Vinyl Chloride Enhances High Fat Diet-Induced Proteome Alterations in the Mouse Pancreas Related to Metabolic Dysfunction: Implications for Individual Susceptibility

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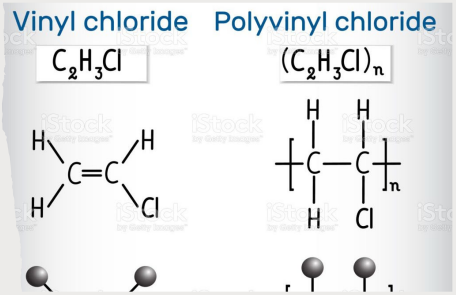
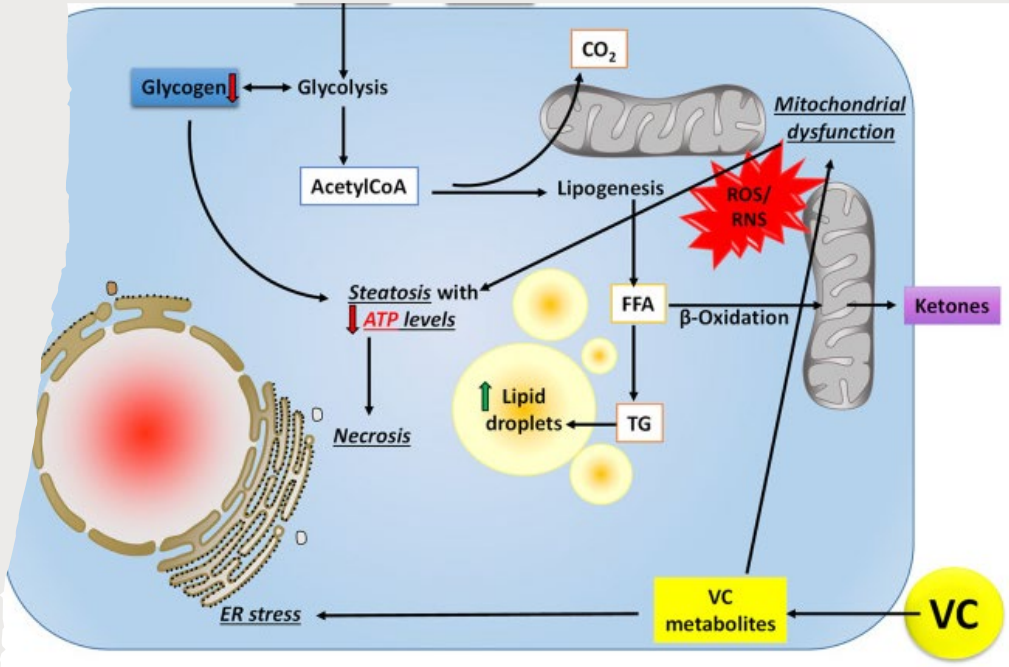
Vinyl Chloride (VC)

- VC is a monomer used by industry to create polyvinyl chloride (PVC) plastic products
- VC is a prevalent environmental chemical and occupational toxicant as well
- VC exerts its main effects on the liver, where it has been shown (at high concentrations) to cause:

- Nonalcoholic fatty liver disease (NAFLD)
- Toxicant-associated steatohepatitis (TASH)
- Hepatocellular carcinoma (HCC)

- VC is a known carcinogen and ranked #4 on the ATSDR Hazardous Substance Priority List
- The Occupational Safety and Health Administration (OSHA) has lowered the acceptable exposure threshold to 1 ppm over an 8 h workday
- The effect of this concentration of VC on human health, such as liver disease is unclear



