**Hunter et al. Male Fetus Susceptibility to Maternal Inflammation: C-reactive Protein and Brain Development: Supplement**

**Mass Spectroscopy.** Stable isotope standards for betaine (N,N,N-trimethylglycine, cat no D-3352) and choline (cat no D-2464) were purchased from CDN Isotopes. Serum samples were thawed on ice, then 20 μL was extracted with 480 μL of ice cold extraction buffer (5:3:2 MeOH:MeCN:H2O) containing 0.1 μM each of N,N,N-trimethylglycine-D9 (betaine) and [1,1,2,2-D4]choline. Extraction was performed by vigorous agitation at 40C for 30 min followed by centrifugation at 12,000 rpm, 40C for 10 min. A 100 μL aliquot of supernatant was transferred to a glass vial, dried under N2 flow, and resuspended in an equal volume of water containing 0.1% (v/v) formic acid. Aqueous extracts were analyzed by ultra high pressure liquid chromatography-mass spectrometry (UHPLC-MS) on a Thermo Vanquish UHPLC (San Jose, CA) coupled to a Thermo Q Exactive mass spectrometer (Bremen, Germany) via positive electrospray ionization. Solvents were water (phase A) and acetonitrile (phase B) supplemented with formic acid (0.1%) and flow rate was 0.25 mL/min. Metabolites were separated using a Kinetex C18 (Phenomenex, Torrance, CA) column (2.1 x 150 mm, 1.7 μm) with a 6 minute gradient of 0-2 min 2% B; 2-2.5 min increase to 25% B; 2.5-4 min hold at 25% B; 4-4.01 min decrease to 2% B; 4.01-6 min hold at 2% B. The Q Exactive mass spectrometer was operated in full scan mode over the range of 65-950 m/z. Samples were randomized and a quality control sample was injected every 10 runs. The coefficient of variation was < 10%. Data analysis was performed using Maven Metabolomic Analysis and Visualization Engine (Princeton University) following file conversion by MassMatrix (Case Western Reserve University). Absolute concentrations were obtained using the following equation:

[light] = (peakarealight/peakareaheavy)[heavy]\*DF

where DF = dilution factor, in this case, 25 (i.e. 20μ of serum in a total 500μ volume).

