.8	3.9	1.8	4.2	1.7	4.9	1.8	0.29, < 0.001
Meat and alternatives	7	2.0	1.2	2.3	1.2	2.6	1.2	3.1	1.3	0.33, < 0.001
Dietary intakes										
Food group <sup>§</sup>	AGHE (serves/dav)									
Vegetables and legumes/ beans		2.6	1.4	3.3	1.7	3.8	1.5	4.4	1.7	0.43, < 0.001
Fruit	2	1.4	1.0	1.8	1.1	2.1	1.1	2.4	1.4	0.32, < 0.001
Grains	8.5	2.8	1.6	2.9	1.4	3.2	1.4	3.3	1.4	0.15, < 0.001
Dairy and alternatives	2.5	1.5	1.2	1.5	1.1	1.7	1.0	1.8	1.1	0.11, < 0.001
Meat and alternatives	3.5	1.8	1.1	2.0	1.2	2.4	1.2	2.8	1.7	0.30, < 0.001
Macro- and micronutrients	NRVs (unit/day)									
Carbohydrate (%E)	AMDR 45-65	46.3	7.1	45.3	6.2	43.8	5.5	42.8	5.7	-0.21, < 0.001
Protein (%E)	AMDR 15-25	17.5	3.4	17.6	3.0	18.1	2.7	18.5	2.8	0.11, < 0.001
Total fat (%E)	AMDR 20-35	36.4	5.3	37.0	4.4	38.1	4.2	38.6	3.8	0.18, < 0.001
SFA (%E)	8-10	14.0	2.8	14.2	2.5	14.6	2.2	14.9	2.1	0.12, < 0.001
Fibre (g)	AI 28	21.7	8.1	25.5	8.0	28.2	7.7	31.5	0.6	0.45, < 0.001
Calcium (mg)	EAR 840	737.1	380.7	804.5	337.9	885.4	306.4	978.2	353.2	0.28, < 0.001
Iron (mg)	EAR 22	8.9	3.4	10.1	3.1	11.0	3.2	12.5	3.6	0.41, < 0.001
Sodium (mg)	AI 460-920, UL 2300	1567.2	637.8	1773.6	580.5	1939.3	582.9	2279.1	737.5	0.41, < 0.001

TABLE 3 | (Continued)

		Quartile 1*	le 1*	Quartile 2	ile 2	Quartile 3	ile 3	Quartile 4 <sup>†</sup>	ile 4 <sup>†</sup>	
		(n = 491,	30.0%)	(n = 388, 23.7%)	23.7%)	(n = 418, 25.5%)	, 25.5%)	(n = 341, 20.8%)	, 20.8%)	
LFES		Mean 10.4	SD 2.4	Mean 15.0	SD 0.8	Mean 18.5	SD 1.1	Mean 23.6	SD 2.6	Correlation, $p^{\ddagger}$
Potassium (mg)	AI 2800	2697.8	932.4	3056.0	821.1	3428.3	789.6	3915.0	1002.7	0.49, < 0.001
Zinc (mg)	EAR 9.0	9.6	3.4	10.7	3.2	11.9	3.2	13.6	4.3	0.43, < 0.001
Iodine (µg)	EAR 160	118.1	60.2	127.3	55.3	137.6	51.5	153.3	56.2	0.24, < 0.001
Magnesium (mg)	EAR 290-335	328.3	100.6	363.4	87.2	401.8	87.8	442.9	101.1	0.45, < 0.001
Phosphorus (mg)	EAR 580-1055	1213.9	442.6	1351.3	406.5	1512.4	383.1	1731.5	500.4	0.44, < 0.001
Thiamin (mg)	EAR 1.2	1.4	0.7	1.5	0.5	1.6	9.0	1.8	0.7	0.27, < 0.001
Riboflavin (mg)	EAR 1.2	1.8	6.0	2.00	0.7	2.1	0.7	2.4	8.0	0.30, < 0.001
Niacin (mg)	EAR 14	32.1	11.3	36.0	10.8	40.4	11.5	46.9	15.9	0.44, < 0.001
Vitamin C (mg)	EAR 40	137.2	9.07	156.6	68.2	176.2	68.2	203.9	78.8	0.33, < 0.001
Dietary folate eq. (ug)	EAR 520	481.4	216.6	523.5	172.3	564.6	185.0	615.0	190.7	0.28, < 0.001
Retinol eq. (µg)	EAR 550	700.7	442,5	873.4	592.2	982	382.6	1234.9	1044.2	0.34, < 0.001
Vitamin E (mg)	EAR 7	8.2	2.9	9.6	2.7	10.8	2.8	12.6	3.3	0.52, < 0.001
Energy intake										
Total energy intake (kJ/day)	y)	7347	2517	8171	2221	9968	2209	10,290	2731	0.44, < 0.001
Energy from nutrient-dense core foods (%E)	e core foods (%E)	65.7	13.4	65.5	12.0	67.5	11.6	65.2	10.2	0.01, 0.676
Energy from energy-dense, nutrient-poor noncore foods (%E)	, nutrient-poor noncore	34.3	13.4	34.5	12.0	32.5	11.6	34.8	10.2	-0.01, 0.672

