Supporting Information

A Metabolomics Study of the Effects of Inflammation, Hypoxia, and High Glucose on Isolated Human Pancreatic Islets

Marta Garcia-Contreras[1,2,3,¶], Alejandro Tamayo-Garcia[1,¶], Kirk L. Pappan[4], Gregory A. Michelotti[4], Cherie L. Stabler[5], Camillo Ricordi[1, 2,*], and Peter Buchwald[1,*]

[1]Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL, USA; [2]Ri.Med, Palermo, Italy; [3]Catholyc University of Valencia, Valencia, Spain; [4]Metabolon, Inc., Durham, NC; [5]Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA. [¶]These authors contributed equally to this work.

[*]Correspondence to Camillo Ricordi (CRicordi@med.miami.edu) or Peter Buchwald (pbuchwald@med.miami.edu), Diabetes Research Institute, University of Miami, Miller School of Medicine, 1450 NW 10th Ave., Miami, FL 33136, USA; Tel. (1) 305 243-3636.

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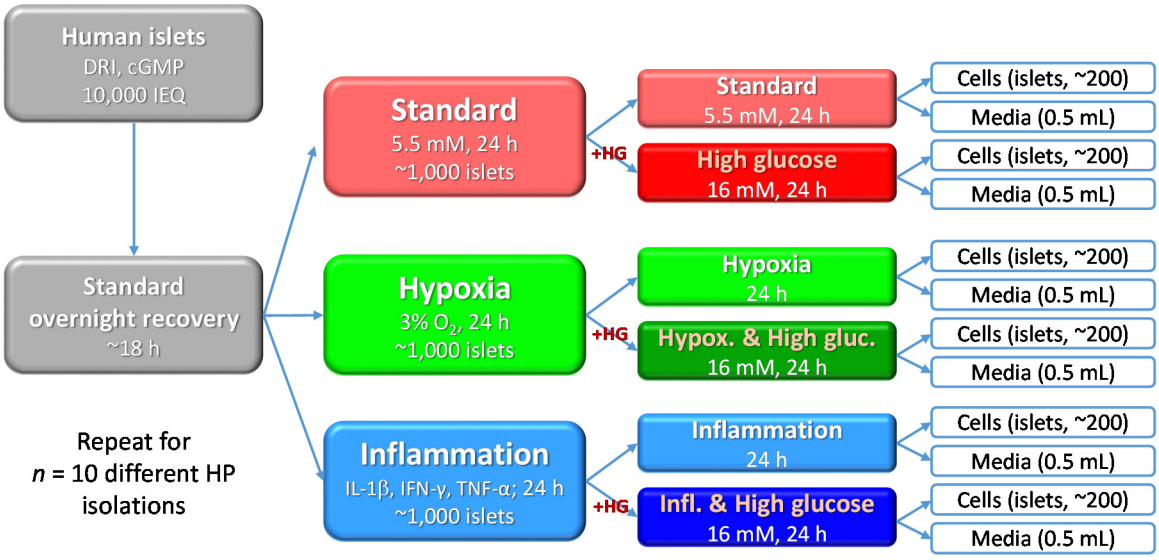
Supplementary Tables

