Supplemental Material

DIFFERENCES BETWEEN HUMAN AND RODENT PANCREATIC ISLETS: LOW PYRUVATE CARBOXYLASE, ATP CITRATE LYASE AND PYRUVATE CARBOXYLATION; HIGH GLUCOSE-STIMULATED ACETOACETATE IN HUMAN PANCREATIC ISLETS

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ENZYME ASSAYS

Enzyme reaction mixtures – All enzymes were measured at 37°. Pyruvate carboxylase (PC), propionyl-CoA carboxylase (PCC), pyruvate dehydrogenase (PDH), mitochondrial glycerol phosphate dehydrogenase (mGPD) and glutamate dehydrogenase activities were measured in a whole cell homogenate prepared as previously described. The activities of other enzymes were measured in the supernatant fraction of the frozen-thawed homogenate so as to include activities of enzymes in the cytosol and mitochondrial matrix. Briefly, islets or cells were washed two or three times with Hank's solution or Hepes (10 mM) fortified Krebs Ringer bicarbonate buffer, pH 7.3, or with phosphate-buffered saline. Cells were homogenized in KMSH solution (220 mM mannitol, 70 mM sucrose, and 5 mM potassium Hepes buffer, pH 7.5) containing 1 mM dithiothreitol and a protease inhibitor mixture (Halt Protease Inhibitor (Pierce)). Islet cell homogenates were obtained by briefly homogenizing islets with a hand held pestle in a microfuge test tube. INS-1 832/13 cells were homogenized with 40 strokes up and down in a mechanical Potter-Elvehjem homogenizer. The supernatant fractions were obtained from centrifuging the whole cell homogenate at 20,800 g for 10 or 20 min.

Pyruvate carboxylase - Pyruvate carboxylase enzyme activity was measured by a CO_2 fixation assay in a reaction mixture containing 20 mM $NaH^{14}CO_3$, 8 mM pyruvate, 2 mM Na_3ATP, 1.6 mM acetyl-CoA, 10 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol, 0.1% Triton X-100, and 100 mM Tris-chloride buffer, pH 7.85, and 10 μl of 2 mg whole-cell homogenate/ml in a final volume of 50 μl incubated at 37° for 30 min. The reaction was stopped by adding 50 μl of 10% trichloroacetic acid, and after 10 min, 80 μl of the mixture was removed and placed in a scintillation vial that was left open for 2 h to eliminate the unincorporated CO_2. Two-tenths milliliter of water and 10 ml of Scintisafe Econ 2 scintillation mixture (Fisher Scientific) were added to the vial, and the carbon fixed was measured. Values from blank test tubes containing no pyruvate were subtracted from test tubes containing the complete reaction mixture to give the rate attributable to pyruvate carboxylase.

Propionyl-CoA carboxylase - The activity of propionyl-CoA carboxylase was measured the same as for pyruvate carboxylase, except 2 mM propionyl-CoA was present instead of pyruvate and there was no acetyl-CoA in the reaction mixture.

Pyruvate dehydrogenase - Pyruvate dehydrogenase (PDH) activity was measured by the release of $^{14}CO_2$ from [1-^{14}C]pyruvate in a reaction mixture containing 1 mM pyruvate, 0.2 μCi [1-^{14}C]pyruvate, 1 mM dichloroacetate, 0.4 mM coenzyme-A, 3 mM NAD, 0.4 mM thiamine pyrophosphate chloride, 5 mM $MgCl_2$, 2 mM DTT, 0.1% Triton X-100, 50 mM Hepes buffer at pH 7.5 and 10-40 μl of whole-cell homogenate in KMSH (along with volume of 40 μl to 10 μl of KMSH to bring volume of the addition to 50 μl) in a final volume of 100 μl in a 1.4 ml microfuge test tube. Prior to starting the reaction the homogenate, which contained 10 mM added $MgCl_2$ to activate phosphatases and 1 mM dichloroacetate to inhibit pyruvate dehydrogenase kinase, was incubated for 15-30 min to activate the pyruvate dehydrogenase complex activity. The test tubes were placed inside 20 ml scintillation vials, sealed with rubber caps and incubated for 30 min at 37°. The reaction was stopped by first adding 0.5 ml tissue solubilizer to the scintillation vials outside of the reaction test tubes and then adding 50 μl 15% TCA to the inside of the reaction test tubes. These additions were made by using a syringe through the rubber caps. After sitting for 3 h at room temperature, the reaction tube was removed and 10

ml of scintillation cocktail was added to the vial and the amount of $^{14}CO_2$ released was measured by liquid scintillation spectrometry. Values from blank tubes containing no acetyl-CoA were subtracted from tubes containing the complete reaction mixture to calculate the release of $^{14}CO_2$ attributable to the enzyme.

ATP citrate lyase - ATP citrate lyase activity was measured in a reaction mixture containing 5 mM citrate, 0.3 mM coenzyme A, 3 mM ATP, 0.15 mM NADH, 10 mM $MgCl_2$, 10 mM dithiothreitol, and 6 units/ml of malate dehydrogenase from pig heart mitochondria in 100 mM Tris chloride buffer, pH 8.5, and whole-cell supernatant fraction at 37° (29). The disappearance of NADH was monitored spectrophotometrically at 340 nm. After a background rate was obtained, the enzyme reaction was started with the addition of ATP. The background rate was subtracted from the rate in the presence of ATP to give the rate attributable to oxaloacetate formation.

