

Supplementary material for

Maternal periconceptional and first trimester protein restriction in beef heifers: effects on placental parameters and fetal and neonatal calf development

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Table S1. Mean normalised hepatic gene expression of all signalling factors in the liver of male and female 98 days post conception (dpc) fetuses exposed to maternal diets low or high in protein during the periconception (PERI; –60 to 23 dpc) and postconception (POST; 24 to 98 dpc) periods of gestation

Data are the mean \pm s.e.m. Within rows and within sex, values with different superscript letters differ significantly ($P < 0.05$). Mean normalised hepatic gene expression is given as the ratio of the ΔC_t for the target gene to the three selected reference genes peptidylprolyl isomerase A (*PPIA*), β_2 -microglobulin (*B2M*) and tyrosine 3-monooxygenase (*YWHAZ*). LL, low level of dietary protein in the PERI and POST periods; LH, low level of dietary protein in the PERI period and high level of dietary protein in the POST period; HL, high level of dietary protein in the PERI period and low level of dietary protein in the POST period; HH, high level of dietary protein in the PERI and POST periods; *IGF1*, insulin-like growth factor 1; *IGF1R*, *IGF1* receptor; *IGF2*, insulin-like growth factor 2; *IGF2R*, *IGF2* receptor; *GLUT1*, glucose transporter 1; *GR*, glucocorticoid receptor; *HSD11B1*, 11 β -hydroxysteroid dehydrogenase type 1 and *HSD11B2*, type 2; *FOXO1*, Forkhead Box O1; *PEPCK-C*, phosphoenolpyruvate carboxykinase-C; *CD36*, cluster of differentiation 36, also known fatty acid translocase; *FATP1*, fatty acid transport protein 1; *PPAR γ* , peroxisome proliferator-activated receptor gamma and *PPAR α* , alpha; *RXR*, retinoid X receptor; *PGC1 α* , peroxisome proliferator activated receptor gamma coactivator 1 alpha; *ACC*, acetyl-CoA carboxylase; *CPT1*, carnitine palmitoyltransferase I; *PDK1*, 3-phosphoinositide-dependent protein kinase-1, *PDK2*, -2 and *PDK4*, -4.

Item	PERI Diet			
	Male		Female	
	Low (LL + LH)	High (HH + HL)	Low (LL + LH)	High (HH + HL)
Total no. fetuses ^A	9	16	10	11
<i>IGF1</i>	0.014 \pm 0.001 ($n = 9$)	0.016 \pm 0.001 ($n = 15$)	0.016 \pm 0.002 ($n = 9$)	0.018 \pm 0.001 ($n = 10$)
<i>IGF2</i>	20.66 \pm 1.69 ($n = 9$)	19.89 \pm 1.66 ($n = 15$)	18.08 \pm 1.56 ^a ($n = 9$)	25.59 \pm 2.25 ^b ($n = 11$)
<i>IGF1R</i>	0.053 \pm 0.004 ($n = 9$)	0.053 \pm 0.005 ($n = 16$)	0.042 \pm 0.006 ^a ($n = 9$)	0.057 \pm 0.006 ^b ($n = 10$)
<i>IGF2R</i>	0.139 \pm 0.010 ($n = 9$)	0.159 \pm 0.016 ($n = 16$)	0.141 \pm 0.018 ($n = 8$)	0.192 \pm 0.025 ($n = 10$)
<i>FOXO1</i>	0.007 \pm 0.001 ($n = 9$)	0.008 \pm 0.001 ($n = 14$)	0.007 \pm 0.001 ^a ($n = 10$)	0.009 \pm 0.001 ^b ($n = 10$)
<i>PDK1</i>	0.014 \pm 0.001 ^a ($n = 9$)	0.019 \pm 0.002 ^b ($n = 16$)	0.014 \pm 0.002 ^a ($n = 9$)	0.021 \pm 0.001 ^b ($n = 10$)