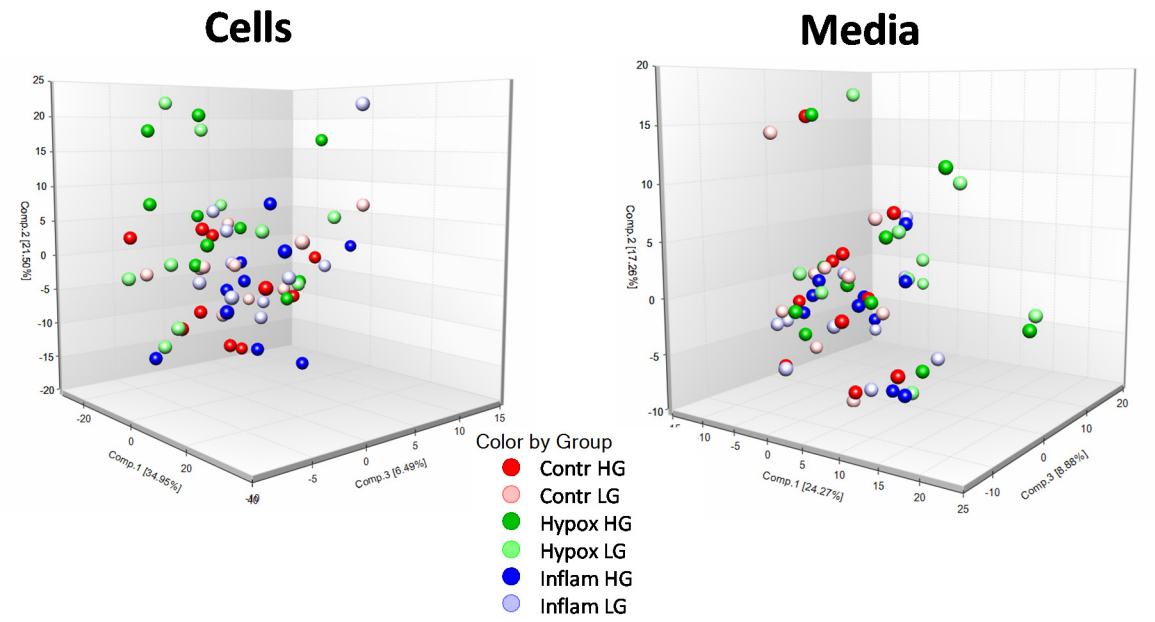
Supplementary Table S1: Summary of the donor characteristics for the human islet samples used in the present study (*n* = 10).

Donor ID:	**2215**	**2217**	**2219**	**2220**	**2221**	**2222**	**2224**	**2226**	**2230**	**2231**
Diabetic	N	N	N	N	N	N	N	N	N	N
Age (years)	38	59	43	51	53	53	49	59	52	20
Gender	M	F	F	F	F	F	M	M	F	F
Race	C	W	W	H	W	C	W	W	B	W
Weight (lb)	191.8	144.2	223	118.2	134.5	180	157	149	166	192
Height (inch)	70	64	71	64	63	61	63	68	66	66
BMI	27.5	24.8	31.1	20.2	24.0	34.0	34.0	22.8	26.8	30.9
ABO/Rh	O	A	A	A	A	AB	O	O	O	A
Cold ischemia time (h)	18.4	24.5	16.1	14.5	21.4	12.5	13.3	19.0	12.5	10.1
Purity (%)	90	90	95	85	90	90	80	80	90	70
Viability (%)	93	95	92	95	90	89	90	90	90	90

Supplementary Figures

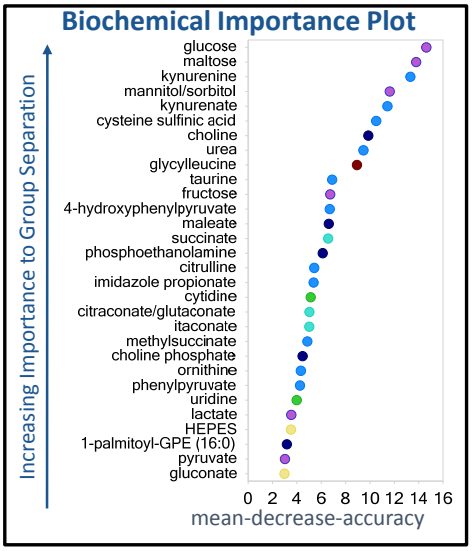


Supplementary Figure S1. Schematic design of the present study. Human islets isolated from *n* = 10 different donors (DRI, cGMP) were divided in six (1,000 IEQ, 3 mL, 6-well plate) and incubated for 24 + 24 h as shown under standard conditions, hypoxia (3% O_2), and inflammatory conditions (in the presence of a cytokine cocktail of IL-1β 50 U/mL, 0.25 ng/mL; IFN-γ 1,000 U/mL, 50 ng/mL; and TNF-α 1,000 U/mL, 21.5 ng/mL). Half of the samples were kept at basal (5.5 mM) and half were exposed to high glucose for the second 24 h (16 mM). From each sample, cells and media were sent to Metabolon for metabolomics analysis for a total of 10×3×2×2 = 120 samples. Per protocol, study needed 100,000 cells (~100 IEQ) and 500 µL per sample.



