

## Supporting Information

### **Maternal caffeine intake during pregnancy, early growth and body fat distribution at school-age. The Generation R Study**

*Running title: Maternal caffeine intake and childhood body fat*

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#### **Content:**

Methods S1	Unbalanced repeated measurement regression models
Table S1	Infant and childhood growth characteristics
Table S2	Correlation coefficients between childhood body fat measures and insulin and c-peptide levels
Table S3	Non-response analysis at baseline
Table S4	Non-response analysis at follow-up measurement
Table S5	Maternal caffeine intake during pregnancy and childhood body fat distribution at 6 years (basic models)
Table S6	Maternal caffeine intake during pregnancy and childhood overweight at 6 years
Table S7	Maternal caffeine intake during pregnancy and childhood insulin and c-peptide levels at 6 years (basic models)

## Supporting Information Methods S1 Unbalanced repeated measurement regression models

We used unbalanced repeated measurement regression models to analyze the infant and childhood growth patterns among children whose mothers consumed 2-3.9, 4-5.9 and  $\geq 6$  units of caffeine per day during their pregnancy, as compared to children whose mothers consumed  $< 2$  units of caffeine per day. These models allow for incomplete outcome data and take the correlation between repeated measurements of the same subject into account by modelling the correlated errors of these measurements (1, 2). To model the correlated errors, a compound symmetry covariance structure was assumed. The models can be written as:

$$\text{Height (SDS)} = \beta_0 + \beta_1 \times \text{caffeine intake category} + \beta_2 \times \text{age} + \beta_3 \times \text{caffeine intake category} \\ \times \text{age}$$

$$\text{Weight (SDS)} = \beta_0 + \beta_1 \times \text{caffeine intake category} + \beta_2 \times \text{age} + \beta_3 \times \text{caffeine intake category} \\ \times \text{age}$$

$$\text{Body mass index (SDS)} = \beta_0 + \beta_1 \times \text{caffeine intake category} + \beta_2 \times \text{age} + \beta_3 \times \text{caffeine intake} \\ \text{category} \times \text{age}$$

In these models, ' $\beta_0 + \beta_1 \times \text{caffeine intake category}$ ' reflects the intercept. The intercept reflects the mean growth characteristic value in SDS for each caffeine intake category. The term ' $\beta_2 \times \text{age}$ ' reflects the change in growth characteristics per month. The term ' $\beta_3 \times \text{caffeine intake category} \times \text{age}$ ', reflects the difference in change in growth characteristics per month between the different caffeine intake categories.

### References:

1. Goldstein H. Multilevel Statistical Methods. 2nd edn. London: Edward Arnold; 1995.
2. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999;28:964–74.