Abbreviations: %E, percentage of energy; µg, microgram; AGHE, Australian Guide to Healthy Eating (applicable to pregnant women aged 19-50 years); Al, adequate intake; AMDR, acceptable macronutrient distribution range; ARFS, Australian Recommended Food Score; EAR, estimated average requirement; eq., equivalent; g, gram; kl, kilojoule; LFES, Listeria Food Exposure Score; mg, milligram; NRVs, nutrient reference values (applicable to pregnant women aged 19-50 years); UL, upper limit.

\*Quartile 1 = lowest quartile of LFES.

†Quartile 4 = highest quartile of LFES.

\*Based on second and third trimesters.

**TABLE 4** | Regression analyses (adjusted and unadjusted) of potential *Listeria monocytogenes* exposure (LFES) and diet quality (ARFS total and subscales).

	Adjuste	ed regression	ı	Unadjusted regression		
Diet quality (ARFS)	Estimate (95% CI)*	<i>p</i> -value	Adjusted R <sup>2</sup>	Estimate (95% CI)	<i>p</i> -value	$R^2$
ARFS (Total)	1.06 (0.99, 1.13)	< 0.001	0.39	1.11 (1.04, 1.19)	< 0.001	0.36
ARFS (Vegetables)	0.47 (0.43, 0.50)	< 0.001	0.27	0.47 (0.44, 0.51)	< 0.001	0.27
ARFS (Fruit)	0.21 (0.18, 0.23)	< 0.001	0.19	0.22 (0.19, 0.24)	< 0.001	0.16
ARFS (Grains)	0.11 (0.09, 0.13)	< 0.001	0.15	0.12 (0.10, 0.14)	< 0.001	0.09
ARFS (Meat)	0.08 (0.07, 0.09)	< 0.001	0.11	0.08 (0.07, 0.09)	< 0.001	0.11
ARFS (Dairy)	0.10 (0.08, 0.12)	< 0.001	0.10	0.10 (0.09, 0.12)	< 0.001	0.08

Abbreviations: ARFS, Australian Food Recommended Score; LFES, Listeria Food Exposure Score;  $R^2$ , coefficient of multiple determination.  $^{\dagger}p < 0.05$ .

nutrients including zinc (r = 0.43, p < 0.001) and iron (r = 0.41, p < 0.001), and significant weak positive correlations were observed with calcium (r = 0.28, p < 0.001) and folate (r = 0.28, p < 0.001).

There was a significant moderate positive correlation between LFES and total energy intake (r = 0.44, p = < 0.001). No significant correlation was observed between LFES and percentage of energy intake from nutrient-dense core foods (r = 0.01, p = 0.676) or energy-dense, nutrient-poor noncore foods (r = -0.01, p = 0.672).

# 4 | Discussion

The current study aimed to examine the relationship between potential *L. monocytogenes* exposure and diet quality and dietary intake in two cohorts of pregnant women in Australia. This analysis extends on previous research [11] showing that more frequent consumption of foods that potentially harbour *L. monocytogenes* are significantly associated with higher diet quality, higher intakes of nutrient-dense core foods, and higher micronutrient intakes.

Regarding the relationship between potential L. monocytogenes exposure and diet quality, findings indicate that of the five nutrient-dense core food groups, vegetable intake has the strongest correlation with LFES. The strong association between the LFES with ARFS vegetables subscale may be attributed to the greater weight from this subscale as it contains the highest number of food items. The finding was also consistent when evaluating the relationship between LFES and AGHE food group, as the vegetables and legumes groups had the strongest associations with the LFES. This is also demonstrated within the LFES, where lettuce and spinach were the most frequently consumed food items by the current sample. Raw vegetables are identified in pregnancy guidelines as highrisk foods for L. monocytogenes with contamination possible due to exposure to decaying vegetation, animal faeces, soil, surface, river and canal waters, or effluent from sewage treatment operations [27]. Moreover, L. monocytogenes are able to survive and multiply during refrigeration temperatures [28]. Ready-toeat salads and raw vegetables carry a greater risk to pregnant women as these foods are often consumed without heating.

