

## SUBMITTED VERSION

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### Development and validation of an iodine-specific FFQ to estimate iodine intake in Australian pregnant women

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**Development and validation of an iodine specific food frequency questionnaire to estimate iodine intake in pregnant women.**

Running title: Iodine specific food frequency questionnaire

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## 1 **Abstract**

2

3 Adequate iodine is important during pregnancy to ensure optimal growth and development of  
4 the offspring. This study aimed to develop and validate an iodine specific food frequency  
5 questionnaire (I-FFQ) for use in pregnant women. A 44-item I-FFQ was developed and  
6 administered to 122 pregnant women at study entry (<20 weeks gestation) and 28 weeks  
7 gestation. Iodine intake estimated from the I-FFQ was compared between the two time points  
8 for reproducibility. Correlation between iodine intake estimated from the I-FFQ and intake  
9 from a four day weighed food record, urinary iodine from a 24 hour and a spot urine sample,  
10 and thyroid function from a blood sample were assessed at 28 weeks gestation. Iodine intake  
11 from the I-FFQ at study entry and at 28 weeks gestation was strongly correlated ( $r=0.622$ ,  
12  $p<0.001$ ). A moderate correlation was shown between intake from the I-FFQ and the four day  
13 weighed food record ( $r=0.349$ ,  $p<0.001$ ) which was strengthened with the addition of iodine  
14 supplements ( $r=0.876$ ,  $p<0.001$ ). There was a strong agreement ( $k=0.799$ ,  $p<0.001$ ) between  
15 the two dietary measures in the ability to classify the women as adequate ( $\geq 220\mu\text{g/day}$ ) or  
16 inadequate ( $<220\mu\text{g/day}$ ) intake but the limits of agreement from the Bland-Altman plot was  
17 low. Iodine intake from the I-FFQ correlated with 24 hour urinary iodine excretion ( $r=0.488$ ,  
18  $p<0.001$ ) but did not correlate with spot urinary iodine concentration. In conclusion, the I-  
19 FFQ provides a valid tool to estimate iodine intake in pregnant women and can be used to  
20 screen women whose iodine intake is below the recommendations.

21

## 22 Introduction

23 Iodine is crucial in the formation of thyroid hormones, triiodothyronine (T3) and thyroxine  
24 (T4), and is essential for mammalian life <sup>(1)</sup>. Worldwide, iodine deficiency has emerged as a  
25 major public health issue because it is one of the most common micronutrient deficiencies,  
26 affecting developing as well as industrialised countries <sup>(2)</sup>. This is of particular concern during  
27 pregnancy as iodine deficiency can lead to spontaneous abortion, premature births, impaired  
28 growth and adverse neurological development as well as cretinism and infant mortality in  
29 severe iodine deficiency <sup>(1,3)</sup>.

30 Iodine requirement is thought to increase during pregnancy with the World Health  
31 Organisation (WHO) recommending that pregnant women increase their intake to 250µg/day  
32 compared with 150µg/day for women of child bearing age <sup>(2)</sup>. This increased requirement is  
33 due to the transfer of thyroid hormone from the mother to the fetus as well as the greater renal  
34 clearance of iodine <sup>(4, 5)</sup>. However, the recommended intake of iodine varies between  
35 industrialised countries, ranging from 140µg/day in the UK (no increment from non-pregnant  
36 women)<sup>(6)</sup> to 220µg/day in Australia, New Zealand and the United States <sup>(7, 8)</sup>. Assessment of  
37 iodine intake is challenging as iodine content of foods is influenced by a number of factors  
38 including fertilisers, irrigation, sanitising and industrial agents, rainfall, season and location  
39 <sup>(9)</sup> and it is difficult to accurately estimate the intake of iodine from the use of iodised salt in  
40 cooking and at the table <sup>(10)</sup>. As a result, urinary iodine concentration is often used as an  
41 indicator of iodine status with the WHO/UNICEF/ICCIDD defining a median urinary iodine  
42 concentration of  $\geq 150$  µg/L, based on the recommended dietary intake, as sufficient iodine  
43 intake in pregnancy <sup>(11)</sup>. However, UIC is reflective of recent iodine intake and has large  
44 intra-individual variation <sup>(12)</sup>, limiting its use as an assessment of usual dietary iodine intake.  
45 Given the importance of iodine during pregnancy, an accurate assessment of habitual iodine  
46 intake is needed.

