Online Supporting Material

Hepatic inflammatory gene expression: Quantitative real-time PCR was performed as described (1). Briefly, total RNA was extracted from liver tissue using Trizol reagent (Invitrogen), according to the manufacturer's protocol. First-strand cDNA was synthesized using oligo-dT primers and Moloney murine leukemia virus (MMLV) reverse transciptase (Superscript II, Invitrogen). We designed real-time PCR porcine-specific primers using the Beacon Design software (Bio-Rad, Hercules, CA); each primer pair was validated by performing electrophoresis and melting temperature analysis of the PCR product. PCR amplification was performed on a My-IQ thermocycler (Bio-Rad, Hercules, CA) using SYBR Green I (Bio-Rad). For PCR, 5 μL each of the standard and sample cDNA dilutions were added to individual tubes. Amplification (40 cycles) was conducted in a total volume of 25 μL containing primer concentrations of 3 pmol and equal volumes (12 μL each) of iQ SYBR Green supermix (Bio-Rad) and nuclease-free water. The iQ SYBR Green supermix contains the SYBR Green I dye, hot-start iTaq DNA polymerase, dNTPs and buffers. Amplification efficiency was controlled by the use of an internal control (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) and external standards, which were homologous to the targets. Relative quantification of target mRNA expression was calculated and normalized to GAPDH expression. The $2^{-\Delta\Delta CT}$-method was used to compare gene expression levels between samples, which were analyzed to determine the fold induction of mRNA expression relative to the amount present in control samples.

Tissue insulin signaling: For western blots, tissue samples were processed as described previously (2). Briefly, frozen liver and muscle tissue samples (100 mg) were homogenized in buffer A containing in $mmol \bullet L^{-1}$: 50 HEPES (pH 7.4), 1 EDTA, 1 dithiothreitol, 0.03 phenylmethylsulfonyl-fluoride, 0.0008 aprotinin, 0.008 chymostatin and 0.007 pepstatin. The homogenate was then sonicated and centrifuged at 12,000 g for 15 min at 4°C. From the resulting extracts, equal amounts (30-120 μg) of total protein were separated via 7-15% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat milk in Tris-buffered saline (TBS, 20 $mmol \bullet L^{-1}$ Tris, 150 $mmol \bullet L^{-1}$ NaOH, pH 7.4). Membranes were incubated with a primary antibody diluted in 5% non-fat milk in TBS + 0.1% Tween-20. Membranes were incubated with a secondary antibody (goat anti-rabbit IgG-HRP, or goat anti-mouse IgG-HRP, 1:5,000, Santa Cruz Biotech Inc.), and the bands were detected as described below. The membranes were probed with phosphatidylinositol 3-kinase (PI3K) (1:1,000 dilution; monoclonal mouse antibody, MBL international Corporation, Watertown, MA), insulin receptor substrate 1 (IRS-1) (1:1,000 dilution; polyclonal rabbit antibody, Cell Signaling Technology, Danvers, MA), or insulin receptor substrate 2 (IRS-2) (1:1,000 dilution; polyclonal rabbit antibody, Upstate, Lake Placid, NY). The protein bands at 85kDa (PI3K) and 180kDa (IRS-1/2) were detected.

Insulin receptor protein abundance in muscle and liver was determined as follows. Frozen liver and muscle tissue (100 mg) was homogenized in buffer A as described above. The resulting extracts were then centrifuged at 100,000 x g for 1 h. High speed pellets were dissolved in 500 μL of buffer A with 1% Triton X-100, and 200 μg protein was separated via 7% SDS-PAGE, transferred to a nitrocellulose membrane and probed with insulin receptor β (1:250 dilution; rabbit polyclonal antibody, Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Protein bands at 90 kDa were detected.

The abundance of phosphorylated insulin receptor was determined after immunoprecipitation. Briefly, tissue (100 mg) was homogenized in 1 mL buffer A for 1 min and centrifuged at 10,000 x g for 10 min. The resulting extract (1 mg protein) was immunoprecipitated with 12 μg of anti phospho-tyrosine agarose conjugate (mouse monoclonal antibody, Upstate, Lake

Placid, NY) overnight at 4ºC and 100 μg of immunoprecipitated products were separated via 7% SDS-PAGE. The membranes were probed with IRS-1 (1:1,000 dilution; polyclonal rabbit antibody, Cell Signaling Technology, Danvers, MA), IRS-2 (1:1000 dilution; polyclonal rabbit antibody, Cell Signaling Technology, Danvers, MA), or insulin receptor β (1:250 dilution; rabbit polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA).

Liver tissue supernatants were also separated via 7% SDS-PAGE, transferred to nitrocellulose membrane and probed with JNK-1 and phospho-JNK-1 (1:1000 dilution; polyclonal rabbit antibody, pThr183/Tyr185, Cell Signaling Technology, Danvers, MA). Protein bands at 46 kDa band were detected.