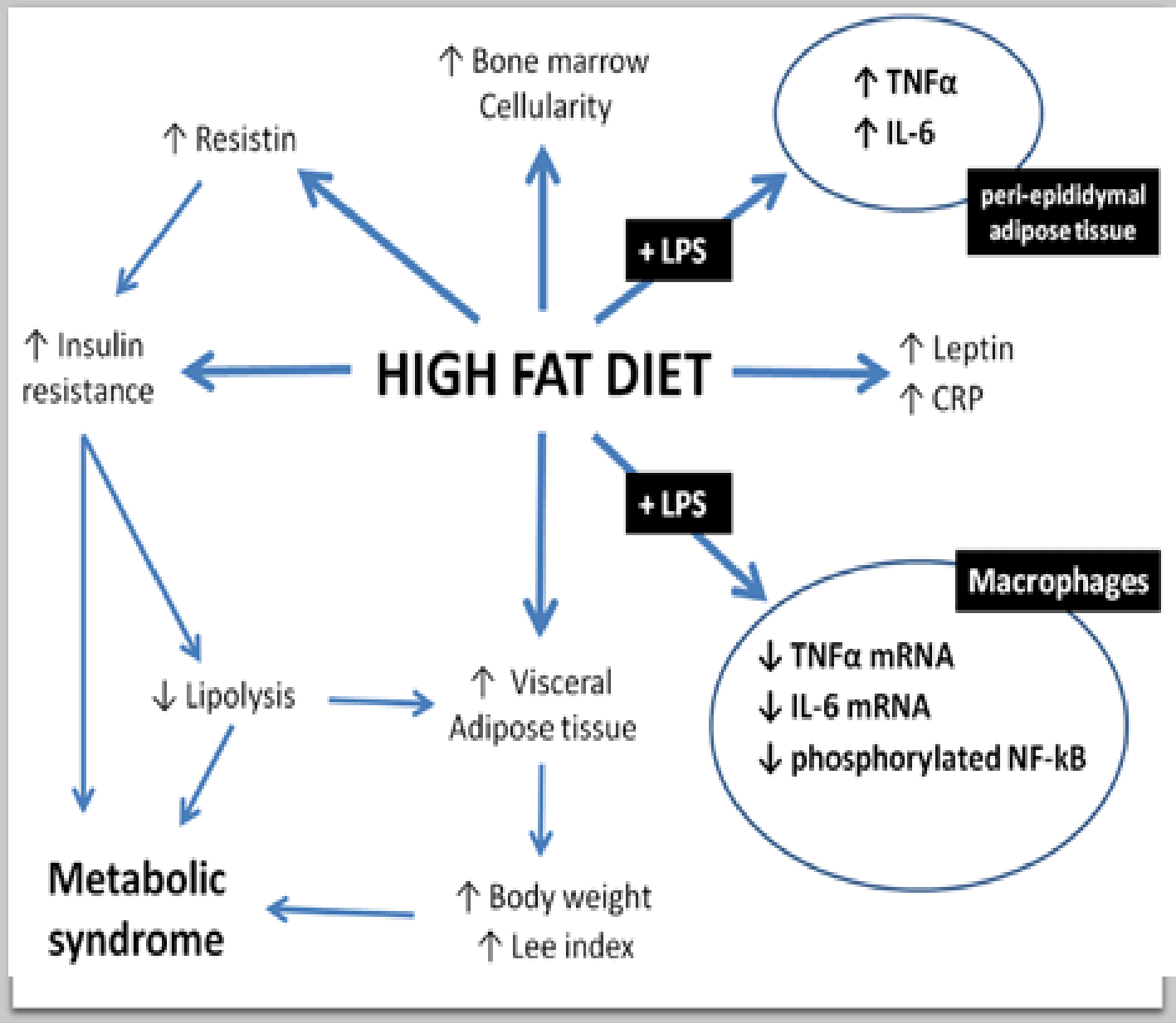


High-Fat Diet (HFD)

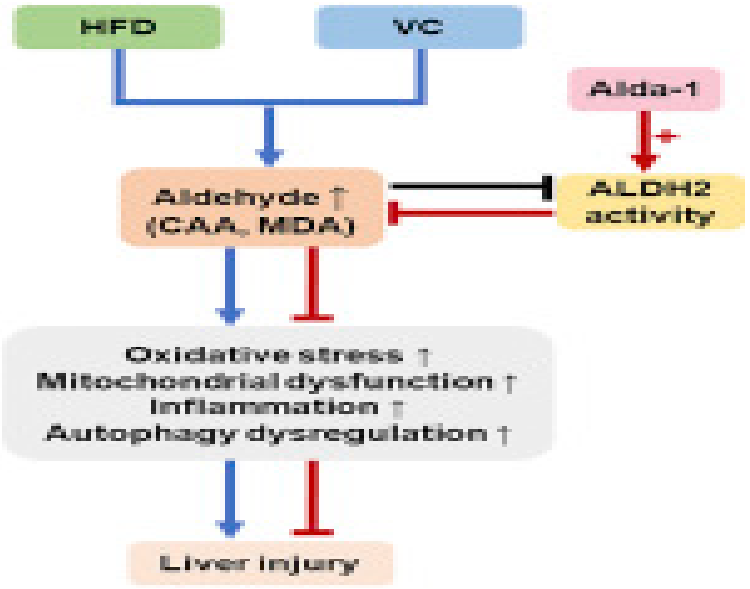
- HFD is often linked to the global pandemic of metabolic diseases:

- Type 2 diabetes (T2D)
- Obesity
- Liver fibrosis
- Steatosis
- Insulin intolerance
- Nonalcoholic fatty liver disease (NAFLD)