47 Dietary assessment poses challenges as many tools rely on memory, accurate estimation of  
48 intake and time commitment <sup>(13)</sup>. Food frequency questionnaires (FFQs) are used to assess  
49 longer term habitual intake, which is useful for nutrients such as iodine that are less common  
50 in the food supply <sup>(13)</sup>. FFQs are less time consuming, have a low burden on participants and  
51 lower cost compared with the more traditional dietary assessment method of weighed food  
52 records <sup>(13)</sup>. However, FFQs must be appropriate for the population in question, considering  
53 usual foods and food patterns. It is known that during pregnancy eating habits often change,

54 which may be a reflection of dietary recommendations, avoidance of foods as well as  
55 pregnancy related sickness <sup>(14)</sup>. Thus, the dietary assessment method must be tailored to suit  
56 this population.

57 Previous studies have developed and validated general FFQs and these have been used to  
58 assess iodine intake in pregnancy <sup>(15, 16)</sup>. However, general FFQs are often long and time  
59 consuming. Additionally, much of the information captured in a general FFQ would not be  
60 relevant when the focus is on iodine intake. We are interested in developing an iodine  
61 specific food frequency (I-FFQ) that can be used in large scale studies to assess iodine intake  
62 as well as a simple tool to identify women with inadequate intake and who may be at risk of  
63 iodine deficiency.

64 To our knowledge there are only three published I-FFQs that have been developed and  
65 validated, two for use in non-pregnant adult women in Denmark <sup>(17)</sup> and the UK <sup>(18)</sup> and one  
66 for use in the elderly <sup>(19)</sup>. These questionnaires were validated for use in those specific  
67 populations, reflecting the common food habits and practices of the population and thus  
68 limiting their use in pregnancy. The aim of this study was to develop an I-FFQ for use in  
69 pregnancy and assess its reproducibility and validity against iodine intake from a weighed  
70 food record; (2) urinary iodine from a 24 hour and a spot urine sample and (3) blood  
71 biomarkers of iodine status.

## 72 **Methods**

### 73 *Subjects*

74 Participants were recruited from women who were participating in the Pregnancy Iodine and  
75 Neurodevelopment in Kids (the PINK study) in Adelaide, Australia. A total of 122 women  
76 from the Women's and Children's Hospital were recruited between August 2011-April 2012  
77 from the antenatal clinic at their first antenatal appointment. Eligible women were less than  
78 20 weeks gestation with no history of thyroid disease. Ethics approval was obtained from the  
79 Women's and Children's Health Network (WCHN) Human Research Ethics Committee and  
80 all women provided written informed consent.

### 81 *Development of the I-FFQ*

82 The I-FFQ was developed to determine the women's average iodine intake over the past  
83 month. The food items were selected based on the most up to date Australian food

84 composition database that is based on analytical data, NUTTAB 2010<sup>(20)</sup>. For food items that  
85 were not listed in NUTTAB, the AUSNUT 2007 was used to supplement the list, which  
86 incorporates nutrient data from a range of sources including recipes, international food  
87 composition tables as well as calculated and imputed data<sup>(21)</sup>.

88  
89 Foods were included in the I-FFQ if they had an iodine content of  $\geq 5\%$  of the recommended  
90 dietary intake (RDI) per serve for Australian pregnant women (10 $\mu$ g/serve). Serving sizes  
91 were based on standard serves using the Australian Guide to Healthy Eating or food labels  
92 and were expressed as measurements (in grams) or convenient household units (cup/tsp  
93 /tbsp). There were some foods that fell just below the 5% RDI criteria per serve, however  
94 were included in the I-FFQ as these foods were considered common in the Australian diet,  
95 including noodles and pasta, rice, cheese, ice cream, cooked broccoli, spinach and bok choy,  
96 chocolate, cashews, cheese flavoured snacks and pizza. For those food items with more than  
97 one variety, such as different types of fish and cheese, the average iodine content was used.  
98 The final questionnaire consisted of 44 food items (See *appendix 1*). The food items were  
99 classified into seven main food groups based on those listed in the NUTTAB database<sup>(20)</sup>  
100 including seafood, cereal products, dairy, egg, vegetables, snacks and sweets and ready made  
101 foods. For each food item, the frequency of intake was recorded as the number of serves per  
102 day, per week or per month. If the food was not consumed on a monthly basis the frequency  
103 of intake was marked as rarely/<1 per month. An additional three questions were included  
104 which related to salt use, including whether salt was added in cooking or at the table, if the  
105 salt added was iodised salt and the individual daily portion used.

