

Supplemental Figure Legends

Figure S1. Metformin Efflux Transporter Expression

MATE1 and *MATE2* expression in fetal sheep tissue (tissues from 2 fetuses) (**A**) and (**B**) fetal rhesus tissue (from 8 fetuses) RT-qPCR was used to examine the expression of genes encoding major metformin transporters. Results for each transporter are shown relative to expression in the liver and on a log-scale. Means \pm SE are shown.

Figure S2. Actin protein abundance and total protein stain.

Hepatocytes were treated with 0-, 250-, or 1000- μ M metformin for 24 hours for western blot analysis in primary hepatocytes collected from fetal sheep, fetal rhesus macaque, and juvenile Japanese macaque. Actin protein abundance (**A**) or total protein stain was used to demonstrate equality of loading. The connected lines and dots represent the means of treatment duplicates within a set of hepatocytes.

Figure S3. Stimulated Glucose Production and *PCK1* Expression in Metformin-Exposed Fetal and Postnatal Hepatocytes

Glucose production was measured under stimulated conditions with 500 nM dexamethasone and 100 μ M db-cAMP (DEX + cAMP) in the presence of substrates (lactate, pyruvate) and in response to metformin at 0-, 250-, and 1000- μ M in fetal sheep (**A**), fetal rhesus macaque (**B**), and juvenile Japanese macaque (**C**) hepatocytes. *PCK1* expression was measured in fetal sheep (**D**), fetal rhesus macaque (**E**), and juvenile Japanese macaque (**F**) hepatocytes in response to metformin at 0-, 250-, and 1000- μ M. Means \pm SE are shown. * P <0.05 compared to basal.

Figure S4. Metabolic Gene Expression in Metformin-Exposed Fetal Hepatocytes

CPT1A expression measured in fetal sheep (**A**) in response to metformin at 0-, 250-, and 1000- μ M. *LDHA* expression measured in fetal sheep (**B**) and fetal rhesus macaque (**C**) hepatocytes in response to metformin at 0-, 250-, and 1000- μ M. TBARS content in fetal sheep hepatocyte

protein lysates collected at end of the glucose production assays (**D**). Means \pm SE are shown. * $P < 0.05$ compared to basal.

Figure S5. CREB Protein Phosphorylation and Abundance in Metformin-Exposed Fetal Hepatocytes

Protein abundance of CREB (total), phospho-CREB (S133) and ratio (ph/total) in fetal rhesus macaque (**A**) and juvenile Japanese macaque (**B**) hepatocytes exposed to metformin at 0-, 250-, and 1000- μ M doses for 24h. Results are expressed relative to basal (0 μ M metformin) levels (n = 3-4). Means \pm SE are shown. * $P < 0.05$ compared to basal.

Figure S6. Expression of apoptosis genes and measures of cellularity.

Relative expression fold-change (metformin exposed versus basal) in fetal sheep hepatocytes for selected genes related to apoptosis in RNA-seq data set (**A**). Measures of cellularity obtained from protein content per well at the end of glucose production assay, protein content per well from western blotting samples, RNA yield per well from gene expression studies, and cell density measured by sulforhodamine B (SRB) content at the end of the seahorse assay, as indicated, in fetal sheep (**B**), fetal rhesus macaque (**C**), and juvenile Japanese macaque (**D**) hepatocytes. The connected lines and dots represent the means of treatment duplicates within a set of hepatocytes. Means are shown. P -values are shown.

Figure S7. Fetal Hepatic Expression of Hepatokines and Cytokines Following Fetal Metformin Infusion

Liver tissue from fetal sheep receiving metformin infusions (MetF, n=4) was compared to fetuses without infusions (n=12). Expression of hepatokines and cytokines was measured (**A**). mTOR (Y2448), and S6 (S235/6) protein phosphorylation and total abundance were measured using western blot analysis (**B**). Means \pm SE are shown. P -values are shown.