**Supporting Information Table S1 Childhood growth characteristics (N=7,857)<sup>1</sup>**

	Maternal caffeine intake during pregnancy <sup>2</sup>					P-value <sup>3</sup>
	Total group N=7,857	< 2 units N=4,804	2 -3.9 units N=2,447	4-5.9 units N=496	>6 units N=110	
<b>Birth</b>						
Gestational age at birth, median (95% range), weeks	40.1 (35.7, 42.3)	40.1 (35.6, 42.3)	40.1 (36.0, 42.3)	40.3 (33.3, 42.4)	40.2 (33.3, 42.5)	0.001
Birth length, mean (SD), cm	50.2 (2.4)	50.2 (2.4)	50.2 (2.4)	50.3 (2.2)	50.6 (2.5)	0.216
Birth weight, median (95% range), g	3435 (2242, 4490)	3420 (2250, 4450)	3460 (2260, 4520)	3490 (2144, 4567)	3380 (1980, 4300)	<0.001
<b>6 months</b>						
Age at follow-up, median (95% range), months	6.2 (5.2, 8.3)	6.2 (5.2, 8.3)	6.2 (5.2, 8.3)	6.2 (5.3, 8.1)	6.2 (5.3, 9.4)	0.530
Length, mean (SD), cm	67.6 (2.7)	67.7 (2.7)	67.6 (2.7)	67.8 (2.7)	67.2 (2.4)	0.375
Weight, median (95% range), kg	7.9 (6.2, 9.8)	7.9 (6.2, 9.8)	7.8 (6.2, 9.8)	7.8 (6.3, 10.0)	7.8 (6.4, 9.8)	0.110
Body mass index, median (95% range), kg/m <sup>2</sup>	17.2 (14.7, 20.3)	17.2 (14.7, 20.3)	17.1 (14.7, 20.3)	17.2 (14.4, 20.4)	17.6 (15.1, 20.0)	0.012
<b>12 months</b>						
Age at follow-up, median (95% range), months	11.1 (10.1, 12.5)	11.1 (10.1, 12.6)	11.1 (10.1, 12.5)	11.1 (10.2, 12.5)	11.1 (10.0, 12.7)	0.705
Length, mean (SD), cm	74.4 (2.7)	74.4 (2.7)	74.3 (2.6)	74.5 (2.9)	74.1 (2.7)	0.329
Weight, median (95% range), kg	9.6 (7.7, 12.0)	9.7 (7.6, 11.9)	9.6 (7.6, 11.9)	9.6 (7.7, 12.4)	9.5 (7.9, 12.1)	0.672
Body mass index, median (95% range), kg/m <sup>2</sup>	17.4 (14.9, 20.3)	17.4 (14.9, 20.2)	17.4 (14.9, 20.5)	17.4 (14.6, 20.6)	17.4 (15.3, 20.7)	0.959
<b>24 months</b>						
Age at follow-up, median (95% range), months	25.0 (23.4, 31.4)	25.0 (23.4, 31.2)	24.9 (23.4, 31.4)	25.1 (23.5, 31.7)	24.6 (23.4, 30.4)	0.374
Height, mean (SD), cm	88.7 (3.8)	88.8 (3.8)	88.6 (3.7)	88.9 (4.0)	88.2 (3.2)	0.216
Weight, median (95% range), kg	12.9 (10.3, 16.5)	12.9 (10.3, 16.5)	12.9 (10.3, 16.6)	13.0 (10.4, 16.3)	13.2 (9.7, 16.1)	0.924
Body mass index, median (95% range), kg/m <sup>2</sup>	16.5 (14.1, 19.6)	16.5 (14.0, 19.5)	16.5 (14.1, 20.0)	16.6 (14.1, 18.9)	16.8 (13.9, 19.0)	0.296

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<b>36 months</b>						
Age at follow-up, median (95% range), months	36.7 (35.4, 40.8)	36.7 (35.3, 40.8)	36.7 (35.4, 40.8)	36.7 (35.4, 40.8)	36.7 (35.2, 41.5)	0.997
Height, mean (SD), cm	97.4 (3.8)	97.4 (3.8)	97.4 (3.8)	97.5 (3.8)	97.1 (3.4)	0.833
Weight, median (95% range), kg	15.1 (12.0, 19.5)	15.0 (12.0, 19.5)	15.1 (12.1, 19.5)	15.3 (12.3, 19.0)	15.5 (12.2, 21.7)	0.549
Body mass index, median (95% range), kg/m <sup>2</sup>	15.9 (13.7, 19.0)	15.9 (13.7, 19.0)	15.9 (13.8, 19.1)	16.0 (14.0, 18.8)	16.6 (14.2, 20.8)	0.149
<b>48 months</b>						
Age at follow-up, median (95% range), months	45.8 (44.4, 48.6)	45.8 (44.5, 48.7)	45.8 (44.4, 48.4)	45.9 (44.4, 48.7)	46.0 (44.3, 49.1)	0.807
Height, mean (SD), cm	103.2 (4.2)	103.2 (4.2)	103.2 (4.3)	103.3 (4.2)	103.1 (3.9)	0.986
Weight, median (95% range), kg	16.7 (13.3, 22.1)	16.7 (13.2, 22.2)	16.7 (13.3, 22.1)	17.0 (13.3, 21.4)	17.3 (12.8, 25.7)	0.269
Body mass index, median (95% range), kg/m <sup>2</sup>	15.7 (13.6, 19.2)	15.7 (13.5, 19.3)	15.7 (13.5, 19.9)	15.9 (13.8, 19.0)	16.1 (14.0, 20.8)	0.068
<b>72 months</b>						
Age at follow-up, median (95% range), months	72.6 (67.8, 95.6)	72.7 (67.9, 96.9)	72.5 (67.6, 93.3)	72.6 (66.7, 88.8)	73.0 (67.7, 90.7)	0.042
Height, mean (SD), cm	119.5 (6.0)	119.5 (6.2)	119.5 (5.9)	119.5 (5.9)	119.1 (5.1)	0.960
Weight, median (95% range), kg	22.6 (17.4, 34.2)	22.6 (14.4, 34.4)	22.4 (17.6, 33.8)	22.8 (17.8, 33.8)	22.8 (16.8, 34.2)	0.071
Body mass index at 6 years, median (95% range), kg/m <sup>2</sup>	15.9 (13.6, 21.3)	15.9 (13.6, 21.3)	15.7 (13.7, 20.9)	16.0 (13.8, 21.4)	16.2 (13.9, 21.0)	0.001