- HFD has been used for decades to treat rodents to generate *in vivo* disease model of obesity and steatosis, which closely resembles human metabolic syndromes



Co-exposure of VC and HFD as a Model of Environment and Obesity Interaction



➢ **The common pathways targeted by both HFD and VC**

- Oxidative stress
- Inflammation
- Energy and lipid metabolism

➢ **The common tissues targeted by both HFD and VC**

- Liver
- Pancreas

➢ The common diseases caused by both HFD and VC

- Nonalcoholic fatty liver disease (NAFLD)
- Other metabolic disorders

➢ VC modifies sensitivity of the liver to nonalcoholic fatty liver disease (NAFLD) caused by HFD

➢ The combination of VC and HFD significantly enhances liver disease

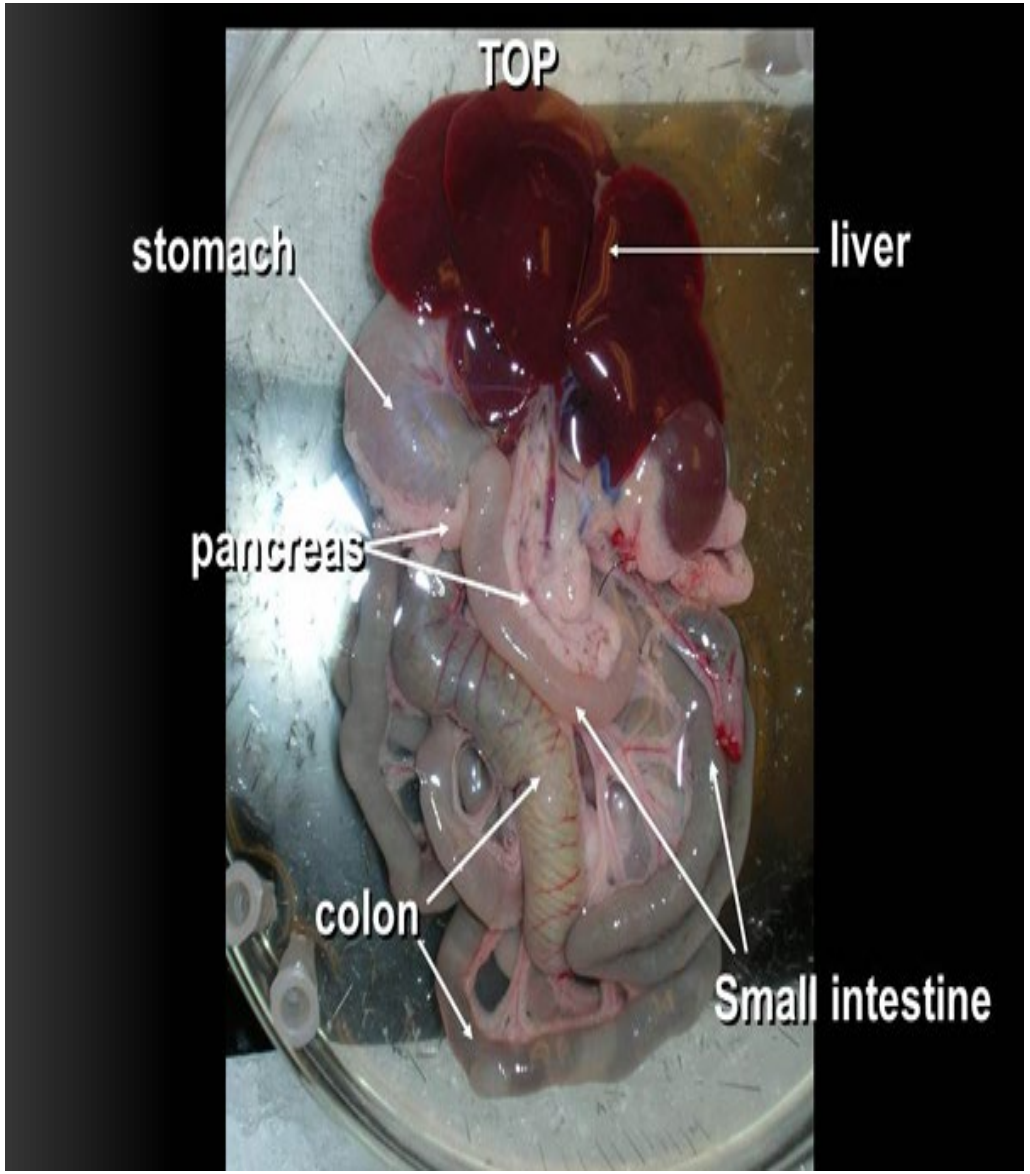
- **Cave, M.,** Falkner, KC., McClain, CJ. 2012. Occupational and Environmental Hepatotoxicity In Zakim and Boyer's Hepatology. Edited by Boyer DT, Manns MP, Sanyal AJ, 476–492, Saunders Philadelphia, PA.
- Lang, A.L., Goldsmith, W.T., Schnegelberger, R.D., Arteel, G.E., and **Arteel, Julian**., 2020. Vinyl chloride and high-fat-diet as a model of environmental and obesity interaction. J. Vis Exp. Doi: 10.3791/60351.

