Flavor sensing in utero and emerging discriminative behaviors in the human fetus

Supplemental materials

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Supplemental Note | Adapted Vegetable Food Frequency Questionnaire (VFFQ)

The following questions are about the foods you USUALLY eat. Please indicate the number of days per week that you eat each item on average.

Please do not include the amount of Kale or Carrot capsules you consume during the study!

VFFQ1: How many times a week nowadays do you eat carrots (raw, cooked or boiled)?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times
- o 4-7 times
- o More than once a day

VFFQ2: How many times a week nowadays do drink carrot juice/smoothie?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times
- o 4-7 times
- o More than once a day

VFFQ3: How many times a week nowadays do you eat kale (raw, cooked or boiled)?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times
- o 4-7 times
- o More than once a day

VFFQ4: How many times a week nowadays do drink kale juice/smoothie?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times
- o 4-7 times
- o More than once a day

VFFQ5: How many times a week nowadays do you eat chips, roast potatoes, boiled, mashed, jacket potatoes, sweet potatoes?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times
- o 4-7 times
- o More than once a day

VFFQ6: How many times a week nowadays do you eat peas, beans, sweetcorn?

- o Rarely or Never
- o Once a fortnight
- o 1-3 times

- 4-7 times
- More than once a day

VFFQ7: How many times a week nowadays do you eat brassica vegetables?

- Rarely or Never
- Once a fortnight
- 1-3 times
- 4-7 times
- More than once a day

VFFQ8: How many times a week nowadays do you eat other green vegetables (lettuce, leeks etc.)?

- Rarely or Never
- Once a fortnight
- 1-3 times
- 4-7 times
- More than once a day

Scoring: The Sheffield Pregnancy Food Frequency Questionnaire (Mouratidou et al., 2006) was used as a reference from which the questions were adapted. Each item is rated on a 5-point Likert-scale ranging from rarely or never (1) to more than once a day (5). The ratings are summed and higher scores indicating a higher frequency of vegetable consumption.

Carrot consumption: VFFQ1 + VFFQ2 (min:1, max:10)

Kale consumption: VFFQ3 + VFFQ4 (min:1, max:10)

Other non-bitter vegetable consumption: VFFQ5 + VFFQ6 (min:1, max:10)

Other bitter vegetable consumption: VFFQ7 + VFFQ8 (min:1, max:10)

Overall non-bitter vegetable consumption: VFFQ1 + VFFQ2 + VFFQ5 + VFFQ6 (min:1, max:20)

Overall bitter vegetable consumption: VFFQ3 + VFFQ4 + VFFQ7 + VFFQ8 (min:1, max:20)

Supplemental Table S1| The mean of codable scan length (in seconds)

	Kale Flavour Group Mean (SE)	Carrot Flavour Group Mean (SE)	Control Group Mean (SE)
At 32 weeks	487.56 (49.786)	425.49 (60.501)	458.25 (49.159)
At 36 weeks	499.85 (60.694)	467.03 (60.255)	508.21 (46.774)

Supplemental Table S2| Chi-Square Test to examine fetal sex differences in the dataset

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.018	2	.991
N of Valid Cases	99		

*Group * Sex Cross tabulation*

Exposure group		Female	Male	Total
Kale	Count	17	17	34
	Expected Count	16.8	17.2	34.0
Carrot	Count	17	18	35
	Expected Count	17.3	17.7	35.0
Control	Count	15	15	30
	Expected Count	14.8	15.2	30.0
Total	Count	49	50	99
	Expected Count	49.0	50.0	99.0

Supplemental Table S3| Descriptive statistics of maternal and fetal information

	Kale Flavour Group	Carrot Flavour Group	Control Group
	N Mean (SE)	N Mean (SE)	N Mean (SE)
Demographics	N=34	N= 35	N=30
Maternal age	29.88 (.774)	31.26 (.792)	28.4 (1.033)
BMI	25.86 (.571)	26.15 (.604)	25.55 (.307)
Level of Education	GCSE (2) College/A level (13) Degree (14) Postgraduate (5)	College/A level (22) Degree (11) Postgraduate (2)	GCSE (3) College/A level (10) Degree (10) Postgraduate (7)
Fetus information			
Fetal head circumference at 20 weeks	168.07 (1.424)	164.35 (1.023)	171.23 (1.493)
Fetal exact age at			
32 weeks	32.09 (.070)	32.12(.096)	32.09 (.100)
36 weeks	35.97 (.089)	36.04 (.098)	35.83 (.095)
Birth outcomes			
Birth Weight (grams)	3346.26 (40.296)	3255.42 (61.727)	3428.12 (99.775)
Gestational Age at Birth (weeks)	39.3 (.170)	39.39 (.185)	39.83 (.182)