Supplementary Figure S2. Principal component analysis of the present metabolomics data in cell (left) and media samples (right) obtained from human islets (*n* = 10) cultured under different conditions as indicated (control, hypoxia, and inflammatory cytokines with low or high glucose). There is relatively poor separation of treatment groups with some clustering of the hypoxic samples especially in the cell samples as noticeable for the corresponding color coded symbols (green).

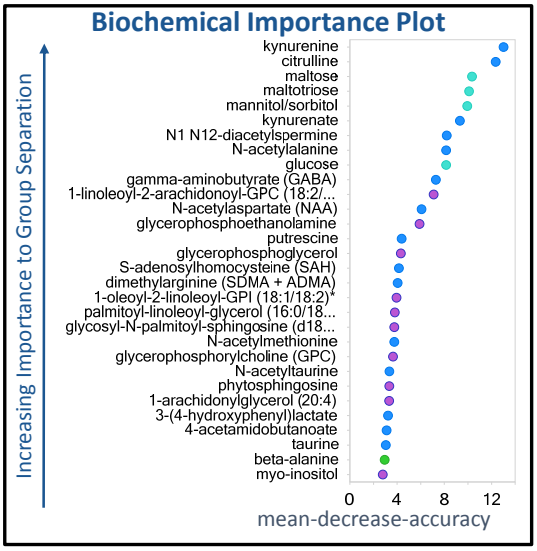
Media – All groups



Random Forest Confusion Matrix

Media		Predicted Group						Class. Error
		Contr LG	Contr HG	Hypox LG	Hypox HG	Inflam LG	Inflam HG	
Actual Group	Contr LG	6	2	0	0	1	0	0.33
	Contr HG	2	8	0	0	0	0	0.20
	Hypox LG	1	0	9	0	0	0	0.10
	Hypox HG	0	2	1	7	0	0	0.30
	Inflam LG	0	0	0	0	9	1	0.10
	Inflam HG	0	0	0	0	0	10	0.00
Predictive Accuracy = 83%								