Mouse Model of Co-Exposure to VC and HFD

- Six-week-old male C57BL/6J mice fed with low fat diet (LFD) or HFD were exposed to VC at 0.085 ppm (± 0.01) in inhalation chambers for 6 hours per day, 5 days per week for 12 weeks in bedding-free cages
- It is a chronic VC exposure at low concentrations
- The co-exposure model is the whole-body exposure, without restraint
- The exposure conditions more closely resemble a very common human situation of a combined exposure to VC with HFD inducing metabolic disorders and diseases
- This model of toxicant-induced liver injury can be used for other volatile organic compounds and other organ systems to study interactive effects of environmental factors

Pancreas as a Model of Organ for Our Proteomic Study

- A key organ for the understanding of metabolic processes and diseases
- Pancreas is the major source for insulin, an important hallmark of metabolism control
- Pancreas interacts with liver in a highly sophisticated network via the released insulin, enzymes, hormones, and cytokines
- The produced cytokines regulate a variety of metabolic pathways:
  - Oxidative stress
  - Inflammation
  - Lipid metabolism
  - Insulin production
  - Hormone release
  - β-cell proliferation



Our Research Interest

Protein attributes in the context of metabolic processes and disfunctions in pancreas of mice exposed to HFD, VC and HFD+VC

Why Proteins or Proteome

- Health states of the exposed cells and tissues are largely determined by the changes of expression, PTMs, and activity levels of thousands of proteins (proteome)
- Effects of HFD, VC or HFD+VC on metabolic processes could be characterized and predicted based on proteome alterations in pancreas of mice exposed to HFD, VC or HFD+VC
- Interactive effects of HFD+VC on proteome changes in pancreas could be identified and characterized by comparing protein changes associated with HFD+VC and HFD and VC

Our Hypothesis

VC potentiates HFD-induced metabolic processes and dysfunctions through alteration of pancreas proteome in mice exposed to HFD+VC, and the potentiation was mediated by selective proteins or pathways

The Major Goals

- Identified pancreas proteome changes linked to exposure of HFD, VC, HFD+VC respectively
- Characterized interactive effect of VC and HFD on pancreas proteome changes
- Predicted potential differential risks of HFD, VC and HFD+VC toward different groups of mice

Experimental Design

- **Cytokine array:** performed cytokine expression profiling of pancreas in mice treated with LFD, HFD, LFD+VC, and HFD+VC to measure concentrations of 200 cytokines in the following experimental groups of mice:

  - **LFD mice** (LFD), mice fed with LFD only, a nonexposed control group
  - **VC mice** (LFD+VC): LFD mice exposed to VC, a VC exposed control group
  - **HFD mice** (HFD): mice fed with HFD only
  - **HFD+VC mice** (HFD+VC): HFD fed mice exposed to VC

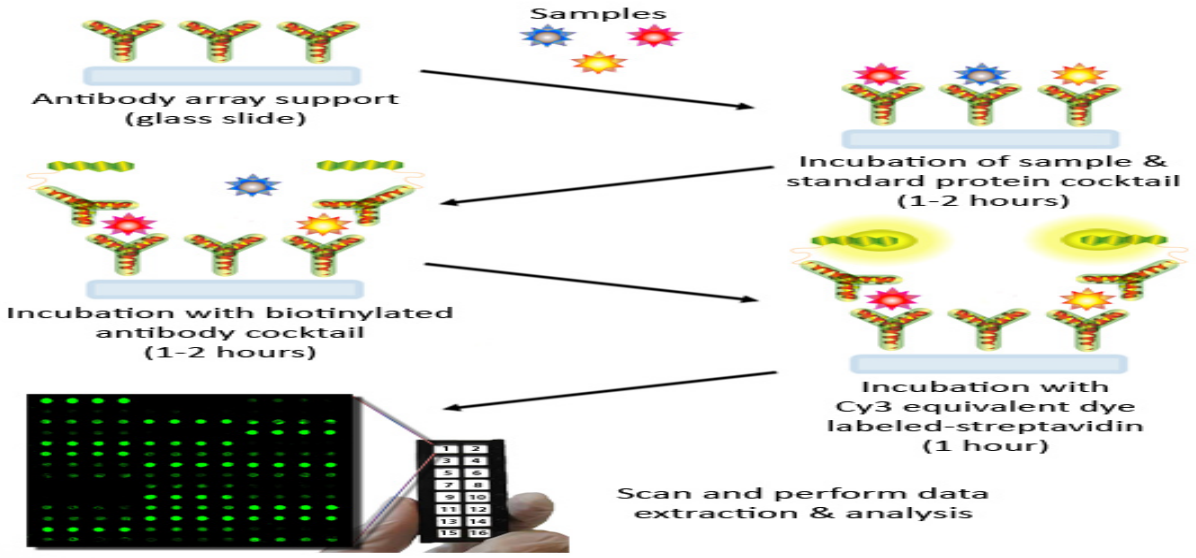
- **Western blot:** expression levels of AKT, GSK3β, CPT1A, and GSTμ, and phosphorylation levels of AKT and GSK3β