Supplemental Table S4| Predictor fetal and maternal variables in relation to fetal movements

Variable	F	Sig. (Between groups)	95% Confidence Interval for Mean	
			Lower	Upper
Maternal Age	2.698	.072	28.914	30.921
Maternal pre-pregnancy BMI	.319	.728	25.268	26.469
Gestational Age at Birth (in weeks)	2.517	.086	39.275	39.693
Birth Weight (in gram)	.405	.668	3295.73	3452.541
Head Circumference (in cm) at 20 weeks[*]	6.781	**.002[*]**	166.123	169.304

[*]included as a covariate.

Supplemental Table S5| Independent sample t-test on the frequency of maternal vegetable consumption

	F	Sig.	t	df	Two-Sided p	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Vegetable consumption at 32 weeks									
Kale	1.910	.172	.458	67	.649	.068	.149	-.229	.365
Carrot	.079	.780	-.054	67	.957	-.010	.185	-.380	.360
Overall bitter	.545	.463	.517	67	.607	.294	.569	-.841	1.429
Overall non-bitter	.748	.390	.011	67	.991	.005	.431	-.855	.865
Vegetable consumption at 36 weeks									
Kale	3.400	.070	2.156	67	.035	.513	.238	.038	.988
Carrot	1.927	.170	-.660	67	.511	-.152	.231	-.613	.309
Overall bitter	1.142	.289	.598	67	.552	.271	.452	-.632	1.174
Overall non-bitter	.029	.864	-.721	67	.473	-.305	.422	-1.147	.538

Supplemental Table S6| Correlations between maternal mental health scores and FMs at 32 weeks

Fetal Movements		Anxiety (HADS) 32 weeks	Depression (HADS) 32 weeks	Stress (PSS) 32 weeks
FM1-Inner-brow-raiser 32 weeks	Pearsons Correlations	.075	.152	.070
	Sig. (2-tailed)	.464	.138	.494
	N	97	97	97
FM2-Outer-brow-raiser 32 weeks	Pearsons Correlations	.112	.015	-.003
	Sig. (2-tailed)	.275	.881	.977
	N	97	97	97
FM4-Brow-lowerer 32 weeks	Pearsons Correlations	-.005	.110	-.008
	Sig. (2-tailed)	.960	.281	.938
	N	97	97	97
FM6-Cheek-raiser 32 weeks	Pearsons Correlations	.043	-.001	-.067
	Sig. (2-tailed)	.679	.989	.513
	N	97	97	97
FM9–Nose-wrinkle 32 weeks	Pearsons Correlations	-.032	-.033	-.074
	Sig. (2-tailed)	.756	.751	.470
	N	97	97	97
FM10–Upper-lip-raiser 32 weeks	Pearsons Correlations	.111	.028	.036
	Sig. (2-tailed)	.280	.782	.723
	N	97	97	97
FM11–Nasolabial-furrow 32 weeks	Pearsons Correlations	.114	.092	.060
	Sig. (2-tailed)	.267	.372	.559
	N	97	97	97
FM12–Lip-corner-puller 32 weeks	Pearsons Correlations	-.098	.002	-.069
	Sig. (2-tailed)	.338	.985	.502
	N	97	97	97
FM16–Lower-lip-depressor 32 weeks	Pearsons Correlations	.092	.011	.066
	Sig. (2-tailed)	.368	.912	.519
	N	97	97	97
FM18–Lip-pucker 32 weeks	Pearsons Correlations	-.180	-.118	-.146
	Sig. (2-tailed)	.078	.251	.154
	N	97	97	97
FM19–Tongue-show 32 weeks	Pearsons Correlations	-.154	-.049	-.155
	Sig. (2-tailed)	.133	.634	.128
	N	97	97	97
FM20–Lip-stretch 32 weeks	Pearsons Correlations	.193	.014	.058
	Sig. (2-tailed)	.058	.892	.574
	N	97	97	97
FM24–Lip-presser 32 weeks	Pearsons Correlations	.080	.036	.051
	Sig. (2-tailed)	.438	.724	.622
	N	97	97	97