All western blots were allowed to react with horseradish peroxidase substrate (ECL-plus, Amersham Biosciences, Piscataway, NJ) and then exposed to X-ray film for 30-300 s, and the image was scanned and quantified by ImageQuant 5.0 software (Molecular Dynamics, Amersham Biosciences, Sunnyvale, CA). All western blots were run with six pigs from each treatment group and used for statistical analysis. Treatments means and standard errors are shown as bar graphs. We expressed the abundances of specific target proteins relative to that of tubulin measured after stripping and reprobing membranes. To quantify the phosphorylated forms of specific proteins, we expressed the calculated densitometric band intensities of the phosphorylated form relative to that of the total protein; the latter was determined after stripping and reprobing the membranes.

Literature Cited

1. Shaik SS, Soltau TD, Chaturvedi G, Totapally B, Hagood JS, Andrews WW, Athar M, Voitenok NN, Killingsworth CR, Patel RP, Fallon MB, Maheshwari A. Low intensity shear stress increases endothelial ELR+ CXC chemokine production via a focal adhesion kinase-p38{beta} MAPK-NF-{kappa}B pathway. J Biol.Chem. 2009;284:5945-5955.
2. Burrin DG, Stoll B, Guan X, Chang X, Hadsell D. GLP-2 rapidly activates divergent signaling pathways involved in intestinal cell survival and proliferation in neonatal piglets. Am J Physiol. 2007;292:E281-E291.

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Supplemental Table 1. Daily macronutrient intake and composition of enteral formula and TPN diets[1].

	EN[2]	TPN[3,4,5,6]
Fluid intake, *mL*	240	240
Energy, *kJ*	816	824
Protein, *g*	12.5	13
Carbohydrate, *g*	25	25
Fat, *g*	5	5

[1]Daily nutrient intakes expressed as units per kg body weight.

[2]Soweena LitterLife, Merricks; Ingredient list includes, dried whey protein concentrate, dried whey product, dried whey, animal plasma, animal and vegetable fat preserved with BHA, lecithin, dicalcium phosphate, vitamin A acetate, d-activated animal sterol (source of vitamin D_3), vitamin e supplement, menadione dimethylpyrimidinol bisulfite (source of vitamin k activity), choline chloride, riboflavin supplement, calcium pantothenate, niacin supplement, vitamin 12 supplement, biotin, ascorbic acid, magnesium sulfate, manganese sulfate, ferrous sulfate, zinc sulfate, cobalt sulfate, copper sulfate, calcium iodate, sodium selenite, dried lactose, natural and artificial flavors. The major component fatty acid composition as percentage of dry matter is stearic (1.00%), oleic (2.98%), linoleic (1.68%), linolenic (0.14%), total saturated fatty acid (2.93%), total unsaturated fatty acid (5.01%).

[3]TPN solution carbohydrate and amino acid composition includes (g/L): dextrose, 104; total amino acids, 54; alanine, 2.70; arginine, 2.34; aspartic acid, 4.15; cysteine•HCl•H_2O, 1.20; glutamic acid, 5.19; glutamine, 4.15; glycine, 2.03; histidine, 1.35; isoleucine, 3.01; leucine, 5.35; lysine•HCl, 5.05; methionine, 1.35; phenylalanine, 2.75; proline, 3.90; serine, 2.91; threonine, 3.26; tryptophan, 0.63; tyrosine, 0.63; valine, 3.28; lipid 5 g (as Intralipid 20%).

[4]Intralipid® contains 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin; the major component fatty acids and their range of content as percentage of total lipid are linoleic (44-62%), oleic (19-30%), palmitic (7-14%), linolenic (4-11%) and stearic (1.4-5.5%).

[5]TPN electrolyte composition includes (mmol/L): sodium chloride, 17.2; potassium acetate, 13.8; potassium phosphate, 19.7; magnesium sulfate, 2.9; calcium gluconate, 4.5; sodium hydroxide, 13.0.

[6]TPN vitamin and trace mineral composition expressed as the daily intake per kg body weight: vitamin A, 234 µg; vitamin D, 1.7 µg; vitamin E, 1.36 mg; vitamin B_6, 0.396 mg; thiamin B_1, 3.96 mg; riboflavin B_2, 0.198 mg; folic acid, 0.016 mg, vitamin B_{12}, 0.158 ppm, niacin B_3, 3.96 mg; pantothenol, 0.396 mg; sulfur, 145 mg; zinc, 1056 µg; copper, 422 µg; manganese, 106 µg; selenium, 21.1 µg; chromium, 4.22 µg.