**Figure S1. Flow of mothers and offspring over the course of gestation and post-birth development.**

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**Table S1. Maternal status during gestation, labor, and delivery, neonatal outcomes stratified by offspring sex.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Males = 76Mean (SD) or N (%) | Females = 74Mean (SD) or N (%) | *P* =(t-test or Fisher's exact test) |
| **Maternal demographics** |
| Maternal age yrs | 27.6 (6.3) | 29.8 (5.7) | 0.88 |
| Maternal education yrs | 13.7 (3.0) | 13.4 (3.1) | 0.54 |
| Pre-pregnancy BMI | 27.0 (6.7) | 27.6 (6.3) | 0.58 |
| Living with biological father N | 56 (69%) | 61 (76%) | 0.38 |
| **Maternal mental illness and drug use** |
| Bipolar Disorder N | 3 (4%) | 4 (5%) | 0.22 |
| Major Depression N | 14 (17%) | 10 (13%) | 0.51 |
| Anxiety Disorder N | 2 (2%) | 5 (6%) | 0.28 |
| Schizophrenia N | 0 | 2 (3%) | 0.24 |
| Alcohol use N | 14 (17%) | 8 (10%) | 0.25 |
| Cannabis use N | 16 (20%) | 9 (11%) | 0.19 |
| Current smoker N | 7 (9%) | 4 (5%) | 0.53 |
| Cocaine N | 7 (9%) | 5 (6%) | 0.27 |
| Opioids N | 2 (2%) | 2 (2%) | 0.89 |
| **Obstetrical history** |  |  |  |
| Gravidity N | 3.25 (1.87) | 2.88 (1.80) | 0.22 |
| Prior preterm delivery N | 12 (15%) | 18 (23%) | 0.23 |
| Miscarriage, ectopic, aborted N | 56 (69%) | 54 (67%) | 0.87 |
| Living children N | 1.52 (1.42) | 1.20 (1.25) | 0.33 |
| **Pregnancy, Labor, Delivery** |
| Prenatal vitamins with folic acid N | 68 (89%) | 66 (89%) | 1.00 |
| Choline μM | 6.31 (1.78) | 6.49 (1.87) | 0.54 |
| Betaine μM | 11.1 (2.9) | 11.9 (4.2) | 0.18 |
| Obesity BMI>30 N | 19 (23%) | 28 (35%) | 0.12 |
| Common infections N | 35 (43%) | 30 (38%) | 0.52 |
| Infection severity | 2.05 (2.74) | 1.91 (2.70) | 0.75 |
| Hypertension N | 4 (5%) | 6 (8%) | 0.53 |
| Gestational diabetes N | 4 (5%) | 4 (5%) | 0.99 |
| Preeclampsia N | 7 (9%) | 6 (8%) | 0.99 |
| Preterm labor N | 8 (10%) | 3 (4%) | 0.21 |
| Preterm birth <37 weeks N | 7 (7%) | 3 (4%) | 0.50 |
| Vaginal delivery N | 64 (67%) | 63 (66%) | 0.99 |
| **Maternal self-ratings** |
| Center for Epidemiological Studies of Depression | 14.5 (10.3) | 13.6 (8.6) | 0.53 |
| State-Trait Anxiety Inventory | 35.6 (12.2) | 35.9 (9.6) | 0.89 |
| Perceived Stress Scale | 23.4 (9.1) | 23.8 (6.8) | 0.75 |
| Parenting Stress Index 3 mos post birth | 26.8 (7.4) | 25.7 (7.2) | 0.81 |
| **Neonatal Status** |
| APGAR 5 min | 8.86 (0.45) | 8.69 (0.80) | 0.32 |
| Small for gestational age N | 3(4%) | 4 (5%) | 0.72 |
| Large for Gestational age N | 11 (14%) | 9 (71%) | 0.81 |
| Meconium fluid N | 16 (20%) | 20 (25%) | 0.57 |
| Nuchal cord N | 24 (30%) | 14 (66%) | 0.09 |
| Days in NICU>1 | 5 (6%) | 5 (6%) | 0.99 |
| Jaundice N | 32 (40%) | 36 (45%) | 0.52 |
| Gestational age at birth days | 273.8 (15.7) | 271.4 (18.6) | 0.38 |
| Birth weight g | 3266.9 (585.0) | 3049.4 (607.7) | 0.022 |
| Birth length cm | 49.7 (4.2) | 48.4 (5.3) | 0.086 |
| Birth head circumference cm | 35.0 (2.7) | 34.1 (2.7) | 0.045 |
| **Maternal inflammation** |
| CRP mg/ml | 7.75 (7.01) | 9.68 (8.00) | 0.12 |
| IL-6 pg/ml | 0.46 (1.6) | 0.63 (1.21) | 0.46 |
| IL-8 pg/ml | 1.18 (1.36) | 1.71 (2.38) | 0.09 |
| TNFα pg/ml | 3.32 (1.59 | 3.52 (1.95) | 0.48 |
| **Newborn cerebral physiology 1 month post birth** |
| P50S1 μV | 1.60 (0.67) | 1.81 (1.00) | 0.10 |
| P50S2μV | 0.83 (0.60) | 0.83 (0.65) | 0.96 |
| **Infant behavior 3 months post birth** | N = 72 | N = 65 |  |
| Surgency | 4.19 (1.11) | 4.30 (1.12) | 0.58 |
| Negativity | 3.09 (0.89) | 3.13 (0.94) | 0.80 |
| Regulation | 5.26(0.68) | 5.21 (0.68) | 0.67 |

**Table S2. Effects of maternal factors on CRP levels at 16 weeks gestation: multiple regression analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Maternal factors | Standardized CoefficientsBeta | 95% Wald Confidence Interval | Wald Chi-Square df1 | *P* |
| CESD 16 weeks | 0.033 | -0.125 | 0.191 | .177 | 0.679 |
| Infection severity 16 weeks | 0.202 | 0.047 | 0.356 | 6.841 | 0.011 |
| Pre-pregnancy BMI | 0.329 | 0.176 | 0.482 | 18.537 | <0.001 |
| Maternal education yrs | -0.050 | -0.208 | 0.108 | -.635 | 0.527 |