<i>PDK2</i>	0.010 ± 0.002 (<i>n</i> = 9)	0.009 ± 0.001 (<i>n</i> = 15)	0.013 ± 0.001 (<i>n</i> = 10)	0.012 ± 0.002 (<i>n</i> = 10)
<i>PDK4</i>	0.0002 ± 0.00003 (<i>n</i> = 9)	0.0002 ± 0.00002 (<i>n</i> = 16)	0.0002 ± 0.00003 (<i>n</i> = 10)	0.0003 ± 0.00002 (<i>n</i> = 9)
<i>PEPCK-C</i>	0.002 ± 0.0003 (<i>n</i> = 8)	0.002 ± 0.0003 (<i>n</i> = 15)	0.002 ± 0.0002 (<i>n</i> = 9)	0.003 ± 0.0004 (<i>n</i> = 10)
<i>GR</i>	0.059 ± 0.002 (<i>n</i> = 7)	0.063 ± 0.06 (<i>n</i> = 16)	0.053 ± 0.005 ^a (<i>n</i> = 10)	0.080 ± 0.008 ^b (<i>n</i> = 11)
<i>HSD11β1</i>	0.020 ± 0.004 (<i>n</i> = 9)	0.022 ± 0.003 (<i>n</i> = 16)	0.019 ± 0.004 (<i>n</i> = 10)	0.017 ± 0.002 (<i>n</i> = 10)
<i>HSD11β2</i>	0.0008 ± 0.0001 (<i>n</i> = 9)	0.0008 ± 0.0002 (<i>n</i> = 16)	0.0007 ± 0.0001 (<i>n</i> = 9)	0.0008 ± 0.0001 (<i>n</i> = 10)
<i>GLUT1</i>	0.499 ± 0.067 (<i>n</i> = 9)	0.490 ± 0.028 (<i>n</i> = 16)	0.439 ± 0.035 ^a (<i>n</i> = 9)	0.598 ± 0.062 ^b (<i>n</i> = 9)
<i>CPT1</i>	0.003 ± 0.0004 (<i>n</i> = 8)	0.004 ± 0.0003 (<i>n</i> = 16)	0.003 ± 0.0005 (<i>n</i> = 9)	0.004 ± 0.0005 (<i>n</i> = 10)
<i>FATP1</i>	0.0007 ± 0.0001 (<i>n</i> = 9)	0.0007 ± 0.0001 (<i>n</i> = 16)	0.0007 ± 0.0001 (<i>n</i> = 9)	0.0009 ± 0.0001 (<i>n</i> = 9)
<i>ACC</i>	0.040 ± 0.008 (<i>n</i> = 8)	0.034 ± 0.004 (<i>n</i> = 15)	0.056 ± 0.006 (<i>n</i> = 10)	0.043 ± 0.005 (<i>n</i> = 10)
<i>CD36</i>	0.096 ± 0.014 (<i>n</i> = 8)	0.106 ± 0.010 (<i>n</i> = 16)	0.118 ± 0.008 (<i>n</i> = 10)	0.105 ± 0.010 (<i>n</i> = 9)
<i>PPARα</i>	0.0006 ± 0.0001 (<i>n</i> = 9)	0.0006 ± 0.0001 (<i>n</i> = 16)	0.0005 ± 0.0001 (<i>n</i> = 9)	0.0008 ± 0.0002 (<i>n</i> = 9)
<i>PPARγ</i>	0.0034 ± 0.0006 (<i>n</i> = 9)	0.0028 ± 0.0003 (<i>n</i> = 16)	0.0046 ± 0.0005 ^a (<i>n</i> = 10)	0.0029 ± 0.0003 ^b (<i>n</i> = 9)
<i>PGC1α</i>	0.009 ± 0.001 (<i>n</i> = 9)	0.010 ± 0.001 (<i>n</i> = 16)	0.008 ± 0.001 (<i>n</i> = 9) ^a	0.012 ± 0.001 (<i>n</i> = 10) ^b
<i>RXR</i>	0.071 ± 0.015 (<i>n</i> = 9)	0.075 ± 0.007 (<i>n</i> = 15)	0.107 ± 0.009 ^a (<i>n</i> = 10)	0.076 ± 0.009 ^b (<i>n</i> = 10)
POST Diet				
	Low	High	Low	High
	(LL + HL)	(HH + LH)	(LL + HL)	(HH + LH)
Item	Male		Female	
Total no.	14	11	12	10
fetuses ^A				
<i>IGF1</i>	0.015 ± 0.001 (<i>n</i> = 13)	0.015 ± 0.001 (<i>n</i> = 11)	0.018 ± 0.002 (<i>n</i> = 10)	0.016 ± 0.001 (<i>n</i> = 9)
<i>IGF2</i>	19.26 ± 1.21 (<i>n</i> = 13)	21.26 ± 2.21 (<i>n</i> = 11)	21.60 ± 1.18 (<i>n</i> = 10)	22.82 ± 3.12 (<i>n</i> = 10)