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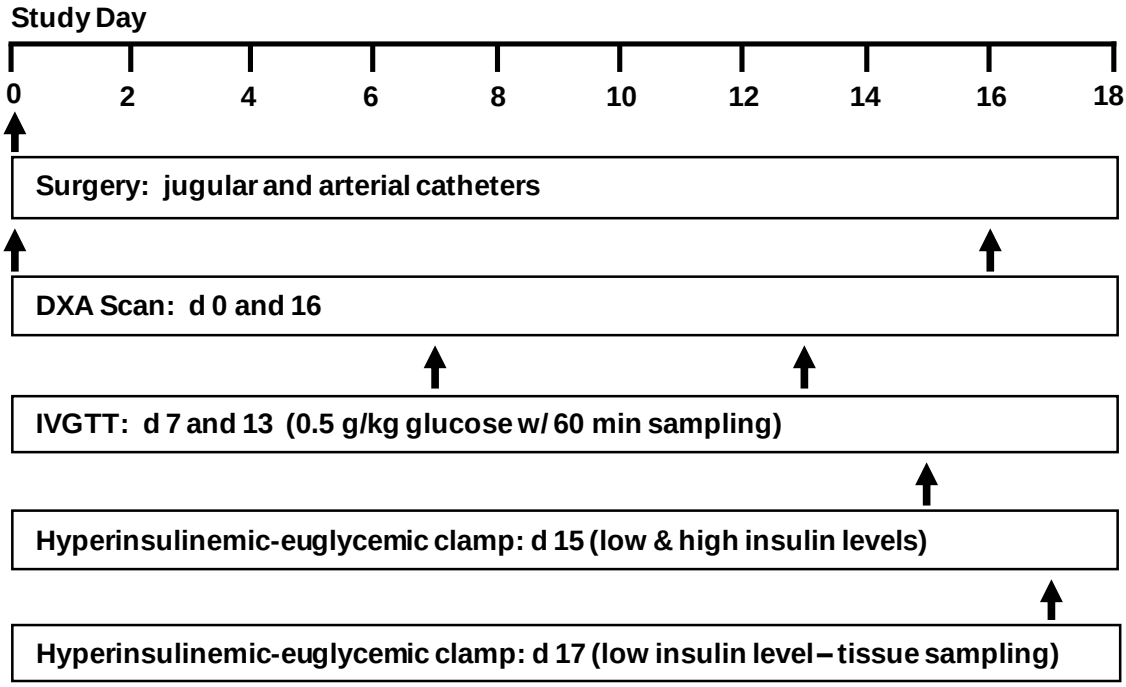

Supplemental Figure 1. Study design. Time line of events during 17-d study of neonatal pigs fed EN or TPN.

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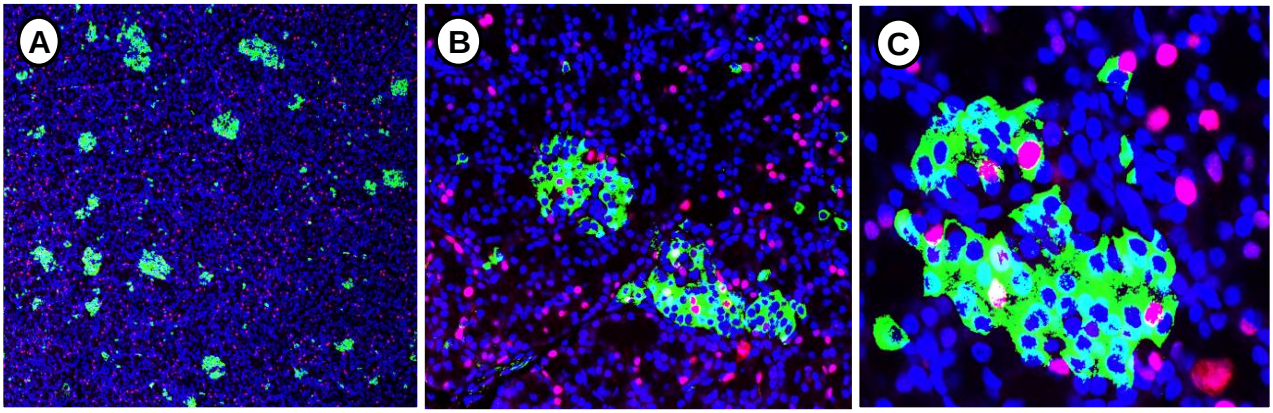

Supplemental Figure 2. Pancreatic morphometry. Quantitative analysis of pancreatic tissue shown in Figure 4 of the article were based on analysis of paraffin-stained sections stained with antibody against Ki-67 (Red color) representing proliferating cells, insulin (Green color) representing β-cells, and Topro-3 (Blue color) representing total cell nuclei. ***A-C:*** Representative images from an EN pig show tissue magnification at ***A:*** 100X, ***B:*** 200X, and ***C:*** 400X.