Electronic Supplementary Information

Dziuba et al. “Low-molecular mass iron complexes in blood plasma of iron-deficient pigs do not originate directly from nutrient iron”

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Appendix I: Additional Surgical Details

Prior to surgery, the animals are induced with 2.2-6.66 mg/Kg tiletamine/zolazepam (Telazol, Zoetis, Florham Park, NJ) intramuscularly. The animals are then intubated and placed on an isoflurane anesthesia. When the animal was under a surgical plane of anesthesia the surgeries were performed.

Caudal Vein: The right external jugular vein was exposed and isolated and a ligature was placed cranial to the proposed catheter insertion site using a 3-0 polydioxanone suture (PDS). A 2 mm incision was made in the right external jugular vein. A 1.57 mm OD polyethylene catheter (catheter-only) or a silicone catheter (VAP) was inserted into the vein and advanced 9-10 cm distally. The catheter was secured in place using a Chinese finger trap suture. The catheter was tunneled subcutaneously leaving several loose bends (to allow for growth of the pig). The catheter was flushed with heparinized saline (100IU/mL). If a catheter-only or a VAP was used, then the following procedure was performed.

For the animals with only a catheter, the tubing was made to exit the skin through a separate 1cm incision inside the 2cm-lateral to the original incision. The jugular incision was closed in two layers using 2-0 Vicryl in a continuous pater and 0.4 mm Vetafil. The small tubing exit incision was closed with 0.4 mm Vetafil in a continuous pattern and an adhesive bandage was placed over the incisions. A blunt 18-gauge needle was inserted into the end of the tubing and attached to an injection cap for flushing and blood draws and flushed with saline.

To insert the VAP, a 5-6 cm curvilinear incision was made just in the mid-cervical region. Subcutaneous tissue was undermined to create a pocket for the port. The catheter and the port were connected, and the port was secured to the underlying musculature using a 3-0 PDS. The VAP was tested via a blood draw before closure. The port and catheter were flushed with heparinized saline and locked with locking solution. Subcutaneous tissues were closed with 3-0 PDS. The skin was closed in an intradermal pattern using a 2-0 polyglyecapore. Tissue glue was applied to the skin incision to create a seal.

For catheter installation into the portal vein, the animal was placed in dorsal recumbency. The ventral midline and right femoral triangle were aseptically prepared using chloroheximide and alcohol and draped with sterile surgical drapes. A 15 cm ventral midline incision was made starting 2 cm behind the xyphoid and extended through the skin, subcutaneous tissues, linea alba and peritoneum. The spleen was localized, exteriorized and a splenic vein was isolated. A 1.57 mm OD polyethylene catheter (for catheter-only) or a silicone catheter (VAP) was inserted into the splenic vein using the Seldinger technique. The catheter was advanced until the tip was located in the portal vein and was verified by palpation; once correct placement was ensured the catheter was secured using a Chinese finger-trap using 2-0 polyglactin 910. If a catheter-only or a VAP was used, then the following procedure was performed.

For the animals with only a catheter, a blunt 18 gauge needle was inserted into the end of the tubing and attached to an injection cap for flushing and blood draws. The catheter was flushed with heparinized saline (100 IU/mL). A stab incision was made in the left flank, and the catheter port was made to exit the abdominal cavity through the stab incision and placed in a pouch that was glued to the pigs back. The abdominal incision was closed in 3 layers: the linea alba, using a simple continuous pater with 0-polyglactin 910; the subcutaneous tissues, using a simple
continuous patter with 3-0 polyglactin 910; and the sin, using the Ford interlocking pattern with 0-polyamine.

For the animal with the VAP, the VAP port was inserted subcutaneously in the paralumbar fossa and tested, in the same manner as the cervical port. The VAP was tested via a blood draw before closure. The port and catheter were flushed with heparinized saline and locked with locking solution. The abdominal incision was closed in 3 layers: the linea alba, using a simple continuous patter with 0-polyglactin 910; the subcutaneous tissues, using a simple continuous patter with 3-0 polyglactin 910; and the sin, using the Ford interlocking pattern with 0-polyamine.

**Gastrostomy Tube:** A gastrostomy tube was installed after installing the PV catheter using the same ventral midline surgical approach as the PV catheter installation. Once the animal is opened the stomach was identified and exteriorized through the ventral midline incision. The location of the gastrostomy tube was identified between the lesser and greater curvature of the stomach. A 1 cm stab incision was made, and the end of the gastrostomy tube was inserted and secured in place using a string suture with 2-0 Vicryl. The stomach was then secured to the body wall using 2 small rows of continuous suture with 2-0 Vicryl. A small stab incision was made through the left body wall and the tube was exteriorized. The right-angle fixation device was inserted to secure the tube to the body wall and the Y-angle adapter was placed on the tube to facilitate deeding. The body was closed as previously described in the portal vein installation procedure.

**Post-OP:** The pigs are administered one dose of tulathromycin (Draxxin) and one dose of flunixin meglumine (Banamine). VAPs and/or catheters were maintained by daily flushing with heparinized saline solution for 5 days; the VAPs were locked with a locking solution (taurodilide citrate) post-flushing for 5 days. Afterwards, the VAPs and catheters are maintained weekly. Animals with VAPs are left to heal for two weeks before experiments are conducted; animals with only catheters were allowed to heal for between 3-6 days before experiments are conducted. To draw blood from the VAP, lidocaine solution is subcutaneously injected to the skin overlying the VAP to desensitize the skin, a Huber needle is inserted into the VAP for blood collection. Post blood-collection the VAP was flushed with heparinized saline and locked with locking solution.

**Euthanasia:** Every 12 hours for 1 day prior to euthanasia, 250IU/kg of heparin was administered subcutaneously. Euthanasia was performed via exsanguination.
Figure S1. Expanded view of P1 HMM in the 700 kDa region. These are the same traces as in Figure 3, top panel. The peaks in 700 kDa region were not enriched with $^{57}$Fe during the experiment.
Figure S2. Selected chromatograms showing the effect of passing blood through the liver of iron-deficient pigs on LMM iron species. Traces A – C refer to the P3 experiment whereas traces D and E refer to P2; PV plasma is in red, CAV plasma is in blue. A, pre-injection; B, ~ 17 min post injection; C, ~ 102 min post-injection; D, pre-injection; E, ~ 104 min post injection.
Figure S3. Representative high-molecular weight LC-ICP-MS chromatograms of select isotopes of PV (red) and CAV/CRV (blue) plasma FTS. Traces of these elements were essentially invariant with time after $^{57}\text{Fe}$ injection. Phosphorus-detected peaks in the 3 kDa region (using the HMM column) were likely due to nucleotide mono-, di-, and triphosphates. We assigned the strong sulfur peak at 130 - 30 kDa as ceruloplasmin (120 kDa) due to coelution with Cu peak in the same region. The intense Mn-detected peaks near the void volume might have been due to alpha-2 macroglobulin (720 kDa), a Mn protein in blood serum.\(^1\) Four Co-detected peaks were observed with apparent masses of 700, 550, 70 and 2 kDa. The 2 kDa peak may be due to coenzyme B$_{12}$.

Filtered plasma exhibited three major Zn-detected peaks at 550, 200, and 60 kDa some of which could be due to albumin, the major known zinc transport protein in the plasma.\(^2\) There were molybdenum-detected peaks at 80 and 2 kDa.


Figure S4. Representative LMM LC-ICP-MS chromatograms of select isotopes in PV (red) vs. CAV/CRV (blue) plasma FTS. Copper-detected LMM traces exhibited minor peaks but they were not reproducible from sample-to-sample.