<i>IGF1R</i>	0.055 ± 0.006 (<i>n</i> = 14)	0.051 ± 0.004 (<i>n</i> = 11)	0.053 ± 0.005 (<i>n</i> = 10)	0.047 ± 0.007 (<i>n</i> = 9)
<i>IGF2R</i>	0.155 ± 0.016 (<i>n</i> = 14)	0.148 ± 0.014 (<i>n</i> = 11)	0.168 ± 0.019 (<i>n</i> = 9)	0.170 ± 0.029 (<i>n</i> = 9)
<i>FOXO1</i>	0.008 ± 0.0006 (<i>n</i> = 12)	0.008 ± 0.0009 (<i>n</i> = 11)	0.008 ± 0.0004 (<i>n</i> = 10)	0.008 ± 0.0012 (<i>n</i> = 10)
<i>PDK1</i>	0.017 ± 0.001 (<i>n</i> = 14)	0.016 ± 0.002 (<i>n</i> = 11)	0.017 ± 0.001 (<i>n</i> = 10)	0.019 ± 0.003 (<i>n</i> = 9)
<i>PDK2</i>	0.008 ± 0.001 (<i>n</i> = 13)	0.011 ± 0.001 (<i>n</i> = 11)	0.015 ± 0.002 (<i>n</i> = 10)	0.011 ± 0.001 (<i>n</i> = 10)
<i>PDK4</i>	0.0002 ± 0.00002 (<i>n</i> = 14)	0.0002 ± 0.00003 (<i>n</i> = 11)	0.0002 ± 0.00002 (<i>n</i> = 9)	0.0002 ± 0.00003 (<i>n</i> = 10)
<i>PEPCK-C</i>	0.002 ± 0.0002 (<i>n</i> = 12)	0.002 ± 0.0003 (<i>n</i> = 11)	0.002 ± 0.0003 (<i>n</i> = 12)	0.002 ± 0.0005 (<i>n</i> = 9)
<i>GR</i>	0.059 ± 0.006 (<i>n</i> = 13)	0.065 ± 0.005 (<i>n</i> = 10)	0.068 ± 0.007 (<i>n</i> = 10)	0.067 ± 0.009 (<i>n</i> = 11)
<i>HSD11β 1</i>	0.023 ± 0.004 (<i>n</i> = 14)	0.019 ± 0.004 (<i>n</i> = 11)	0.017 ± 0.003 (<i>n</i> = 10)	0.019 ± 0.003 (<i>n</i> = 10)
<i>HSD11β 2</i>	0.0008 ± 0.0001 (<i>n</i> = 14)	0.0008 ± 0.0002 (<i>n</i> = 11)	0.0009 ± 0.0001 (<i>n</i> = 9)	0.0006 ± 0.0001 (<i>n</i> = 10)
<i>GLUT1</i>	0.514 ± 0.043 ^a (<i>n</i> = 14)	0.467 ± 0.040 ^b (<i>n</i> = 11)	0.455 ± 0.032 (<i>n</i> = 9)	0.581 ± 0.068 (<i>n</i> = 9)
<i>CPT1</i>	0.004 ± 0.0003 (<i>n</i> = 13)	0.003 ± 0.0004 (<i>n</i> = 11)	0.004 ± 0.0004 (<i>n</i> = 9)	0.003 ± 0.0006 (<i>n</i> = 10)
<i>FATP1</i>	0.0007 ± 0.0001 (<i>n</i> = 14)	0.0007 ± 0.0001 (<i>n</i> = 11)	0.0007 ± 0.0001 (<i>n</i> = 9)	0.0009 ± 0.0001 (<i>n</i> = 9)
<i>ACC</i>	0.038 ± 0.006 (<i>n</i> = 13)	0.034 ± 0.004 (<i>n</i> = 10)	0.055 ± 0.006 (<i>n</i> = 10)	0.044 ± 0.005 (<i>n</i> = 10)
<i>CD36</i>	0.095 ± 0.009 (<i>n</i> = 13)	0.111 ± 0.014 (<i>n</i> = 11)	0.123 ± 0.011 (<i>n</i> = 9)	0.102 ± 0.005 (<i>n</i> = 10)
<i>PPARα</i>	0.0006 ± 0.0001 (<i>n</i> = 14)	0.0006 ± 0.0001 (<i>n</i> = 11)	0.0005 ± 0.0001 (<i>n</i> = 9)	0.0009 ± 0.0002 (<i>n</i> = 9)
<i>PPARγ</i>	0.0029 ± 0.0003 (<i>n</i> = 14)	0.0032 ± 0.0005 (<i>n</i> = 11)	0.0044 ± 0.0005 ^a (<i>n</i> = 9)	0.0032 ± 0.0004 ^b (<i>n</i> = 10)
<i>PGC1α</i>	0.010 ± 0.001 (<i>n</i> = 14)	0.009 ± 0.001 (<i>n</i> = 11)	0.009 ± 0.001 (<i>n</i> = 10)	0.011 ± 0.002 (<i>n</i> = 9)
<i>RXR</i>	0.076 ± 0.011 (<i>n</i> = 13)	0.070 ± 0.006 (<i>n</i> = 11)	0.097 ± 0.013 (<i>n</i> = 10)	0.087 ± 0.007 (<i>n</i> = 10)

^ATotal numbers included in each group are given. Where animals were excluded from analyses, this is indicated in the appropriate cell per respective gene.