However, avoiding the consumption of vegetables, especially green leafy vegetables, has implications for pregnancy outcomes as they are important dietary sources of folate, fibre, and nonheme iron [29]. Therefore, to reduce listeriosis risk and also ensure adequate nutrient intake, pregnant women are recommended to consume cooked vegetables [7]. In addition, salad leaves should be washed before eating and consumed immediately [7]. Apart from green leafy vegetables, pregnant women are also recommended to consume a variety of vegetables to support overall nutrient needs. A previous study compared the growth potential of L. monocytogenes on 12 salad products and found that carrot, celery and corn salads do not promote the growth of L. monocytogenes compared to other salad products including lettuce, arugula leaves, parsley, radish green, beetroot and cabbage [30]. This shows that there are lower risk vegetable alternatives for women to consume to achieve the recommended daily core food group serves and nutrient reference values during pregnancy.

In examining the relationship between potential L. monocytogenes exposure and macronutrient intakes, similar to a previous study [11], the current analysis found that as the LFES increased, the percentage of total energy intake from protein and fats increased, while the percentage of energy from carbohydrate decreased. This may suggest that a dietary pattern with higher protein and fat intake, and lower carbohydrate intake is associated with a higher risk of contracting listeriosis, compared to a dietary pattern with lower protein and fat, and higher carbohydrate intake. Similar trends of macronutrient intakes are also observed in other Australian pregnancy cohorts [31, 32], where women's percentage of energy from total fat and carbohydrate ranged between 36% and 36.5% and 45%-45.7%, respectively. These intakes are slightly higher than those of the general Australian population for carbohydrate (43.6%) and substantially lower for fat (39.1%) [33]. In terms of the positive correlation observed between LFES and percentage of energy from protein, a systematic review that assessed the association between foods and listeriosis reported that mothers with listeriosis during pregnancy were more likely to have consumed unpasteurised dairy products, ready-to-eat, semi-cooked, smoked or processed meat products [34]. These protein-rich foods are considered high-risk foods for pregnant women as L. monocytogenes can survive the food production process and these foods are often consumed without heating [35]. Based on

<sup>\*</sup>Estimates adjusted for parity, maternal age, BMI, smoking during pregnancy, and SES (SEIFA IRSAD deciles).

these findings, pregnant women should aim to meet their protein requirements by consuming lower risk protein foods such as home-cooked lean red meat, poultry and eggs, hard cheese, and nuts, and should also aim to meet their carbohydrate requirements with at least 45% of their total energy intake from carbohydrate [29].

In terms of micronutrient intakes, the findings were consistent with a previous study [11], which showed that greater consumption of foods potentially harbouring L. monocytogenes was significantly associated with higher micronutrient intakes. Maternal micronutrient status during pregnancy can influence foetal health, birth outcomes and the longer-term health of the offspring [36]. Avoiding the consumption of nutritious but also high-risk foods such as cottage cheese, ricotta, and prepackaged salads without replacing with safer alternatives such as hard cheeses and freshly prepared homemade salads would likely have negative implications on overall diet quality, consequently affecting maternal and foetal health due to suboptimal nutrient status. Currently, it could be argued that dietary guidelines for pregnant women may overly focus on the exclusion of certain foods, while lacking information on the inclusion of safer nutrient-rich alternatives. A previous study examining women's awareness of pregnancy-specific recommendations suggested that women consciously eliminated foods reported in guidelines as high risk for Listeria from their diet [37]. In contrast, a study in 407 pregnant women reported that 77% experienced difficulty avoiding high listeriosis risk foods as they perceived these foods to be rich in calcium, iron, and folic acid, which are important to support pregnancy needs [47]. This suggests that while women may be aware of the importance of nutrition during pregnancy, they are also confused with regard to balancing their dietary needs with food safety risks. Therefore, it is important for pregnant women to be educated about making safer food choices that will concurrently reduce their risk of contracting listeriosis and address their nutritional requirements [7, 11]. A strength of the current study is the large sample included in the analyses. In addition, the evaluation between potential listeriosis exposure and dietary intakes was conducted in a contemporary sample of pregnant women, which is important given that the dietary data from the previous publication was collected in 2003 [11]. Further, the education levels of participants in the current analysis are reflective of the broader Australian population, where the proportion of women with post-school and university degree qualifications was similar to the latest national data, respectively [48]. Moreover, the LFES used in the current study was developed based on a contemporary list of potential higher-risk food sources of Listeria from FSANZ [7] within a validated FFQ [39]. That is, the current AES collected data about the types of cheese and icecreams consumed (i.e., cottage cheese, soft serves, sundae cones) that are more prone to harbouring L. monocytogenes, which the questionnaire used in the previous study did not specify [49]. This research should also be interpreted in the context of several limitations. Firstly, the current LFES did not include other high Listeria-risk foods listed in the FSANZ recommendations such as, raw or uncooked seafood, pre-packaged and pre-cut vegetables and fruit, bean sprouts or alfalfa sprouts [7]. Future studies could consider including other contemporary food items that may possess foodborne illness risks among Australian childbearing age women. Additionally, while diet quality and dietary intake were assessed using a validated FFQ, self-reported dietary measures have inherent limitations, such as recall bias and social desirability bias [50]. Further, energy intake in this cohort may be underreported, as previous studies have shown that pregnant women in the overweight and obese BMI categories tend to underreport their dietary intake [51–53]. A further limitation of this study is that pregnant women's food handling practices and behaviours were not measured, which should be considered in future research. Finally, this study did not collect data on the incidence of listeriosis among participants from the study participants. Future research could address this gap by investigating the association between dietary intake and listeriosis incidence in pregnant women.

