|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 1 – DEXA body composition analysis of offspring exposed to maternal low protein or normal protein diet** | | | | | | | | | | | | |
|  | **PN21** | | | | **PN180** | | | | **PN360** | | | |
|  | NP  (Nlitter=10) | | LP  (Nlitter=4) | | NP  (Nlitter=13) | | LP  (Nlitter=9) | | NP  (Nlitter=14) | | LP  (Nlitter=9) | |
|  | Female  (8 pups) | Male  (8 pups) | Female  (4 pups) | Male  (2 pups) | Female  (10 pups) | Male  (10 pups) | Female  (9 pups) | Male  (8 pups) | Female  (10 pups) | Male  (13 pups) | Female  (8 pups) | Male  (7 pups) |
| **Fat (%)** | 21.1 ± 1.2 | 19.5 ± 1.2 | 16.2 ± 1.8 | 21.4 ± 2.5 | 22.1 ± 1.5 | 24.1 ± 1.5 | 21.5 ± 1.5 | 20.0 ± 1.6 | 34.7 ± 2.2 | 33.7 ± 1.9 | 31.3 ± 2.5 | 27.4 ± 2.5 |
| **Muscle (%)** | 76.9 ± 1.3 | 78.4 ± 1.3 | 81.6 ± 1.8 | 76.4 ± 2.5 | 74.6 ± 1.5 | 73.1 ± 1.5 | 75.1 ± 1.5 | 77.1 ± 1.6 | 62.0 ± 2.2 | 63.4 ± 1.9 | 65.5 ± 2.4 | 69.7 ± 2.4 |
| **BMC (%)** | 2.0 ± 0.1 | 2.0 ± 0.1 | 2.2 ± 0.1 | 2.2 ± 0.1 | 3.4 ± 0.1 \*\* | 2.8 ± 0.1 \*\* | 3.4± 0.1 \*\* | 2.9 ± 0.1 \*\* | 3.3 ± 0.1 \* | 2.8 ± 0.1 \* | 3.2 ± 0.1 \* | 2.9 ± 0.1 \* |

Percentage composition of fat, muscle, and bone mineral content (BMC) in LP and NP offspring at PN21, PN180 and PN360. Values are mean ± SEM (Nlitter represents the number of litters). Data analysed by a mixed linear model incorporating least means square regression to account for litter representation. \*\* Psex<0.001, \*Psex<0.05.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 2 – PN180 organ weights from offspring exposed to maternal low or normal protein diet** | | | | | | | |
|  | **NP (Nlitter=7)** | | **LP (Nlitter=5)** | | **P Values** | | |
|  | Female  (12 pups) | Male  (9 pups) | Female  (6 pups) | Male  (5 pups) | Pdiet | Psex | Pdiet\*sex |
| **Left kidney (g)** | 0.93 ± 0.06 | 1.55 ± 0.08 | 0.82 ± 0.07 | 1.62 ± 0.08 | 0.80 | **<0.001** | 0.24 |
| **Left kidney/body weight (mg/g)** | 2.6 ± 0.1 | 2.4 ± 0.1 | 2.5 ± 0.1 | 2.5 ± 0.1 | 0.77 | 0.47 | 0.51 |
| **Heart (g)** | 1.07 ± 0.07 | 1.45 ± 0.12 | 0.91 ± 0.06 | 1.65 ± 0.07 | 0.86 | **<0.001** | **0.05** |
| **Heart/body weight (mg/g)** | 2.6 ± 0.1 | 2.2 ± 0.1 | 2.8 ± 0.1 | 2.5 ± 0.1 | **0.009** | **<0.001** | 0.52 |
| **Liver (g)** | 12.52 ± 1.22 | 19.06 ± 2.17 | 9.83 ± 1.15 | 21.13 ± 1.33 | 0.84 | **<0.001** | 0.13 |
| **Liver/body weight (mg/g)** | 30.8 ± 1.4 | 29.2 ± 2.5 | 30.5 ± 1.3 | 31.4 ± 1.5 | 0.59 | 0.83 | 0.49 |