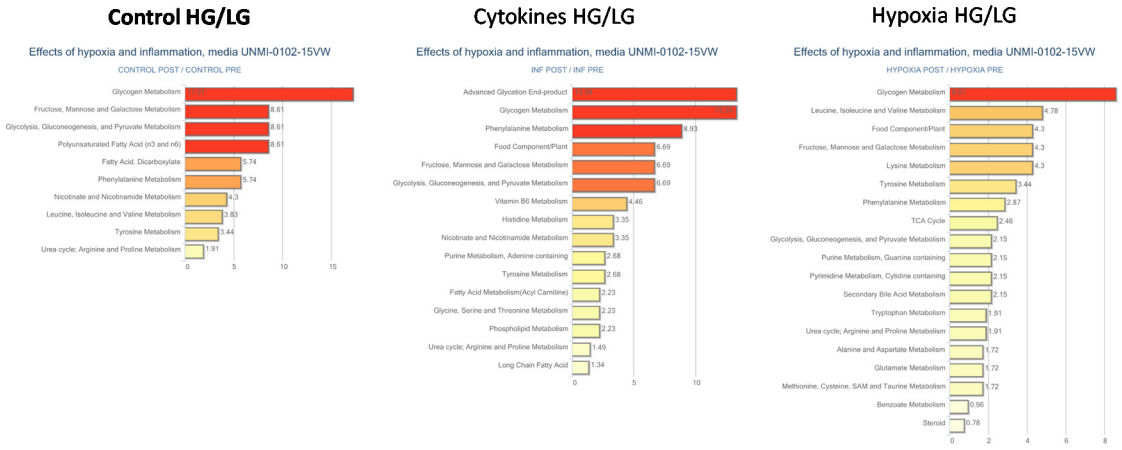
Cells – All groups



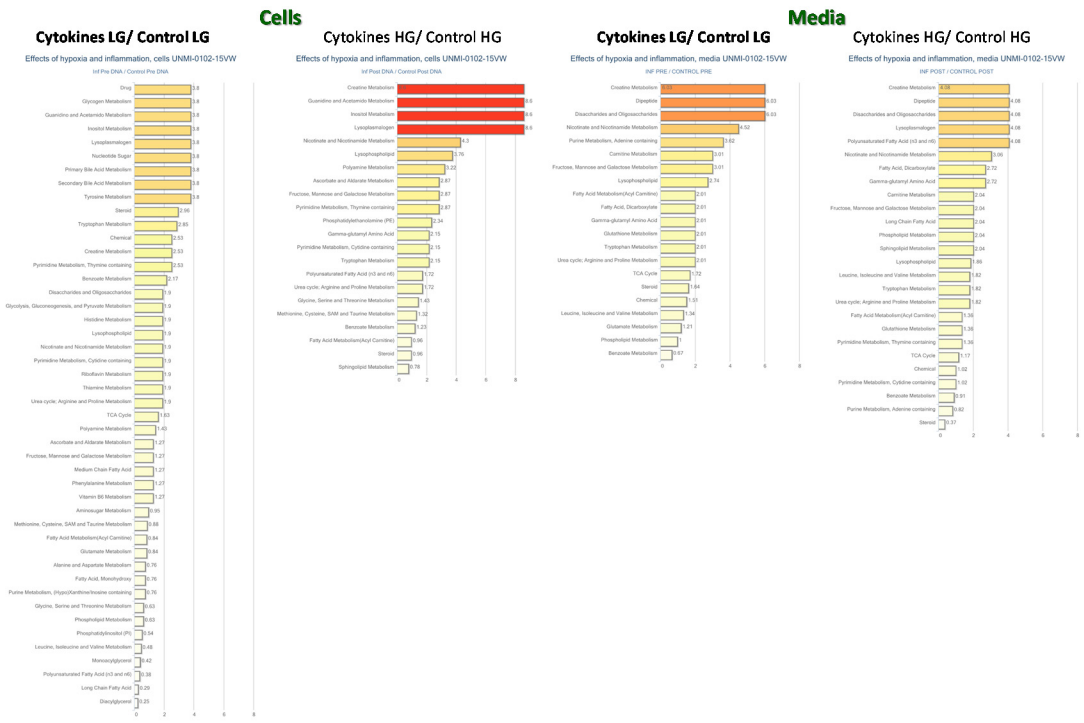
Random Forest Confusion Matrix

Cells		Predicted Group						Class. Error
		Contr LG	Contr HG	Hypox LG	Hypox HG	Inflam LG	Inflam HG	
Actual Group	Contr LG	2	5	1	1	1	0	0.80
	Contr HG	5	3	0	0	1	1	0.70
	Hypox LG	0	1	3	6	0	0	0.70
	Hypox HG	0	0	8	2	0	0	0.80
	Inflam LG	1	0	0	1	6	2	0.40
	Inflam HG	0	1	0	0	4	5	0.50
Predictive Accuracy = 35%								