- **IPA:** Identification of protein interacting networks

- **Statistical analysis of proteomic data:** Identification of critical proteins underlying metabolic and pathological processes

Mouse Cytokine Antibody Array

(A) How It Works



(B) Protocol

- Completely Air Dry The Glass Slide
- Prepare Cytokine Standard Dilutions
- Blocking & Incubation
- Incubation with Biotinylated Antibody Cocktail & Wash
- Incubation with Cy3 Equivalent Dye-Streptavidin & Wash
- Fluorescence Detection
- Data Analysis

(C) Key features

- **Simplicity**: With a simple copy and paste process, the cytokine concentration is determined
- **Normalization**: allows for intra- and inter-slide normalization for large numbers of samples
- **Two Positive Controls**: utilizes the two positive controls in each
- array for normalization
- **Two Data Outputs**: standard curves and digital concentration
- **Lower and Upper Limits Determination**: automatically marks out the values below or above the detection range
- **Standard Deviation**: the standard deviations of the quadruplicate spots for data accuracy

(D) Array Map & Standard Curves

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Amphiregulin			
B	Axl				CD27 Ligand				CD30 (TNFRSF8)			
C	CD40 (TNFRSF5)				CXCL16				EGF			
D	E-Selectin				Fractalkine				GITR (TNFRSF18)			
E	HGF				IGFBP-2				IGFBP-3			
F	IGFBP-5				IGFBP-6				IGF-1			
G	IL-12 p70				IL-17E (IL-25)				IL-17F			
H	IL-1 ra (IL-1 F3)				IL-2 R alpha				IL-20			
I	IL-23 p19				IL-28A				I-TAC (CXCL11)			
J	MDC (CCL22)				MIP-2				MIP-3 alpha (CCL20)			
K	Osteopontin (SPP1)				Osteoprotegerin				Prolactin			
L	Pro-MMP-9				P-Selectin				Resistin			
M	SCF				SDF-1 alpha				Thrombopoietin (TPO)			
N	VCAM-1 (CD106)				VEGF-A				VEGF-D			

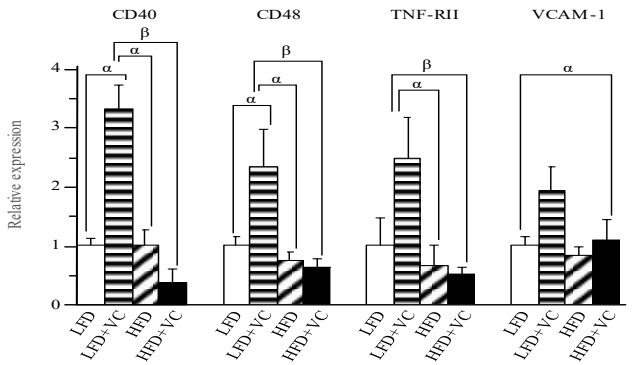
Overview on Pancreas Proteome Alterations

- Twenty-six proteins with 1.2-fold or greater ($p < 0.05$) changes compared to LFD mice or VC mice were identified
- The altered proteins are largely linked to pancreas-mediated metabolic processes, stresses, and diseases
- Seven categories of proteins involved in pancreatic function were identified:

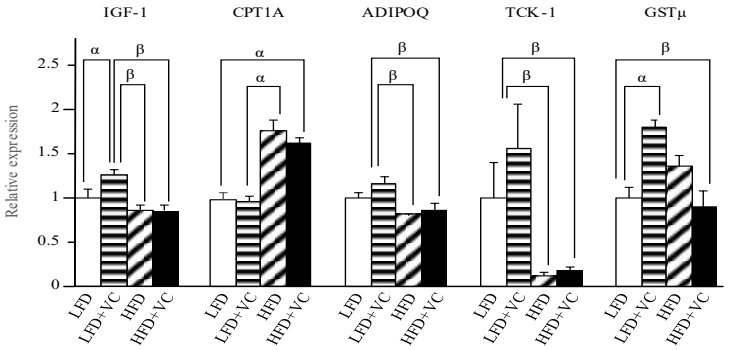
- carbohydrate, lipid, and energy metabolism: CPT1A
- oxidative stress and detoxification: GSTμ
- insulin secretion and regulation: ADIPO
- cell growth, development, and communication: IGF-1
- immunological responses: TNFRII
- Inflammation: CCL-11
- biomarkers of pancreatic diseases and cancers: DCN