| **Brain (g)** | 1.90 ± 0.06 | 1.99 ± 0.11 | 1.86 ± 0.06 | 2.03 ± 0.07 | 0.86 | 0.12 | 0.69 |
| **Brain/body weight (mg/g)** | 4.7 ± 0.2 | 3.0 ± 0.3 | 5.8 ± 0.2 | 3.1 ± 0.2 | **0.014** | **<0.001** | **0.03** |
| **Pancreas (g)** | 0.72 ± 0.06 | 0.87 ± 0.10 | 0.70 ± 0.05 | 0.91 ± 0.06 | 0.92 | **0.02** | 0.67 |
| **Pancreas/body weight (mg/g)** | 1.8 ± 0.1 | 1.3 ± 0.2 | 2.2 ± 0.1 | 1.4 ± 0.1 | 0.20 | **0.002** | 0.35 |
| **Mesenteric fat (g)** | 5.66 ± 0.67 | 7.18 ± 1.22 | 4.51 ± 0.68 | 7.29 ± 0.73 | 0.55 | **0.02** | 0.47 |
| **Mesenteric fat/body weight (mg/g)** | 13.7 ± 1.0 | 10.9 ± 1.9 | 13.5 ± 1.1 | 11.1 ± 1.1 | 0.99 | 0.07 | 0.92 |
| **Peri-renal fat (g)** | 9.08 ± 1.42 | 14.21 ± 2.51 | 5.23 ± 1.34 | 17.20 ± 1.55 | 0.80 | **<0.001** | 0.07 |
| **Peri-renal fat/body weight (mg/g)** | 21.9 ± 2.1 | 21.8 ± 3.7 | 15.6 ± 1.9 | 25.4 ± 2.3 | 0.60 | 0.08 | 0.07 |
| **Abdominal fat (g)** | 8.95 ± 1.27 | 6.18 ± 2.24 | 6.32 ± 1.29 | 9.70 ± 1.38 | 0.79 | 0.85 | 0.07 |
| **Abdominal fat/body weight (mg/g)** | 21.2 ± 2.1 | 9.6 ± 3.8 | 18.6 ± 2.2 | 14.1 ± 2.3 | 0.73 | **0.007** | 0.20 |
| **Total fat (g)** | 23.70 ± 3.31 | 27.79 ± 5.99 | 14.56 ± 3.12 | 34.18 ± 3.62 | 0.75 | **0.01** | 0.08 |
| **Total fat/body weight (mg/g)** | 56.8 ± 5.5 | 42.7 ± 10.0 | 43.3 ± 5.2 | 50.6 ± 6.0 | 0.69 | 0.63 | 0.14 |

Absolute and relative (to body weight) organ and fat pad weights from male and female offspring exposed to maternal low or normal protein diet at PN180. Values are mean ± SEM, Nlitter represents the number of litters, data analysed by a mixed linear model incorporating least means square regression to account for litter representation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 3 – PN360 organ weights from male and female offspring exposed to maternal low or normal protein diet** | | | | | | | |
|  | **NP (Nlitter=18)** | | **LP (Nlitter=6)** | | **P Values** | | |
|  | Female  (9 pups) | Male  (20 pups) | Female  (7 pups) | Male  (3 pups) | Pdiet | Psex | Pdiet\*sex |
| **Left Kidney (g)** | 1.32 ± 0.11 | 1.90 ± 0.05 | 1.17 ± 0.08 | 1.74 ± 0.14 | 0.13 | **<0.001** | 0.96 |
| **Left kidney/body weight (mg/g)** | 3.2 ± 0.2 | 2.6 ± 0.1 | 2.6 ± 0.1 | 2.5 ± 0.2 | **0.017** | 0.06 | 0.12 |
| **Heart (g)** | 1.16 ± 0.09 | 1.69 ± 0.04 | 1.26 ± 0.06 | 1.80 ± 0.12 | 0.21 | **<0.001** | 0.99 |
| **Heart/body weight (mg/g)** | 2.7 ± 1.0 | 2.8 ± 0.5 | 2.9 ± 0.7 | 2.7 ± 1.3 | 0.99 | 0.94 | 0.91 |
| **Liver (g)** | 12.73 ± 1.17 | 21.20 ± 0.74 | 13.69 ± 1.24 | 19.85 ± 2.22 | 0.90 | **<0.001** | 0.47 |