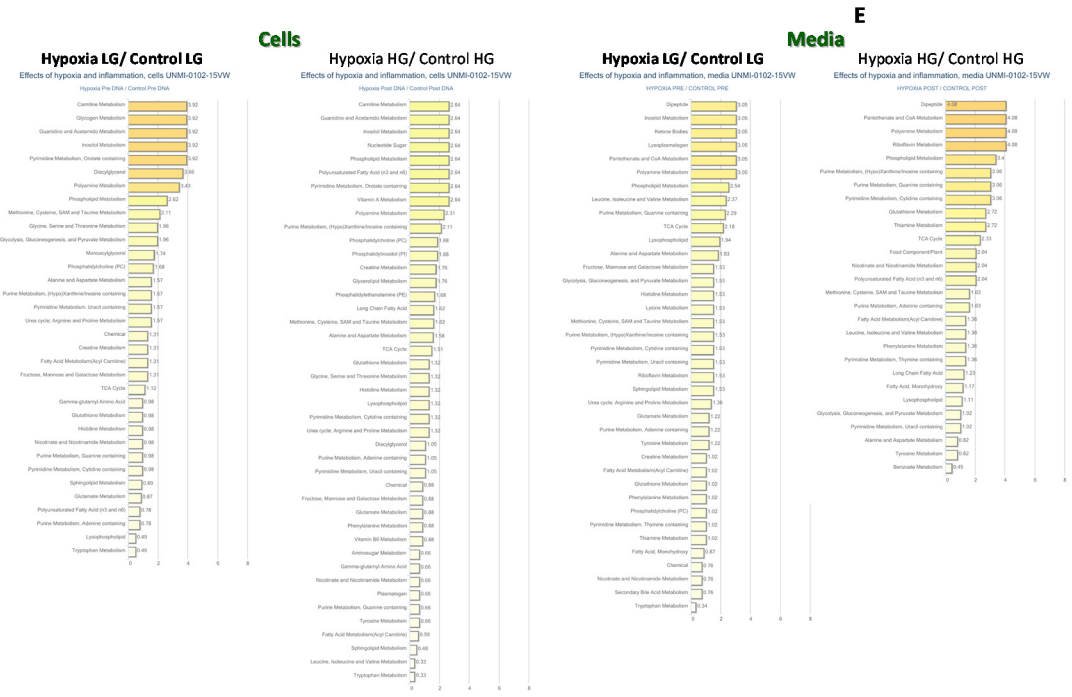
Supplementary Figure S3. Random forest analysis of the present metabolomics data in media (left) and cell samples (right) obtained from human islets (*n* = 10) cultured under different conditions as indicated (control, hypoxia, and inflammatory cytokines with low or high glucose). The *y*-axis represents the molecules in order of importance for group classification in decreasing order from top to bottom, and the tables below show the corresponding confusion matrices. The overall prediction accuracy of this random forest method (predicted vs. actual group) was 83% for media and 35% for cellular samples (to be compared with a 16.7% random chance for six groups).



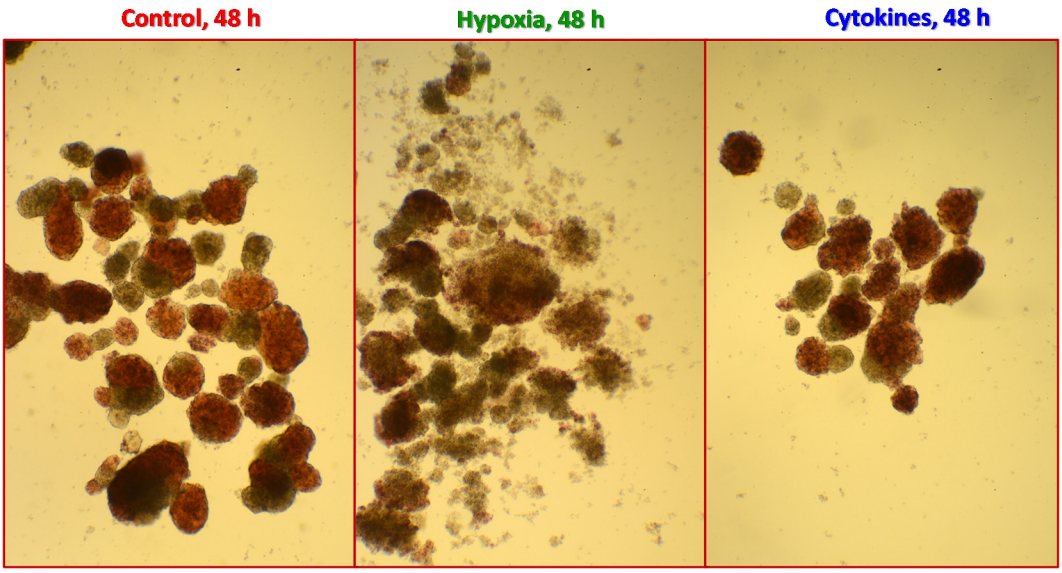
Supplementary Figure S4. Metabolic Pathway Enrichment (PE) values showing the effect of high (HG) versus basal (LG) glucose under control, inflammatory cytokine, and hypoxic conditions in media. Affected pathways are shown on the vertical axis in decreasing order of the unadjusted *p* value from PE analysis (most significant *p*-values in red, less significant ones in yellow and white). The *x*-axis represents increasing metabolic pathway impact according to the betweenness centrality from pathway topology analysis. PE represent the number of experimentally regulated compounds relative to all detected compounds in a pathway for the selected pair-wise comparison as compared to the total number of experimentally regulated compounds relative to all detected compounds in the study. Values larger than one indicate that the pathway contains more experimentally regulated compounds relative to the study overall suggesting that the pathway may be a target of interest for the experimental perturbation. For example, if 10 compounds are significant in a study of 100 detected compounds, then the ratio of experimentally regulated compounds relative to all detected compounds in the study is 10/100 = 0.1. If 8 compounds are significant in a pathway of 20 detected compounds, then the ratio of experimentally regulated compounds relative to detected compounds in this pathway is 8/20 = 0.4, and this makes PE for this pathway in this example to be 0.4 / 0.1 = 4.