106

### 107 ***Validation of the I-FFQ***

108

109 The validity of the I-FFQ was assessed in the following ways:

- 110 1. The comparison of iodine intake estimated from the I-FFQ with the four day weighed  
111 food record at 28 weeks gestation.
- 112 2. The reproducibility of the I-FFQ during pregnancy (<20 weeks and 28 weeks  
113 gestation).
- 114 3. The correlation between iodine intake from the I-FFQ and urinary iodine from a 24  
115 hour urine sample and spot urine sample at 28 weeks gestation.
- 116 4. The correlation between iodine intake and thyroid function (TSH, Tg, fT3 and fT4) at  
117 28 weeks gestation.

118

119 *Assessment of iodine intake*

120 The women completed the I-FFQ at enrolment (<20 weeks gestation) and at 28 weeks  
121 gestation. The questionnaire was checked for completeness by a dietitian. To calculate the  
122 mean daily iodine intake, all frequencies of consumption (per week and per month) were  
123 converted to per day assuming that there were seven days in a week and 30 days in a month.  
124 The frequency of consumption per day was multiplied by the average iodine content of the  
125 specific food. This calculation was completed for each individual food item and was added  
126 together to give the total mean daily iodine intake. The use of iodised salt was not quantified  
127 and therefore not included in the total iodine intake.

128

129 Women were asked to keep a weighed food record for four consecutive days including one  
130 weekend day between 26-28 weeks gestation. They were given oral and written instructions  
131 and were provided with digital kitchen scales and measuring cups. The women were asked to  
132 weigh and record details of the food consumed as well as weigh and record any left overs of  
133 each food item. If eating out the women were asked to record details about their meal. A  
134 separate space was provided to record any home cooked recipes including the amount (in  
135 grams or units) of raw ingredients used, the number of serves the recipe yields and the  
136 number of serves consumed. Foodworks with the NUTTAB 2010 and AUSNUT 2007  
137 (Version 7, 2012) was used to assess dietary intake from the weighed food records. Food  
138 items not listed in the database were entered as the closest resembling food item or the  
139 nutritional information derived from the food label or company website was added to the  
140 database. These food items were kept in a log for consistency of data entry.

141

142 Information regarding supplement usage, including brand name, dose and frequency was  
143 gathered from women at 28 weeks gestation. Iodine intake from these supplements was  
144 calculated based on the manufacturer's information and this was added to the iodine amount  
145 estimated from the I-FFQs and weighed food records as the total iodine intake.

146

147 *Assessment of urinary iodine*

148 Urinary iodine excretion from 24 hour urine collections were used to validate the I-FFQ as  
149 urinary iodine is determined from a pooled 24 hour sample and therefore is seen to better  
150 reflect an individual's iodine excretion when compared to a spot urine sample<sup>(22)</sup>. The  
151 women were asked to collect the 24 hour urine sample after completing the weighed food

152 record and within two days of their 28 week gestation appointment. The first urine passed on  
153 the day of collection was not saved and was recorded as the start time and date of the 24 hour  
154 collection. All urine passed for the next 24 hours was collected. The last sample was  
155 collected 24 hours later from the start time and was recorded as the end time and date.

156 Women were provided with written instructions and with the necessary equipment, including  
157 a 4L container to store the total urine collected and a 1L measuring jug to assist with  
158 collecting each sample, both of which had been tested and cleared for iodine contamination.