| **Liver/body weight (mg/g)** | 31.2 ± 2.5 | 29.3 ± 1.1 | 28.9 ± 1.8 | 28.6 ± 3.2 | 0.52 | 0.64 | 0.73 |
| **Brain (g)** | 2.03 ± 0.19 | 2.10 ± 0.05 | 1.99 ± 0.07 | 2.19 ± 0.17 | 0.87 | 0.33 | 0.66 |
| **Brain/body weight (mg/g)** | 5.4 ± 1.3 | 3.2 ± 0.3 | 4.4 ± 0.5 | 3.2 ± 1.2 | 0.63 | 0.08 | 0.61 |
| **Pancreas (g)** | 0.74 ± 0.10 | 1.06 ± 0.04 | 0.90 ± 0.08 | 1.06 ± 0.19 | 0.50 | **0.041** | 0.51 |
| **Pancreas/body weight (mg/g)** | 1.8 ± 1.0 | 1.8 ± 0.5 | 1.9 ± 0.8 | 1.6 ± 1.90 | 0.95 | 0.88 | 0.88 |
| **Mesenteric fat (g)** | 6.23 ± 0.94 | 7.26 ± 0.41 | 6.96 ± 0.69 | 7.31 ± 1.73 | 0.72 | 0.52 | 0.75 |
| **Mesenteric fat/body weight (mg/g)** | 15.3 ± 1.2 | 10.1 ± 0.5 | 14.6 ± 0.9 | 10.8 ± 2.1 | 0.98 | **0.001** | 0.60 |
| **Peri-renal fat (g)** | 9.96 ± 3.60 | 18.78 ± 1.57 | 10.21 ± 2.65 | 18.75 ± 6.62 | 0.98 | **0.04** | 0.97 |
| **Peri-renal fat/body weight (mg/g)** | 24.3 ± 4.4 | 25.9 ± 1.9 | 20.6 ± 3.3 | 27.7 ± 8.2 | 0.85 | 0.40 | 0.58 |
| **Abdominal fat (g)** | 8.43 ± 1.36 | 12.02 ± 0.59 | 8.12 ± 1.00 | 15.13 ± 2.50 | 0.37 | **0.001** | 0.27 |
| **Abdominal fat/body weight (mg/g)** | 20.7 ± 1.7 | 16.4 ± 0.7 | 17.2 ± 1.2 | 22.3 ± 3.1 | 0.53 | 0.84 | **0.02** |
| **Total fat (g)** | 24.63 ± 5.42 | 38.06 ± 2.37 | 25.29 ± 3.99 | 41.19 ± 9.96 | 0.76 | **0.02** | 0.84 |
| **Total fat/body weight (mg/g)** | 60.4 ± 6.1 | 52.0 ± 2.7 | 52.4 ± 4.5 | 60.8 ± 11.3 | 0.95 | 0.99 | 0.23 |

Absolute and relative (to body weight) organ and fat pad weights from male and female offspring exposed to maternal low or normal protein diet at PN360. Values are mean ± SEM, Nlitter represents the number of litters, data analysed by a mixed linear model incorporating least means square regression to account for litter representation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 4 – Food and water intake at PN360** | | | | | | | |
|  | **NP (Nlitter=8)** | | **LP (Nlitter=6)** | | **P Values** | | |
|  | Female  (3 pups) | Male  (8 pups) | Female  (5 pups) | Male  (2 pups) | Diet | Sex | Interaction |
| **Food Intake (g/24 h)** | 26.9 ± 2.1 | 43.4 ± 1.4 | 24.8 ± 1.6 | 46.1 ± 2.3 | 0.87 | **<0.001** | 0.21 |
| **Energy Intake (kJ/g/24 h)** | 402.5 ± 69.7 | 672.2 ± 81.0 | 390.4 ± 48.4 | 725.0 ± 86.0 | 0.61 | **<0.001** | 0.42 |
| **Water Intake (ml/24 h)** | 36.3 ± 17.8 | 58.6 ± 11.5 | 56.9 ± 12.9 | 47.0 ± 19.4 | 0.77 | 0.67 | 0.32 |