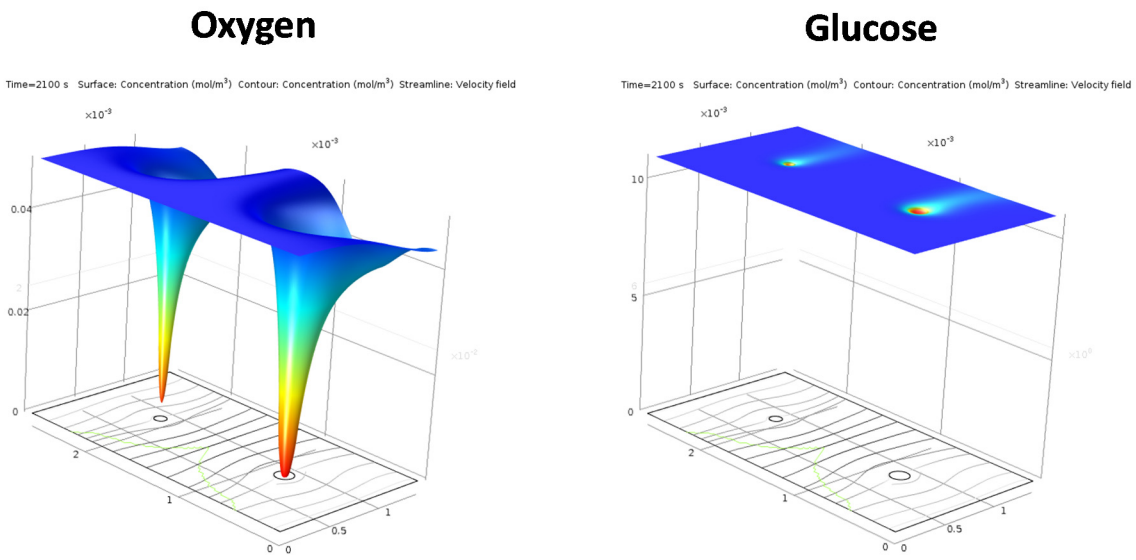
Supplementary Figure S5. Metabolic Pathway Enrichment (PE) values showing the effect of inflammatory cytokines under basal (LG, 5.5 mM) and high (HG, 16 mM) glucose in cells and media. Affected pathways are shown on the vertical axis in decreasing order of the unadjusted *p* value from PE analysis (most significant *p*-values in red, less significant ones in yellow and white). See caption of Supplementary Figure S4 for further details.



Supplementary Figure S6. Metabolic Pathway Enrichment (PE) values showing the effect of hypoxia under basal (LG, 5.5 mM) and high (HG, 16 mM) glucose in cells and media. Affected pathways are shown on the vertical axis in decreasing order of the unadjusted *p* value from PE analysis (most significant *p*-values in red, less significant ones in yellow and white). See caption of Supplementary Figure S4 for further details.



Supplementary Figure S7. Comparison of dithizone (DTZ) stained islets from one representative sample incubated for 24 + 24 h under the present conditions as indicated. All islets shown are from the LG glucose group.



Supplementary Figure S8. Comparison of model-calculated [15, 39] oxygen (left) and glucose (right) concentrations during high glucose (G11) exposure in two representative islets (diameters of 100 and 150 μm; see circles on bottom of the graphs) under tissue oxygen (5%) conditions. Data are concentrations shown as surface plots that are also color-coded from blue for high to red for low to illustrate that oxygen diffusion is indeed the main limiting factor as it decreases much more severely inside the islets than glucose. Consumptions rates for oxygen and glucose are similar, but oxygen is less soluble and much more strongly diffusion limited [15].