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<sup>1</sup>Values represent means (SD), median (95% range) or number of subjects (valid %). <sup>2</sup>1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).

<sup>3</sup>Differences in subject characteristics between the groups were tested using One-Way ANOVA for continuous variables and Chi-square tests for proportions.

**Supporting Information Table S2 Correlation coefficients between childhood body fat measures and insulin and c-peptide levels (N=5,562)<sup>1</sup>**

<i>Spearman r<sub>s</sub></i>	Body mass index	Total body fat mass	Android/gynoid fat mass ratio	Preperitoneal fat area	Insulin	C-peptide
<b>Body mass index</b>	1	0.57 P<0.001	0.47 P<0.001	0.42 P<0.001	0.13 P<0.001	0.09 P<0.001
<b>Total body fat mass</b>	0.57 P<0.001	1	0.55 P<0.001	0.55 P<0.001	0.09 P<0.001	0.06 P=0.001
<b>Android/gynoid fat mass ratio</b>	0.47 P<0.001	0.55 P<0.001	1	0.40 P<0.001	0.09 P<0.001	0.08 P<0.001
<b>Preperitoneal fat area</b>	0.42 P<0.001	0.55 P<0.001	0.40 P<0.001	1	0.09 P<0.001	0.06 P=0.001
<b>Insulin</b>	0.13 P<0.001	0.09 P<0.001	0.09 P<0.001	0.09 P<0.001	1	0.88 P<0.001
<b>C-peptide</b>	0.09 P<0.001	0.06 P=0.001	0.08 P<0.001	0.06 P=0.001	0.88 P<0.001	1

<sup>1</sup>Values are correlation coefficients using Spearman's rho tests for skewed variables.