159  
160 Once completed, the samples were refrigerated and delivered to the laboratory at the  
161 Women's and Children's Hospital within two days of collection. The total volume was  
162 measured and aliquots of 10ml were taken and stored at  $-20^{\circ}\text{C}$  for analysis. The method for  
163 the analysis of UIC was modified from the WHO 'Method A' procedure<sup>(2, 23)</sup>, using  
164 ammonium persulfate digestion and microplate reading. The analytical value for the external  
165 iodine standard was  $284.5 \pm 12.2\mu\text{g/L}$  compared with the certified value of  $304 \pm 44\mu\text{g/L}$ .  
166 The percent relative standard deviation of the assay was 4.3%.

167  
168 As part of the PINK study participants also provided a spot urine sample at 28 weeks. Similar  
169 to the 24 hour urine sample, UIC from the spot urine sample was analysed and used as an  
170 additional reference measure.

#### 171 172 *Blood Biomarkers*

173 At 28 weeks gestation a blood sample was taken via venepuncture for analysis of thyroid  
174 stimulating hormone (TSH), thyroglobulin (Tg), free T3 (fT3) and free T4 (fT4). The analysis  
175 was conducted by SA Pathology, a National Association of Testing Authorities (NATA)  
176 accredited diagnostic laboratory in Adelaide. TSH, fT3) and fT4 were determined using an  
177 ADVIA Centaur automatic chemiluminescence immunoassay (Siemens Healthcare  
178 Diagnostics, US). Tg was determined using the Immulite 2000 chemiluminescent  
179 immunometric assay (Siemens Healthcare Diagnostics, UK). The coefficients of variability  
180 for TSH, fT3, fT4 and Tg were 5%, 7%, 4.5% and 8%, respectively.

#### 181 182 *Sample size and Statistics*

183



184 At the time that the study was conducted there was limited data on total dietary iodine intake  
185 in pregnant women. Therefore, sample size calculations were based on iodine intake data  
186 from a previous iodine FFQ validation study in females of child bearing age<sup>(17)</sup>. Assuming a  
187 median iodine intake of 115µg<sup>(17)</sup>, we estimated that 84 women would be required to detect a  
188 minimum difference of 20µg (10% of the RDI) in reported iodine intake between the two  
189 dietary assessment methods with 90% power and a correlation of 0.5 ( $p < 0.05$ ). A difference  
190 of  $< 10\%$  RDI was considered clinically insignificant.

191

192 Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS)  
193 V16.0.0 (SPSS Inc. Chicago IL, USA). Results were reported as the mean  $\pm$  SD for  
194 continuous variables and number and percentage for categorical variables. Paired *t* tests were  
195 conducted to compare mean iodine intakes between the I-FFQ and weighed food records as  
196 well as iodine intake from the I-FFQ between the two time points ( $< 20$  weeks vs 28 weeks  
197 gestation). Pearson's correlation coefficient was used to determine the correlation between  
198 iodine intake from the I-FFQ and weighed food record (food only and food plus supplements)  
199 as well as the correlation in iodine intake estimated from the I-FFQ (food only) at the two  
200 time points. Agreement between the two dietary methods was assessed using the Bland–  
201 Altman method. Limits of Agreement (LOA), defined as the mean difference  $\pm 2$  standard  
202 deviations between the methods were calculated<sup>(24)</sup>.

203

204 Iodine intake from the I-FFQ and weighed food record was also categorised into adequate  
205 ( $\geq 220\mu\text{g/day}$ ) and inadequate ( $< 220\mu\text{g/day}$ ) based on the Australian RDI. Weighted kappa  
206 coefficient *k* was used to assess the agreement in the categorisation between both dietary  
207 assessment methods. The following guide was used to describe the strength of agreement: *k*  
208  $< 0.20$  = poor agreement; *k*: 0.21-0.40 = fair agreement; *k*: 0.41-0.60 = moderate agreement;  
209 *k*: 0.61-0.80 = good agreement; *k*: 0.81-1.0 = very good/strong agreement<sup>(25)</sup>.