Food, energy and water intake were measured over a 24-hour period at PN360 in male and female offspring from both dietary groups. Values are mean ± SEM, Nlitter represents the number of litters, data analysed by a mixed linear model incorporating least means square regression to account for litter representation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 5 – Offspring blood glucose levels at PN360** | | | | | | | |
|  | **NP (Nlitter=9)** | | **LP (Nlitter=12)** | | **P Values** | | |
|  | Female  (11 pups) | Male  (25 pups) | Female  (16 pups) | Male  (24 pups) | Diet | Sex | Interaction |
| **Blood Glucose Level (mmol/L)** | 6.4 ± 0.2 | 6.3 ± 0.1 | 6.3 ± 0.2 | 6.3 ± 0.1 | 0.98 | 0.67 | 0.89 |

Blood glucose levels in male and female offspring exposed to maternal LP or NP diets. Values are mean ± SEM, Nlitter represents the number of litters, data analysed by a mixed linear model incorporating least means square regression to account for litter representation.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 6 - PN180 plasma and urine electrolyte concentration and, excretion values from male and female offspring exposed to maternal low protein or normal protein diet.** | | | | | | | | | | | | | |
|  |  | **Plasma** | | | | **Urine** | | | | **Excreted values** | | | |
| **Diet** | Sex | Na (mmol/l) | K (mmol/l) | Cl (mmol/l) | Osmolality (mOsm/kg) | Na (mmol/l) | K (mmol/l) | Cl (mmol/l) | Osmolality (mOsm/kg) | Na (mmol/l/min) | K (mmol/l/min) | Cl (mmol/l/min)) | TTKG |
| **NP (Nlitter=9)** | Female  (5 pups) | 128.4 ± 10.1 | 3.3 ± 0.3 | 103.1 ± 6.6 | 378.2 ± 50.9 | 93.5 ±18.6 | 119.7 ± 25.6 | 157.3 ± 24.7 | 1252.0 ± 224.3 | 3.2 ± 1.1 | 96.9 ± 30.4 | 5.3 ± 1.7 | 12.1 ± 2.8 |
| Male  (7 pups) | 142.3 ± 8.4 | 3.7 ± 0.2 | 112.3 ± 5.7 | 374.2 ± 50.7 | 38.2 ± 15.6 | 143.8 ± 22.2 | 120.8 ± 20.3 | 1684.2 ± 202.1 | 1.1 ± 1.0 | 58.1 ± 28.3 | 2.3 ± 1.5 | 10.0 ± 2.8 |
| **LP (Nlitter=4)** | Female  (6 pups) | 157.0 ± 9.9 | 3.6 ± 0.3 | 116.2 ± 6.9 | 321.5 ± 67.8 | 92.5 ± 18.6 | 87.3 ± 27.3 | 136.4 ± 23.6 | 1071.1 ± 257.0 | 1.4 ± 1.3 | 43.0 ± 36.7 | 2.3 ± 1.9 | 7.4 ± 3.7 |
| Male  (5 pups) | 135.6 ± 11.0 | 3.5 ± 0.3 | 105.5 ± 7.5 | 305.0 ± 67.9 | 35.1 ± 20.6 | 94.3 ± 29.4 | 135.3 ± 26.6 | 1327.3 ± 269.0 | 0.7 ± 1.4 | 28.2 ± 37.8 | 1.3 ± 2.0 | 7.4 ± 3.7 |
| **P Values** | Pdiet | 0.38 | 0.79 | 0.71 | 0.47 | 0.93 | 0.25 | 0.91 | 0.42 | 0.51 | 0.38 | 0.43 | 0.45 |
| Psex | 0.63 | 0.67 | 0.87 | 0.13 | **0.002** | 0.35 | 0.33 | **0.02** | **0.04** | 0.08 | **0.04** | 0.11 |