Table S3. Effects of maternal CRP at 16 weeks gestation on labor and the neonate.

|  |  |  |  |
| --- | --- | --- | --- |
| **Pregnancy Complications** | CRP mg/ml complicationmean (SD) | CRP mg/mlno complication | *P* |
| Pre-eclampsia N = 12 | 8.38 (8.07) | 9.07 (7.56) | 0.77 |
| Gestational diabetes N =6 | 9.05 (9.76) | 9.01 (7.51) | 0.99 |
| Pre-mature delivery <37 wks N = 7 | 10.34 (13.2) | 8.94 (7.24) | 0.64 |
| Infant small for gestational age N = 7 | 7.37 (5.31) | 9.10 (7.69) | 0.56 |
| Chorioamniotis N = 10 | 8.44 (6.94) | 9.06 (7.65) | 0.81 |
| **Newborn birth parameters** | CRP Pearson’s r | *P* |
| Birth weight gm | .030 | 0.72 |
| Birth head circumference cm | -.063 | 0.45 |
| Birth length cm | .085 | 0.30 |
| Gestational age at birth | .029 | 0.73 |
| APGAR 5 min | -.07 | 0.40 |

Table S4. Effects of maternal cytokines on newborn P50S2 amplitude

|  |  |
| --- | --- |
| Source Tamp | Type III |
| Wald Chi-Square | df | Sig. |
| Model | 83.420 | 10 | <.001 |
| (Intercept) | 2.591 | 1 | .107 |
| Sex | 3.312 | 1 | .069 |
| Maternal CRP 16 wks | .068 | 1 | .794 |
| Maternal IL6 16 wks | .102 | 1 | .749 |
| Maternal IL8 16wks | .049 | 1 | .826 |
| Maternal TNF 16 wks | 1.925 | 1 | .165 |
| P50S1 μV | 100.741 | 1 | <.001 |
| Sex\*CRP | 6.522 | 1 | .011 |
| Sex\*IL6 | .048 | 1 | .827 |
| Sex\*IL8 | .001 | 1 | .975 |
| Sex\*TNF | 1.146 | 1 | .284 |

Table S5. Effects of maternal cannabis, alcohol, and cigarette smoking on the effect of maternal CRP on newborn P50S2 amplitude.

|  |  |
| --- | --- |
| Source | Type III |
| Wald Chi-Square | df | Sig. |
| Model | 84.182 | 7 | <.001 |
| (Intercept) | .878 | 1 | .349 |
| Sex | 2.981 | 1 | .084 |
| Maternal CRP 16 wks | .019 | 1 | .891 |
| P50S1 μV | 93.165 | 1 | .000 |
| Maternal tobacco smoking 16 wks | .773 | 1 | .379 |
| Maternal cannabis 16 wks | 1.045 | 1 | .307 |
| Maternal alcohol use 16 wks | .872 | 1 | .350 |
| Sex\* CRP | 9.372 | 1 | .002 |