210

211 Linear regression analysis was used to assess the relationship between the I-FFQ (food +  
212 supplements) and biomarkers including urinary iodine excretion (UIE), UIC and thyroid  
213 function as well as the relationship between UIE from the 24 hour urine sample and UIC  
214 from the spot urine sample, adjusted for potential confounding factors including BMI, age,  
215 gestational age, parity, smoking status and education. Subgroup analyses were conducted to  
216 compare iodine supplement vs. non-iodine supplement users and iodised salt vs. non-iodised  
217 salt users. Statistical significance was set at  $P < 0.05$ .

218

**219 Results**

220

221 One hundred and twenty-two women were recruited for the validation study and 96 women  
222 completed the study. Characteristics of the participants are shown in Table 1. These women  
223 were aged between 18-41 years with a gestational age at study entry between 11-19.5 weeks.  
224 Seventy five percent of women were taking iodine supplements and 44% were using iodised  
225 salt. Demographic characteristics of non-completers (n=26) compared to completers (n=96)  
226 did not differ (data not shown). Reasons for women not completing the study included lack of  
227 time (n=17), withdrawal from the PINK study (n=7), miscarriage (n=1) and illness (n=1).

228

**229 *Iodine intake from the I-FFQ and four day weighed food record***

230

231 Mean iodine intakes from the I-FFQ and four day weighed food record were  $144 \pm 52 \mu\text{g/d}$   
232 and  $160 \pm 54 \mu\text{g/d}$ ,  $p < 0.001$  (food only) and  $281 \pm 124 \mu\text{g/d}$  and  $297 \pm 124 \mu\text{g/d}$ ,  $p < 0.001$   
233 (food + supplement). As shown in Figure 1, a significant correlation was found between the  
234 estimated iodine intake from the I-FFQ and weighed food record ( $r=0.349$ ,  $p < 0.001$ ) that was  
235 strengthened once supplements were added ( $r=0.876$ ,  $p < 0.001$ ). The limits of agreement  
236 (LOA) for the Bland-Altman plot was between -102 and 134  $\mu\text{g}$  across the range of iodine  
237 intake reported from food (Figure 2). There was a strong agreement ( $k=0.799$ ,  $p < 0.001$ )  
238 between the two dietary measures in the ability to classify the women as adequate or  
239 inadequate intake based on RDI with 92% of women classified into a same category.

240

**241 *Reproducibility of the I-FFQ in pregnancy***

242

243 There was no difference in the mean iodine intake estimated from the I-FFQ completed at  
244 enrolment ( $< 20$  weeks gestation) and at 28 weeks gestation ( $153 \pm 70 \mu\text{g/d}$  vs.  $144 \pm 52$   
245  $\mu\text{g/day}$  respectively,  $p=0.338$ ). A significant positive correlation ( $r=0.622$ ,  $p < 0.001$ ) was  
246 shown in the estimated iodine intake from the I-FFQ completed at the two time points (Figure  
247 3).

248

**249 *Correlation between iodine intake estimated from the I-FFQ and UIC***

250

251 Median UIC (interquartile range) from the 24 hour urine sample and spot urine sample was  
252 178 (38-586)  $\mu\text{g/L}$  and 212 (7-881)  $\mu\text{g/L}$ , respectively. Urinary iodine excretion (UIE) from  
253 the 24 hour urine sample was 332 (49-799)  $\mu\text{g/day}$ , calculated using UIC from the 24 hour  
254 urine multiply by the total volume of 24 hour urine. The percent of women with UIC <150  
255  $\mu\text{g/L}$  was 39% from the 24 hour urine sample and 37% from the spot urine sample. Iodine  
256 intake from the I-FFQ was positively correlated with iodine concentration from the 24 hour  
257 urine sample expressed either as UIC ( $\mu\text{g/L}$ ) or UIE ( $\mu\text{g/day}$ ), with adjustment for BMI, age,  
258 gestational age, parity, smoking status and education ( $r=0.321$  and  $r=0.448$ ,  $p<0.001$ ,  
259 respectively) or without adjustment ( $r=0.299$  and  $r=0.477$ ,  $p<0.001$ ). There was no  
260 correlation between iodine intake from the I-FFQ and the spot urine sample (Table 2) or  
261 between UIE ( $\mu\text{g/day}$ ) from the 24 hour urine sample and UIC ( $\mu\text{g/day}$ ) from the spot urine  
262 sample ( $r=0.112$ ,  $p=0.281$ ).