| Pdiet\*sex | **0.04** | 0.27 | **0.04** | 0/32 | 0.93 | 0.60 | 0.36 | 0.47 | 0.25 | 0.39 | 0.25 | 0.11 |

Values are mean ± SEM, Nlitter represents the number of litters, data analysed using a mixed linear model taking into account litter representation. Sodium (Na), potassium (K) and chloride (Cl) plasma, sodium and excretory values and trans-tubular potassium gradient (TTKG) for male and female offspring exposed to maternal low protein (LP) or normal protein (NP) diet.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 7 – PN360 plasma and urine electrolyte concentration and, excretion values from male and female offspring exposed to maternal low protein or normal protein diet.** | | | | | | | | | | | | | |
|  |  | **Plasma** | | | | **Urine** | | | | **Excreted Values** | | | |
| **Diet** | Sex | Na (mmol/l) | K (mmol/l) | Cl (mmol/l) | Osmolality (mOsm/kg) | Na (mmol/l) | K (mmol/l) | Cl (mmol/l) | Osmolality (mOsm/kg) | Na (mmol/l/min) | K (mmol/l/min) | Cl (mmol/l/min) | TTKG |
| **NP (Nlitter=12)** | Female  (12 pups) | 133.0 ± 7.8 | 10.9 ± 5.8 | 108.5 ± 3.2 | 471.9 ± 70.7 | 80.7 ± 9.9 | 62.3 ± 15.2 | 129.2 ± 16.3 | 1366.9 ± 172.3 | 0.6 ± 0.3 | 23.1 ± 5.1 | 1.4 ± 0.4 | 5.4 ± 1.2 |
| Male  (12 pups) | 139.2 ± 7.8 | 11.2 ± 5.8 | 111.6 ± 3.2 | 459.2 ± 70.9 | 48.1 ± 10.3 | 83.7 ± 15.9 | 88.8 ± 16.8 | 1473.0 ± 172.3 | 0.6 ± 0.3 | 21.1 ± 5.4 | 1.0 ± 0.4 | 6.3 ± 1.2 |
| **LP (Nlitter=10)** | Female  (7 pups) | 128.0 ± 10.2 | 3.6 ± 7.2 | 105.5 ± 4.2 | 323.4 ± 88.5 | 47.3 ± 13.8 | 98.8 ± 21.2 | 153.2 ± 22.4 | 1293.0 ± 225.6 | 0.4 ± 0.3 | 34.0 ± 7.1 | 1.6 ± 0.5 | 8.9 ± 1.6 |
| Male  (10 pups) | 133.6 ± 8.6 | 3.4 ± 7.2 | 104.4 ± 3.5 | 310.3 ± 88.1 | 30.7 ± 11.3 | 115.6 ± 17.4 | 157.7 ± 18.4 | 1531.4 ± 188.7 | 0.2 ± 0.3 | 21.3 ± 5.9 | 0.9 ± 0.4 | 6.8 ± 1.4 |
| **P Values** | Pdiet | 0.54 | 0.43 | 0.16 | 0.20 | 0.06 | 0.10 | **0.04** | 0.97 | 0.22 | 0.41 | 0.89 | 0.19 |
| Psex | 0.50 | 0.75 | 0.78 | 0.19 | **0.02** | 0.22 | 0.31 | 0.37 | 0.70 | 0.16 | 0.19 | 0.67 |
| Pdiet\*sex | 0.97 | 0.29 | 0.55 | 0.98 | 0.43 | 0.88 | 0.21 | 0.73 | 0.58 | 0.30 | 0.64 | 0.13 |

Values are mean ± SEM, Nlitter represents the number of litters, data analysed using a mixed linear model taking into account litter representation. Sodium (Na), potassium (K) and chloride (Cl) plasma, sodium and excretory values and trans-tubular potassium gradient (TTKG) for male and female offspring exposed to maternal low protein (LP) or normal protein (NP) diet.