Table S6. Effects of maternal CRP at 16 weeks gestation on Infant Behavior Questionnaire-R indices at 3 months of age.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | Dependent Variable | Type III Sum of Squares | df | F | Sig. |
| Model | Surgency | 20.041a | 9 | 1.907 | .058 |
| Negativity | 18.340b | 9 | 2.588 | .010 |
| Regulation | 11.362c | 9 | 3.374 | .001 |
| Intercept | Surgency | 4.808 | 1 | 4.118 | .045 |
| Negativity | 2.013 | 1 | 2.556 | .113 |
| Regulation | 5.775 | 1 | 15.436 | <.001 |
| Sex\*Maternal CRP 16 wks gestation | Surgency | .106 | 1 | .091 | .763 |
| Negativity | .541 | 1 | .687 | .409 |
| Regulation | 1.605 | 1 | 4.291 | .041 |
| Maternal CRP 16 wks gestation | Surgency | 5.424 | 1 | 4.645 | .033 |
| Negativity | 1.183 | 1 | 1.502 | .223 |
| Regulation | .397 | 1 | 1.062 | .305 |
| Sex | Surgency | .473 | 1 | .405 | .526 |
| Negativity | .424 | 1 | .538 | .465 |
| Regulation | 1.354 | 1 | 3.618 | .060 |
| Maternal education yrs | Surgency | 1.771 | 1 | 1.517 | .221 |
| Negativity | .909 | 1 | 1.154 | .285 |
| Regulation | 2.886 | 1 | 7.713 | .006 |
| Gestational age at birth | Surgency | .493 | 1 | .422 | .517 |
| Negativity | .263 | 1 | .334 | .564 |
| Regulation | .091 | 1 | .243 | .623 |
| Center Epidemiological Studies Depression-R 6 wks postpartum | Surgency | 4.367 | 1 | 3.740 | .056 |
| Negativity | 5.265 | 1 | 6.685 | .011 |
| Regulation | .339 | 1 | .905 | .343 |
| State-Trait Anxiety Inventory 6 wks postpartum | Surgency | .120 | 1 | .103 | .749 |
| Negativity | 4.642 | 1 | 5.894 | .017 |
| Regulation | .069 | 1 | .185 | .668 |
| Perceived Stress Scale 6 wks postpartum | Surgency | 3.785 | 1 | 3.241 | .075 |
| Negativity | .028 | 1 | .035 | .851 |
| Regulation | 3.643 | 1 | 9.738 | .002 |
| Parenting Stress Index6 wks postpartum | Surgency | .281 | 1 | .241 | .625 |
| Negativity | .764 | 1 | .970 | .327 |
| Regulation | .013 | 1 | .036 | .850 |
| Error | Surgency | 128.451 | 110 |  |  |
| Negativity | 86.624 | 110 |  |  |
| Regulation | 41.156 | 110 |  |  |
| Total | Surgency | 148.492 | 119 |  |  |
| Negativity | 104.964 | 119 |  |  |
| Regulation | 52.518 | 119 |  |  |

a. R Squared = .135

b. R Squared = .175

c. R Squared = .216

Table S7. Effects of newborn P50S2 amplitude on infant IBQ-R Regulation at 3 months of age

|  |  |
| --- | --- |
| Source  | Type III |
| Wald Chi-Square | df | Sig. |
| Model | 41.191 | 6 | <.001 |
| (Intercept) | 208.479 | 1 | <.001 |
| Sex | .096 | 1 | .756 |
| P50S1 μV | .173 | 1 | .677 |
| P50S2 μV | 3.196 | 1 | .074 |
| IBQ-R Surgency | 46.269 | 1 | <.001 |
| Sex\*50S1 μV | 2.469 | 1 | .116 |
| Sex\*50S2 μV | 7.052 | 1 | .008 |

Table S8. Effects of maternal choline and CRP at 16 weeks gestation on newborn P50S2 amplitude

|  |  |
| --- | --- |
| Source | Type III |
| Wald Chi-Square | df | Sig. |
| Model | 80.598 | 6 | <.001 |
| (Intercept) | 5.607 | 1 | .018 |
| Sex | 1.493 | 1 | .222 |
| Maternal choline 16 wks | 4.758 | 1 | .029 |
| Maternal CRP16 wks | 2.017 | 1 | .156 |
| P50S1 μV | 97.699 | 1 | <.001 |
| Sex\*choline\*CRP | 7.888 | 2 | .019 |

Table S9. Effects of maternal choline and CRP at 16 weeks gestation on infant IBQ-R Regulation at 3 months of age

|  |  |
| --- | --- |
| Source | Type III |
| Wald Chi-Square | df | Sig. |
| Model | 57.997 | 7 | <.001 |
| (Intercept) | 63.025 | 1 | <.001 |
| Sex\* CRP\*Choline  | 7.663 | 2 | .022 |
| Sex | .964 | 1 | .326 |
| Maternal CRP 16 wks | 4.089 | 1 | .043 |
| Maternal choline 16 wks | 2.036 | 1 | .154 |
| IBQ-R Surgency | 55.171 | 1 | <.001 |
| Maternal education yrs | 17.103 | 1 | <.001 |