263

#### 264 ***Correlation between iodine intake from the I-FFQ and thyroid function***

265

266 No correlation was found between total iodine intake (food + supplement) from the I-FFQ  
267 and any markers of thyroid function including TSH, fT3, fT4 and Tg with or without  
268 adjustment for BMI, age, gestational age, parity, smoking status and education (Table 2).

269

#### 270 ***Subgroup analysis***

271 There were no differences in iodine intake (food only) estimated from the I- FFQ and  
272 weighed food record between subgroups (iodine supplement vs. non-supplement users or  
273 iodised salt vs. non-iodised salt users).

274

275 Iodine-supplement users showed a correlation between iodine intake from the I-FFQ and the  
276 weighed food record (food only) ( $r=0.721$ ,  $p<0.001$ ), and between iodine intake from I-FFQ  
277 and UIC ( $\mu\text{g/L}$ ) ( $r=0.362$ ,  $p=0.004$ ) or UIE ( $\mu\text{g/day}$ ) ( $r=0.313$ ,  $p=0.008$ ) from the 24 hour  
278 urine sample, while no correlation was shown in non-iodine supplement users.

279

280 Non-iodised salt users also showed a positive correlation between the I-FFQ and weighed  
281 food record ( $r=0.576$ ,  $p<0.001$ ) and between iodine intake from I-FFQ and UIC ( $\mu\text{g/L}$ ) from  
282 the 24 hour urine sample ( $r=0.491$ ,  $p<0.001$ ) while no correlation was observed in iodised salt

283 users. UIE ( $\mu\text{g}/\text{day}$ ) from the 24 hour urine sample was positively correlated with the I-FFQ  
284 in both iodised salt ( $r=0.331$ ,  $p=0.028$ ) and non-salt users ( $r=0.605$ ,  $p<0.001$ ).

285

286 With the exception of  $\text{fT}_4$  in non-iodine supplement users, no correlation was shown between  
287 the I-FFQ and UIC ( $\mu\text{g}/\text{L}$ ) from spot urine samples or thyroid function in all subgroups (data  
288 not shown).

289

## 290 **Discussion**

291 To the best of our knowledge this is the first study to develop and validate an iodine specific  
292 FFQ for assessing iodine intake in pregnant women, using both dietary assessment and  
293 functional biomarkers. Our results suggest that the I-FFQ can be used as a valid tool in  
294 estimating iodine intake in pregnant women as the I-FFQ had a good correlation with the four  
295 day weighed food record and UIE from the 24 hour urine sample, and showed strong  
296 reproducibility. Additionally, our results suggest that the I-FFQ can be useful in screening  
297 women that may be at risk of inadequate dietary intake.

298

299 Our results show that the correlation between the I-FFQ and weighed food record was  
300 strengthened once supplements were added which is likely a result of the increased range of  
301 iodine intake. The correlation coefficient in our study compared well with other iodine FFQ  
302 validity studies in adults with four day weighed food records ( $r$  ranging from 0.45 to 0.52)<sup>(17,</sup>  
303 <sup>18)</sup> and repeated 24 hour dietary recalls ( $r=0.377$ )<sup>(19)</sup>. Other validation studies in pregnancy  
304 have assessed multiple nutrients including iodine, and not surprisingly the findings were  
305 inconsistent with energy adjusted correlation coefficients ranging from 0.4 to 0.66 between  
306 FFQ and four day weighed food records<sup>(15, 26)</sup> to -0.03 between FFQ and a 24 hour diet recall  
307 <sup>(14)</sup>, which may be a reflection of the reference method and FFQ used, including the length  
308 and food items included. Other single nutrient validation studies reported similar correlations  
309 to our study including an iron specific checklist with diet history interview ( $r=0.69$ , iron from  
310 food and supplement) during pregnancy<sup>(27)</sup> and a calcium specific FFQ with six day  
311 weighed food record in women of child bearing age ( $r=0.42$ )<sup>(28)</sup>.