**Supporting Information Table S3 Non-response analysis at baseline (N=8,879)<sup>1</sup>**

	<b>Maternal caffeine intake during pregnancy available</b>	<b>Excluded because of missing information on maternal caffeine intake during pregnancy</b>	<b>P-value<sup>2</sup></b>
	<b>N= 8,099</b>	<b>N= 780</b>	
<b>Maternal characteristics</b>			
Age, median (95% range), years	30.4 (19.3, 39.3)	28.4 (18.7, 39.3)	<0.001
Height, mean (SD), cm	167.4 (7.4)	164.3 (7.3)	<0.001
Pre-pregnancy weight, median (95% range), kg	64.0 (48.0, 99.0)	63.0 (46.6, 100.0)	0.418
Pre-pregnancy body mass index, median (95% range), kg/m <sup>2</sup>	22.6 (17.9, 35.0)	23.3 (17.6, 35.6)	0.048
Education, No. (%)			
Primary	436 (6.0)	47 (14.1)	<0.001
Secondary	3160 (43.2)	179 (53.6)	
Higher	3712(50.8)	108 (32.3)	
Parity, No. nulliparous (%)	4536 (56.4)	386 (54.3)	0.001
Ethnicity, No. European (%)	4618 (58.4)	150 (31.5)	<0.001
Folic acid supplementation use, No. Yes (%)	4504 (71.2)	128 (53.8)	<0.001
Smoking during pregnancy, No. Yes (%)	1381 (18.6)	59 (18.6)	0.997
Alcohol consumption during pregnancy, No. Yes (%)	2737 (37.3)	49 (16.1)	<0.001
Gestational diabetes, No. Yes (%)	82 (1.1)	9 (1.3)	0.600
Pre-eclampsia, No. Yes (%)	169 (2.3)	18 (2.5)	0.633
Gestational hypertension, No. Yes (%)	301 (4.0)	17 (2.4)	0.039
<b>Child characteristics</b>			
Males, No. (%)	4024 (50.4)	377 (51.3)	0.627
Birth weight, median (95% range), g	3425 (2200, 4482)	3340 (1948, 4460)	<0.001
Gestational age at birth, median (95% range), weeks	40.1 (35.4, 42.3)	40.0 (32.3, 42.4)	<0.001
Ever breastfeeding, No. Yes (%)	5336 (91.9)	275 (95.5)	0.028
Introduction of solid foods, No. before 6 months (%)	3899 (89.4)	123 (89.8)	0.900

<sup>1</sup>Values represent means (SD), median (95% range) or number of subjects (valid %). <sup>2</sup>Differences in subject characteristics between the groups were tested using Independent Samples T-tests for continuous variables and Chi-square tests for proportions.

**Supporting Information Table S4 Non-response analysis at follow-up measurement (N=7,902)<sup>1</sup>**

	Follow-up at 6 years N= 5,562	Lost to follow-up at 6 years N= 2,340	P-value <sup>2</sup>
<b>Maternal characteristics</b>			
Age, median (95% range), years	30.9 (19.8, 39.4)	28.8 (18.5, 38.5)	<0.001
Height, mean (SD), cm	167.7 (7.4)	166.6 (7.3)	<0.001
Pre-pregnancy weight, median (95% range), kg	64.0 (49.0, 98.0)	63.0 (47.0, 100.0)	0.145
Pre-pregnancy body mass index, median (95% range), kg/m <sup>2</sup>	22.6 (18.1, 34.6)	22.6 (17.7, 35.6)	0.300
Caffeine intake during pregnancy, median (95% range), units	1.5 (0, 5.0)	1.3 (0, 5.0)	<0.001
Caffeine intake during pregnancy, No. (%)			
<2 units	3,295 (59.2)	1,545 (66.0)	<0.001
2 – 3.9 units	1,819 (32.7)	635 (27.1)	
4 – 5.9 units	368 (6.6)	130 (5.6)	
≥ 6 units	80 (1.4)	30 (1.3)	
Education, No. (%)			
Primary	272 (5.4)	152 (7.2)	<0.001
Secondary	1,976 (39.3)	1,116 (53.1)	
Higher	2,780(55.3)	833 (39.6)	
Parity, No. nulliparous (%)	3,195 (57.7)	1,238 (53.5)	0.001
Ethnicity, No. European (%)	3,412 (62.1)	1,091 (49.2)	<0.001
Folic acid supplementation use, No. Yes (%)	3,287(75.2)	1,105 (61.6)	<0.001
Smoking during pregnancy, No. Yes (%)	871 (17.1)	478 (22.2)	<0.001
Alcohol consumption during pregnancy, No. Yes (%)	2,056 (40.9)	630 (29.6)	<0.001
Gestational diabetes, No. Yes (%)	55 (1.0)	25 (1.1)	0.679
Pre-eclampsia, No. Yes (%)	99 (1.9)	55 (2.6)	0.082
Gestational hypertension, No. Yes (%)	228 (4.3)	66 (3.1)	0.012
<b>Child characteristics</b>			
Males, No. (%)	2,772 (49.8)	1,207 (51.6)	0.147
Birth weight, median (95% range), g	3,450 (2260, 4460)	3,400 (2205, 4528)	0.002
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.0 (35.0, 42.4)	<0.001
Ever breastfeeding, No. Yes (%)	4,114 (92.5)	1,168 (90.1)	0.005
Introduction of solid foods, No. before 6 months (%)	3,105 (89.4)	755 (89.8)	0.754

<sup>1</sup>Values represent means (SD), median (95% range) or number of subjects (valid %). <sup>2</sup>Differences in subject characteristics between the groups were tested using Independent Samples T-tests for continuous variables and Chi-square tests for proportions .