Pancreas Proteome Alterations in Response to HFD, VC and HFD+VC

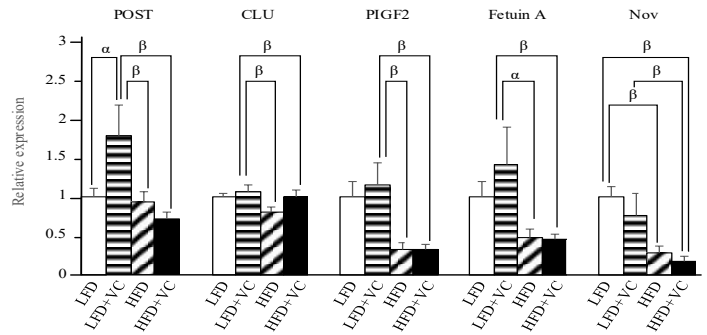
(A) Immunity and immune responses



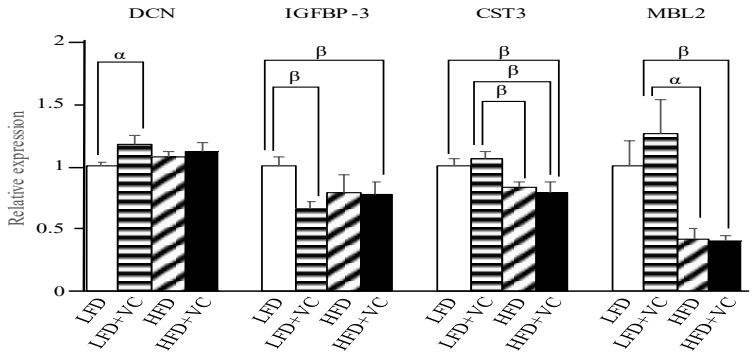
(B) Metabolic events



(C) Cell growth and development, cellular communications and signaling

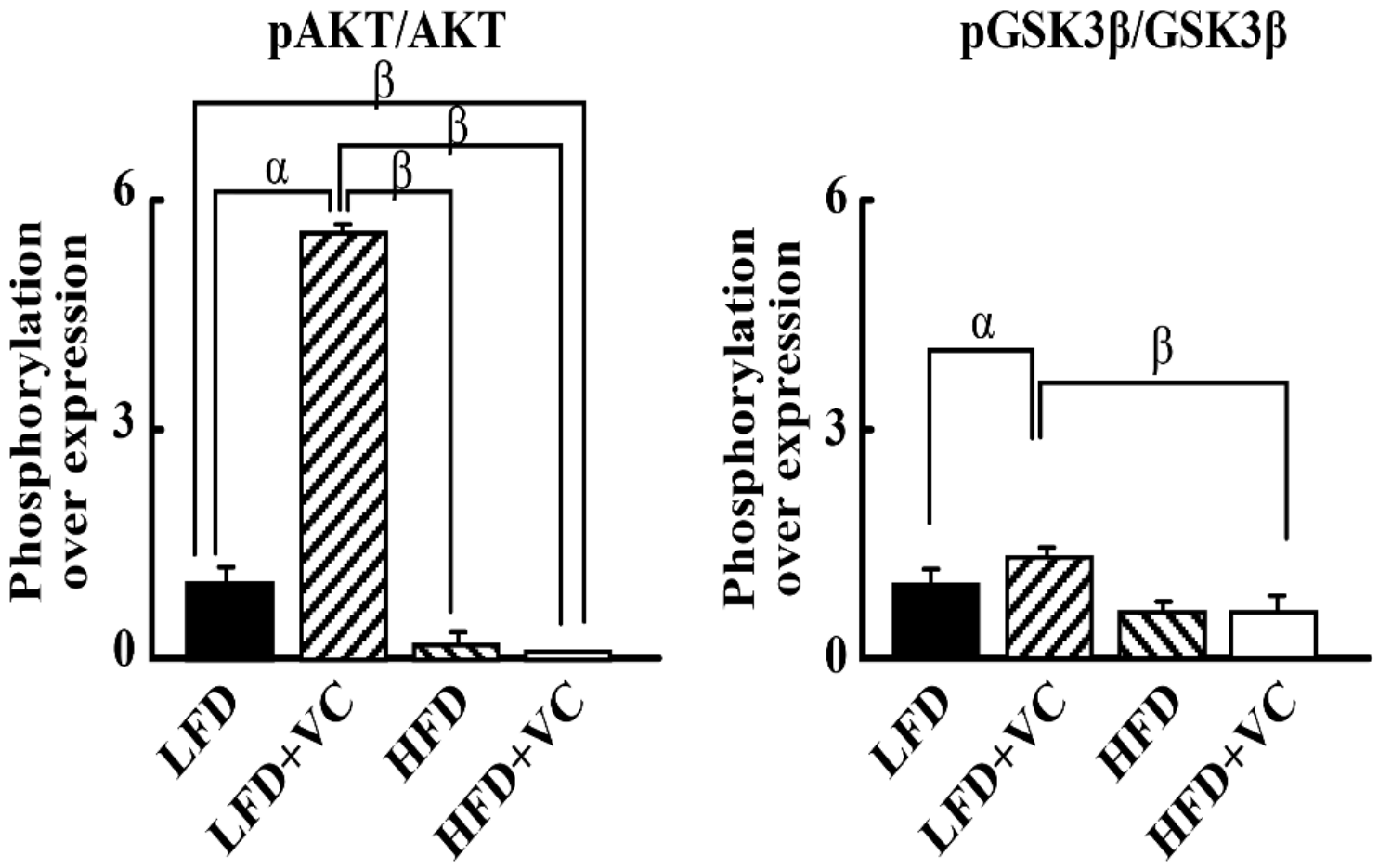


(D) Clinical biomarkers of pancreatic diseases and cancers



α means increased expression and β means decreased expression in the comparisons

Phosphorylation levels of AKT and GSK3β

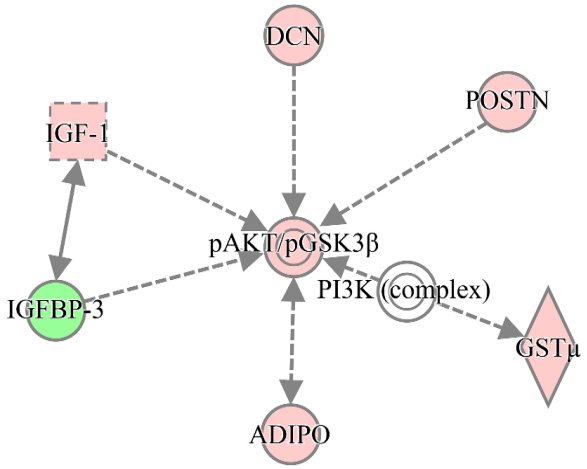


α means increased expression and β means decreased expression in the comparisons

Altered Proteins Due to VC Exposure Only

(LFD+VC vs LFD):