# 4.1 | Implications of Findings for Future Research and for Clinical Practice or Policy

Poor diet quality and listeriosis during pregnancy are risk factors for adverse health outcomes for both the mother and the foetus may result in increased utilisation of healthcare resources throughout the delivery period and throughout an infant's life [11, 54]. The current study suggested that women with higher diet quality simultaneously had a higher intake of foods that may potentially harbour L. monocytogenes, however, the implications and cost of potential L. monocytogenes exposure related perinatal health outcomes are currently underexplored. As such, future research should focus on the association between the consumption of high-risk foods for listeriosis and the risk of adverse maternal and infant health outcomes. In addition, the development of an FFO that includes contemporary food sources high in foodborne illnesses risk during pregnancy with the relevant safe food handling practices would be recommended in future research to assess pregnant women's overall diet quality.

The current findings highlight that a balanced approach is needed to improve dietary quality that aims to achieve adequate nutrient intake while reducing the risk of listeriosis in pregnant women. Healthcare practitioners need to shift the focus from promoting total exclusion of foods that may harbour *L. monocytogenes* to educating pregnant women about safer food alternatives and food preparation, handling, and storage practices. Assessments for pregnant women and women of childbearing age that combine both dietary intakes and safe food handling practices simultaneously, would potentially be useful.

#### 5 | Conclusion

The current analysis found that pregnant women with more frequent consumption of foods potentially harbouring *L. monocytogenes* were significantly associated with higher diet quality, higher intakes of nutrient-dense core foods and higher micronutrient intakes. These findings highlight the importance to balance adequate nutrient intake with minimising listeriosis risk during pregnancy. To address this, healthcare practitioners require further training and resources to guide pregnant women in selecting lower-risk alternatives to high-risk foods, instead of the avoidance of entire food groups, to prevent potential

nutritional inadequacy during pregnancy. Additionally, integrating education on safe food handling practices into routine antenatal care is essential to empower and support pregnant women to prepare food safely and reduce the risk of contracting listeriosis.

#### **Author Contributions**

Clare Collins, Melinda J. Hutchesson, Rachael Taylor, Sasha Fenton contributed to study conceptualisation. Clare Collins, Rachael Taylor, Sasha Fenton obtained funding for the study. Sasha Fenton and Melinda J. Hutchesson developed the analysis plan. Melinda J. Hutchesson performed statistical analysis and provided statistical advice. Kee June Ooi prepared the draft manuscript. All authors contributed to the interpretation of study results and critical revision of the manuscript, and all have approved the final version of the manuscript for submission.

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#### **Ethics Statement**

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Hunter New England Human Research Ethics Committee (2019/ETH00954) and University of Newcastle Human Research Ethics Committee (H-2023-0239) for the John Hunter Hospital cohort in New South Wales, Australia and Human Research Ethics Committee of Joondalup Health Campus cohort (2023/ETH/0001) for the ORIGINS cohort in Western Australia, Australia. Written informed consent was obtained from all study participants.

#### **Conflicts of Interest**

The authors declare no conflicts of interest.

# **Data Availability Statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

#### Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jhn.70032.

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# **Supporting Information**

Additional supporting information can be found online in the Supporting Information section.