Supplemental Table 1. Enzyme reaction mixture volumes and volumes and protein concentrations of enzyme sources used. The protein concentrations of the human islet, rat islet and INS-1 832/13 whole cell homogenates used were 1-2.8 mg protein/ml, 1.1-1.4 mg protein/ml and 3-6 mg protein/ml, respectively and the protein concentrations of the frozen-thawed homogenate supernatant fractions were 0.5-2.0 mg protein/ml, 0.4-0.8 mg protein/ml and 1.2-4.4 mg protein/ml, respectively. These extracts were diluted so that the final concentrations of protein from non-human samples were similar to those of the human samples in the reaction mixtures for each enzyme assay.

Enzyme Assay	Enzyme Source	Volume of Enzyme Source (μl)	Final Volume of Reaction Mixture
Pyruvate Carboxylase	H[a,d]	10 μl	50 μl
Propionyl-CoA Carboxylase	H[a,d]	10 μl	50 μl
Pyruvate Dehydrogenase Complex	H[c]	15-40 μl	100 μl
ATP Citrate Lyase	S[b]	20[e], 40[f], 10[g] μl	1 ml

[a]H, whole-cell homogenate in KMSH solution containing 1 mM dithiothreitol. [b]S, supernatant fraction from centrifuging the frozen and thawed and vigorously vortexed whole-cell homogenate at 20,800 x g for 20 min. [c]Whole-cell homogenate in KMSH, 1 mM dithiothreitol, 10 mM $MgCl_2$ and 1 mM dichloroacetate. [d]Each PC and each PCC assay always contained 10 μl of 2 mg whole-cell homogenate protein/ml. [e]Human islet. [f]Rat islet. [g]INS-1 832/13.

Further evidence indicating the low PC in human islets is intrinsic to the islets and not an artifact –

Islet quality and viability - Although the viability of almost all the human islets preparations we received was described as 90-99%, there are reports of variability among human islet preparations in respect to ATP content, ATP/ADP ratios and quality

of glucose-stimulated insulin release. To cite illustrations of the lack of correlation of islet quality with PC levels, two of the human islet preparations were rated as 90% pure and showed very good ATP/ADP ratios (7.1 and 7.8) and glucose-stimulated:unstimulated insulin secretion ratios of >12 (incubated with 16.7 mM glucose vs with 3.3 mM glucose (GSIS ratio)) as determined by the supplying laboratory. These two preparations possessed some of the highest PC activities we measured. However, these PC activities were still only 20% and 29% of the same-assay PC activities of control INS-1 832/13 cells or rat islets. In two other preparations the purities were 80% and 95%. The ATP/ADP ratios (> 10) and the GSIS ratios (20 and 13.5) were even higher. Although, according to these ratios, the quality of these latter two islet preparations were even more superior than the other two preparations, their PC enzyme activities were only 5% of the same-assay rat islet or INS-1 832/13 PC activities. (Incidentally, these two low PC values and several other very low human islet PC values were not used in calculating the PC values shown in Table 1.) Thus, even the most excellent quality human islets possess low PC levels.

Supplemental Table 2. Primer sequences for qPCR.

	Gene	Sequence
Human	PC1 789 for:	5'- CGCTGTTTGTGGAGAAGTTCATC -3'
	PC1 864 rev:	5'- ATGTTCCCATACTGGTCCCCCAAG -3'
	PC2 2042 for:	5'-GGCTACACCAACTACCCAGACAAC-3'
	PC2 2131 rev:	5'-GGAGTCAAACACACGGAAGACATC-3'
	ME1 for:	5'-CAACAATATAGTTTGGTGTTTCG-3'
	ME1 rev:	5'-CGCTCTCCATCAGTCACCACAATGG -3'
	GPD2 for:	5'-TGGTAGGAGCAATTGTCTACTATG -3'
	GPD2 rev:	5'-GTCTGGGGGTCTGTCTTCTTGAG-3'
	Idh1 for:	5'- TCATGCTTATGGGGATCAATAC-3'
	Idh1 rev:	5'- CAACACCACCACCTTCTTCAAAG-3'
	Idh3a for:	5'- AACAGGTGACCAGAGGTTTTACTG-3'
	Idh3a rev:	5'- ATAGGTGCTTTGGCAGCATC-3'
	FAS for:	5'-GCTGGGTGGAGTCTCTGAAG-3'
	FAS rev:	5'-TGCAACACCTTCTGCAGTTC-3'
	Glud1 1212 for:	5'- CAAATCCAACGCACCCAGAG-3'
	Glud1 1365 rev:	5'- CTGTCACTCCTCCAGCATTCAAG-3'
Rat	PC for:	5'-GCACACGGTCACTGAGGAGATTAC-3'
	PC rev:	5'-GCACAACCATTGATTCGGATG-3'
	ME1 for:	5'- CCAGCAATACAGTTTGGCATTCC-3'
	ME1 rev:	5'- CGCTCTCCATCAGTCACCACAATAG -3'
	GPD2 for:	5'- TGGTCGGAGCCATTGTCTACTATG-3'
	GPD2 rev:	5'- GTTTCGGGGTCTGTCTTCTTGAG-3'
	Idh1 for:	5'- ACACGCCTATGGGGACCAATAC-3'
	Idh1 rev:	5'- CAACACCACCACCTTCTTCAAAG-3'
	Idh3a for:	5'- AACAGGTGACAAGAGGTTTTGCTG-3'
	Idh3a rev:	5'- ATAGGTGCTTTGGCGGCATC-3'
	FAS for:	5'- GACAGTGGCAACCTGATAGTGAGC-3'
	FAS rev:	5'- ATACTTCTCCCTGCGTCAAGCG -3'
	Glud1 1212 for:	5'- CGAGAAGCAGTTGACCAAATCC-3'
	Glud1 1365 rev:	5'- CACTCCTCCAGCATTCAGGTAGAG-3'

Supplemental Figure 1. Linear regression plot showing no correlations of human islet donor body mass index with pancreatic islet pyruvate carboxylase enzyme activity.