312

313 Although correlation analysis is commonly used, this does not indicate the agreement  
314 between two methods. The Bland-Altman method is often viewed as the preferred technique  
315 to assess agreement and hence to determine validity of a new method<sup>(24)</sup>. The results of this

316 study showed large Limits of Agreement, indicating low agreement between the I-FFQ and  
317 the four day weighed food record. Many dietary validation studies have found similar results  
318 (14, 17-19, 27, 29, 30). This is likely to be a reflection of the differences between the dietary  
319 measures, as FFQs are commonly used to estimate longer term, habitual intake while diet  
320 records or 24 hour recalls estimate recent intake. It should therefore be questioned whether  
321 assessing agreement using the Bland-Altman method is appropriate for dietary validation  
322 studies as this technique was originally designed to compare similar methods (24).

323

324 Our study is the only validation study which used both 24 hour urine and spot urine samples  
325 as reference markers to validate I-FFQ. The correlation between iodine intake from the I-  
326 FFQ and 24 hour UIE in our study is comparable to one (17) of the two validation studies that  
327 examined this relationship in non-pregnant women but in contrast to the other study (18),  
328 which showed no correlation between iodine intake from I-FFQ and 24 hour UIE. This is  
329 perhaps not surprising because although a 24 hour sample is less variation when compared to  
330 a spot urine sample (22, 31), it is still subjected to day-to-day variation in iodine intake and  
331 therefore it is not a reliable marker of iodine status for individuals. Furthermore, there was no  
332 correlation between the 24 hour UIE ( $\mu$ /day) and the spot UIC, demonstrating that UIC from a  
333 spot urine sample is a poor indication of iodine intake and status. UIC based on spot urine  
334 adjusted for creatinine (expressed as iodine to creatinine ratio) has been suggested as a more  
335 accurate measure of iodine excretion and better reflection of iodine intake than spot UIC  
336 alone (12, 19, 22, 31, 32). However, it has been shown that 10 repeated spot urine samples are  
337 needed to assess individual iodine status (12), which is cumbersome and impractical similar to  
338 the 24 hour urine collection. Due to these limitations of UIC as a marker of individuals'  
339 iodine status, a simple I-FFQ like the one developed in our study would be a better and  
340 practical tool to assess iodine intake and status in pregnant women.

341

342 No relation between iodine intake from the I-FFQ and any of the blood biomarkers was  
343 shown. It is known that thyroid function is tightly regulated and adaptive mechanisms are in  
344 place to ensure that the functional needs are met, even in times of mild iodine deficiency (33).  
345 Therefore, it may be that changes in blood biomarkers as a result of inadequate iodine intake  
346 will only occur in severely deficient populations, which is not the case for this population,  
347 explaining the lack of correlation shown here. This may also be similar to other biomarkers of  
348 nutrient intake as single nutrient validation studies in pregnancy that have used blood  
349 biomarkers as reference measures also found no or very weak correlations with FFQs (27, 34).

350 Additionally, there are a number of modifications in the regulation of thyroid function that  
351 occur during normal pregnancy, with not all of these entirely well understood. These normal  
352 changes may also contribute to the lack of correlation with dietary iodine intake.

353

354 Within this population there were a similar number of women who used iodised salt  
355 compared to those that did not. Interestingly, non-salt users showed a stronger correlation  
356 between the I-FFQ and both the weighed food record and UIE ( $\mu\text{g}/\text{day}$ ), while iodised salt  
357 users showed no correlation. Although not statistically significant, the non-iodised salt users  
358 had a higher iodine intake of approximately  $20\mu\text{g}$  (10% RDI). It may be a possibility that  
359 those women who add no salt to cooking or at the table are more health conscious and  
360 therefore include foods that are higher sources of iodine, resulting in stronger correlations  
361 between the I-FFQ and weighed food record. Furthermore, iodised salt was not quantified  
362 from the I-FFQ which may explain the poor correlation between the I-FFQ and UIE in  
363 iodised salt users compared to non-iodised salt users. However these results should be  
364 interpreted with caution as this is a secondary analysis and the sample size within the  
365 subgroups may be inadequate.