Fetal Movements (*continued*)		Anxiety (HADS) 32 weeks	Depression (HADS) 32 weeks	Stress (PSS) 32 weeks
FM25–Lips-parting 32 weeks	Pearsons Correlations	-.152	-.037	-.136
	Sig. (2-tailed)	.137	.720	.183
	N	97	97	97
FM26–Jaw-drop 32 weeks	Pearsons Correlations	-.099	-.065	-.109
	Sig. (2-tailed)	.332	.526	.289
	N	97	97	97
FM27-Mouth-stretch 32 weeks	Pearsons Correlations	-.151	-.014	-.097
	Sig. (2-tailed)	.141	.891	.344
	N	97	97	97
FM28-Lip-suck 32 weeks	Pearsons Correlations	-.033	-.043	-.083
	Sig. (2-tailed)	.750	.679	.417
	N	97	97	97

Supplemental Table S7| Correlations between maternal mental health scores and FMs at 36 weeks

Fetal Movements		Anxiety (HADS) 36 weeks	Depression (HADS) 36 weeks	**Stress (PSS)** **36 weeks**
FM1-Inner-brow-raiser 36 weeks	Pearsons Correlations	-.086	-.170	-.080
	Sig. (2-tailed)	.440	.124	.472
	N	83	83	83
FM2-Outer-brow-raiser 36 weeks	Pearsons Correlations	.053	.004	-.021
	Sig. (2-tailed)	.634	.968	.853
	N	83	83	83
FM4-Brow-lowerer 36 weeks	Pearsons Correlations	-.040	-.079	.006
	Sig. (2-tailed)	.723	.477	.958
	N	83	83	83
FM6-Cheek-raiser 36 weeks	Pearsons Correlations	.180	.063	.083
	Sig. (2-tailed)	.103	.574	.457
	N	83	83	83
FM9–Nose-wrinkle 36 weeks	Pearsons Correlations	-.066	-.066	-.123
	Sig. (2-tailed)	.554	.555	.266
	N	83	83	83
FM10–Upper-lip-raiser 36weeks	Pearsons Correlations	-.096	-.185	-.038
	Sig. (2-tailed)	.386	.094	.733
	N	83	83	83
FM11–Nasolabial-furrow 36 weeks	Pearsons Correlations	.102	.024	.165
	Sig. (2-tailed)	.357	.828	.136
	N	83	83	83

Fetal Movements (*continued*)		Anxiety (HADS) 36 weeks	Depression (HADS) 36 weeks	**Stress (PSS) 36 weeks**
FM12–Lip-corner-puller 36 weeks	Pearsons Correlations	.141	-.013	.108
	Sig. (2-tailed)	.202	.906	.331
	N	83	83	83
FM16–Lower-lip-depressor 36 weeks	Pearsons Correlations	.138	.113	**.220***
	Sig. (2-tailed)	.213	.309	**.045**
	N	83	83	83
FM18–Lip-pucker 36 weeks	Pearsons Correlations	.102	-.180	.031
	Sig. (2-tailed)	.360	.104	.779
	N	83	83	83
FM19–Tongue-show 36 weeks	Pearsons Correlations	.132	.189	.108
	Sig. (2-tailed)	.234	.087	.333
	N	83	83	83
FM20–Lip-stretch 36 weeks	Pearsons Correlations	.042	.061	.197
	Sig. (2-tailed)	.706	.587	.074
	N	83	83	83
FM24–Lip-presser 36 weeks	Pearsons Correlations	-.074	-.133	-.026
	Sig. (2-tailed)	.507	.232	.813
	N	83	83	83
FM25–Lips-parting 36 weeks	Pearsons Correlations	.028	-.032	-.014
	Sig. (2-tailed)	.803	.777	.898
	N	83	83	83
FM26–Jaw-drop 36 weeks	Pearsons Correlations	.031	-.007	-.008
	Sig. (2-tailed)	.783	.946	.946
	N	83	83	83
FM27-Mouth-stretch 36 weeks	Pearsons Correlations	.021	.015	-.002
	Sig. (2-tailed)	.848	.894	.983
	N	83	83	83
FM28-Lip-suck 36 weeks	Pearsons Correlations	.128	-.110	.078
	Sig. (2-tailed)	.248	.321	.483
	N	83	83	83

*Correlation is significant at the 0.05 level (2-tailed)

Reference:

Mouratidou, T., Ford, F. & Fraser, B. (2006). Validation of a food-frequency questionnaire for use in pregnancy. *Public Health Nutrition,* 9(4), 515–522. https://doi.org/10.1079/PHN2005876