**Supporting Information Table S5 Maternal caffeine intake during pregnancy and childhood body fat distribution at 6 years (basic models) (N=5,562)**

	Body mass index (SDS)	Total body fat mass (SDS)	Android/gynoid fat mass ratio (SDS)	Abdominal preperitoneal fat area (SDS)
	N=5,562	N=5,407	N=5,405	N=4,508
<b>Maternal caffeine intake categories</b>				
< 2 units	Reference N=3,295	Reference N=3,199	Reference N=3,197	Reference N=2,658
2 -3.9 units	-0.10 (-0.15, -0.04)* N=1,819	-0.08 (-0.13, -0.03)* N= 1,771	-0.02 (-0.08, 0.04) N=1,771	-0.12 (-0.18, -0.06)* N=1,475
4- 5.9 units	0.03 (-0.08, 0.13) N= 368	-0.01 (-0.11, 0.09) N=357	-0.04 (-0.15, 0.07) N=357	-0.13 (-0.24, -0.02)* N=306
≥ 6 units	0.08 (-0.13, 0.29) N=80	0.09 (-0.11, 0.29) N=80	0.24 (0.02, 0.46)* N=80	-0.23 (-0.45, 0)* N=69
<b>P-value for trend</b>	0.245	0.202	0.852	<0.001

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The models were adjusted for child's sex, age at follow-up measurement and height at follow-up measurement (fat mass outcomes only). P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. \*P-value <0.05.



**Supporting Information Table S6 Maternal caffeine intake during pregnancy and childhood overweight at 6 years (N=5,562)**

<b>Maternal caffeine intake categories</b>	<b>Odds Ratio (95% Confidence Interval)</b>
< 2 units	Reference <i>N</i> =3,295
2 -3.9 units	0.91 (0.77, 1.08) <i>N</i> =1,819
4 – 5.9 units	1.09 (0.80, 1.49) <i>N</i> =368
≥ 6 units	1.25 (0.68, 2.30) <i>N</i> =80
<b>P-value for trend</b>	<b>0.937</b>

Values are Odds Ratio's (95% confidence interval) that reflect the risk of overweight/obesity in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The model was adjusted for the child's sex, age at follow-up measurement, maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, folic acid supplementation use, smoking and alcohol consumption during pregnancy, gestational diabetes, gestational hypertensive disorders, birth weight, gestational age at birth, breastfeeding, timing of introduction of solid foods and television-watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable.

**Supporting Information Table S7 Maternal caffeine intake during pregnancy and childhood insulin and c-peptide levels at 6 years (basic models) (N=3,667)**

	<b>Insulin (SDS)</b>	<b>C-peptide (SDS)</b>
	<b>N=3,654</b>	<b>N=3,667</b>
<b>Maternal caffeine intake categories</b>		
< 2 units	Reference <i>N=2,116</i>	Reference <i>N=2,128</i>
2 – 3.9 units	-0.02 (-0.09, 0.05) <i>N= 1,239</i>	0.01 (-0.09, 0.06) <i>N=1,237</i>
4 – 5.9 units	0.16 (0.02, 0.29)* <i>N=241</i>	0.16 (0.03, 0.29)* <i>N=244</i>
≥ 6 units	-0.19 (-0.45, 0.07) <i>N=58</i>	-0.10 (-0.36, 0.16) <i>N=59</i>
<b>P-value for trend</b>	0.738	0.415

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The models were adjusted for child's sex and age at follow-up measurement. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. \*P-value <0.05.