366

367 This study has a number of strengths. The most updated food composition data was used  
368 when estimating iodine intake from the two dietary measures, and the time allocated for the  
369 collection of the reference methods was well controlled and the sample size was adequate.  
370 Additionally, both subjective (the gold standard for dietary assessment) as well as objective  
371 measures were used to assess the validity of the I-FFQ. However, we did not include iodine  
372 intake from iodised salt due to the issues associated with quantifying this. As half of the  
373 women reported the use of iodised salt, this is likely to have increased the mean iodine intake  
374 and therefore effect the relationship between the iodine intake from the I-FFQ and reference  
375 measures.

376

### 377 **Conclusion**

378

379 The validity of the I-FFQ to estimate habitual iodine intake in Australian pregnant women  
380 has been demonstrated by strong correlations with four day weighed food records and  
381 moderate correlation with UIE from 24 hour urine samples as well as strong reproducibility.  
382 Furthermore, the results of our validation study indicate that the I-FFQ can be used as a  
383 simple clinical tool to screen pregnant women at risk of inadequate iodine intake. However

384 the I-FFQ has limited ability to predict thyroid function. This I-FFQ could be modified to  
385 assess iodine intake in other populations.

386

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### 392 **Conflict of interest**

393 None

### 394 **Authorship**

395 The authors contributions are as follows: D.C, M.M, S.S and S.J. Z designed the study; D.C  
396 collected the data and performed statistical analysis; D.C drafted the manuscript with  
397 contributions from all authors. All authors reviewed and approved the manuscript submitted.

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399

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480

**Table 1: Demographic characteristics of the study population**

	<i>Participants</i>	
	<i>(n=96)</i>	
	<b>Mean</b>	<b>SD</b>
Age <sup>a</sup> (year)	31.5	5.1
Gestational Age <sup>a</sup> (week)	15.3	2.3
Weight <sup>a</sup> (kg)	70.1	16.1
BMI <sup>a</sup> (kg /m <sup>2</sup> )	26.2	5.8
Primiparous <sup>b</sup>	55% (53)	
Completed secondary education <sup>b</sup>	85% (82)	
Currently smoking <sup>b</sup>	7% (7)	
Smoking 3 months prior to pregnancy <sup>b</sup>	13% (12)	
Taking iodine containing supplements <sup>b</sup>	75% (72)	
Using iodised salt <sup>b</sup>	45% (43)	

BMI: Body mass index

<sup>a</sup>Data are Mean  $\pm$  SD

<sup>b</sup>Data are % (number)

**Table 2: Association between I-FFQ (Food plus Supplement) and biomarkers**

	Unadjusted (n=96)			Adjusted <sup>a</sup> (n=96)		
	B	SE	P	B	SE	P
<b>Spot urine UIC</b>	0.213	0.128	0.095	0.239	0.143	0.098
<b>FT3</b>	-0.001	0.000	0.052	0.000	0.000	0.318
<b>FT4</b>	-0.001	0.001	0.529	0.000	0.001	0.097
<b>TSH</b>	0.000	0.001	0.900	0.000	0.001	0.798
<b>TG</b>	-0.005	0.011	0.691	-0.009	0.012	0.488

I-FFQ: Iodine specific food frequency questionnaire

B: coefficient

SE: standard error of the coefficient

UIC: urine iodine concentration

FT3: Free Triiodothyronine

FT4: Free thyroxin

TSH: Thyroid stimulating hormone

TG: thyroglobulin

<sup>a</sup>Adjusted for BMI, age, gestational age, parity, smoking status and education

**Figure Legends**

Figure 1: Iodine intakes ( $\mu\text{g}/\text{day}$ ) measured from the I-FFQ at baseline (<20 weeks) and 28 weeks gestation ( $r=0.622$ ,  $p<0.001$ ).

Figure 2: Iodine intakes ( $\mu\text{g}/\text{day}$ ) measured from the I-FFQ and weighed food diary with a) no added supplements ( $r=0.349$ ,  $p<0.001$ ) and b) added supplements ( $r=0.876$ ,  $p<0.001$ ).

Figure 3: Agreement between the I-FFQ and weighed food diary ( $\mu\text{g}/\text{day}$ ) in estimates of iodine intake assessed by the Bland-Altman technique- mean difference ( $\pm 2SDs$ )