10 proteins altered
2: down-regulated
8: up-regulated

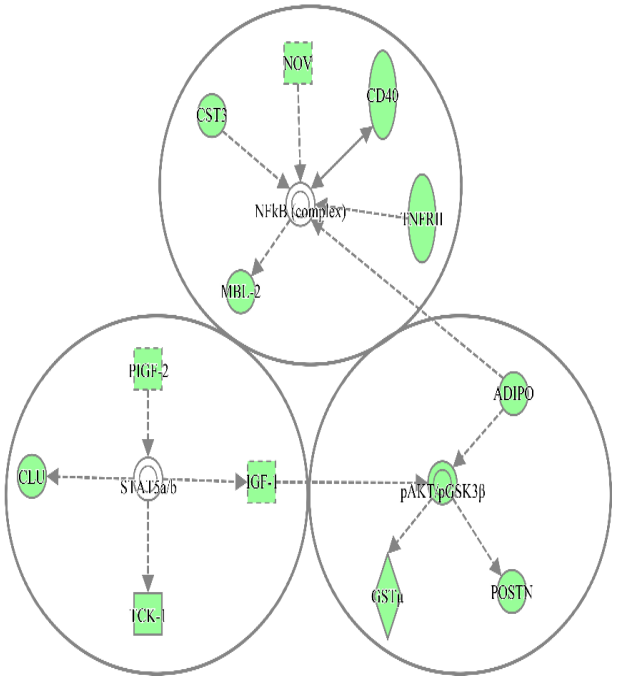


LFD+VC vs LFD					
	Cluster of differentiation 40	CD40	3.31	0.01	TNF receptor superfamily member, pancreatic cancer immunotherapy target
	Cluster of differentiation 48	CD48	2.33	0.04	Adhesion and activation of immune cell, pancreatic graft rejection
	Decorin	DCN	1.20	0.02	Sequester and inhibit growing malignant cells in pancreatic cancer
	Fc fragment of IgG receptor IIb	FcgRIIB	-1.59	0.03	Mediate inhibition of glucose delivery, IgG ligand, inhibition of cell activity
	Insulin-like growth factor 1	IGF-1	1.26	0.02	Normal growth cell proliferation, pancreas β-cell development, protect against diabetes
	Insulin-like growth factor binding protein 3	IGFBP-3	-1.51	0.01	Regulation of β-cell mass and activities
	Periostin	POSTN	1.79	0.04	Regeneration of pancreatic β-cell, potential application to the treatment of diabetes
	Antioxidant glutathione S-transferase μ	GSTμ	1.82	0.01	Detoxification enzyme, oxidative alteration and the pathophysiology of diabetes
	Phosphorylated protein kinase B	pAKT	4.11	0.02	Pancreatitis cell proliferation, protection from apoptosis, insulin secretion, glucose metabolism etc
	phosphorylated glycogen synthase kinase 3 β	pGSK3β	1.35	0.01	Suppressed by insulin, insulin resistance, beta cell mass and function

- VC upregulated proteins known to be involved in insulin production and beta cell proliferation e.g., DCN, IGF-1, POSTN, pAKT and pGSK3β, and promoted proteome changes associated with metabolic dysfunctions
- AKT kinase was identified as a hub in the protein interaction network of the altered proteins
- AKT might play critical role in regulating VC-mediated proteome changes and underlying metabolic syndromes through coordination of the associated proteins presented in the network

Protein Differences Between HFD and LFD with VC Exposures

(HFD+VC vs LFD+VC)



HFD+VC vs LFD+VC				
Adiponectin	ADIPO	-1.35	0.01	Stimulates insulin secretion, cell survival, therapeutic target for obesity, diabetes, and endothelial dysfunction
Cluster of differentiation 40	CD40	-9.09	0.01	TNF receptor superfamily member, pancreatic cancer immunotherapy target
Cluster of differentiation 48	CD48	-3.76	0.01	Adhesion and activation of immune cells, pancreatic graft rejection
Clusterin	CLU	-1.43	0.01	Apolipoprotein, regulate cell growth and death, induced in pancreatic cancer as well as chronic pancreatitis
Cystatin C	CST3	-1.34	0.01	Strongly expressed in most endocrine gastro-entero-pancreatic tumors, cancer biomarker
Alpha-2-HS-glycoprotein	Fetuin A	-3.19	0.02	Secreted from pancreatic β-cells, involved in β-cell dysfunction, obesity, and insulin resistance
Insulin-like growth factor 1	IGF-1	-1.5	0.01	Normal growth cell proliferation, pancreas β-cell development, protect against diabetes
Mannose binding lectin 2	MBL-2	-3.11	0.01	Mediate insulin resistance and inflammation, activate the complement cascade, and promote pancreatic oncogenesis
Nephroblastoma-overexpressed gene protein homolog	NOV	-4.18	0.04	Decreases β-cell proliferation, involved in inflammation, apoptosis, and various disease processes
Periostin	POSTN	-2.48	0.01	Regeneration of pancreatic β-cells, potential application to the treatment of diabetes
Placenta growth factor 2	PlGF-2	-3.64	0.01	Exclusively produced by beta cells, essential role in gestational β-cell growth
Thymus chemokine 1	TCK-1	-8.75	0.03	Energy metabolism, elevated activity is associated with acute pancreatitis
Tumor necrosis factor receptor superfamily member 1B	TNFRII	-4.84	0.02	Initiates immune modulation and tissue regeneration, immunopathogenesis of pancreatitis
Antioxidant glutathione S-transferase μ	GSTμ	-1.99	0.01	Detoxification enzyme, oxidative alterations, and the pathophysiology of diabetes
Phosphorylated protein kinase B	pAKT	-11.2	0.01	Pancreatitis cell proliferation, protection from apoptosis, insulin secretion, glucose metabolism
phosphorylated glycogen synthase kinase 3 β	pGSK3β	-1.49	0.01	Suppressed by insulin, insulin resistance, beta cell mass and function

- 16 proteins involved in insulin secretion, immune response, pancreatic β-cells growth and dysfunction, energy metabolism and gene transcription pathways were altered and down-regulated in pancreas of mice treated with HFD and VC
- AKT/GSK3β, NFkB, and STAT5 were identified as the three main hubs of the protein interaction networks

VC Potentiated HFD-Induced Metabolic Dysfunction

- There was a “ crosstalk” or “interplay “ between VC and HFD
- VC exposure modified HFD-induced metabolic processes through the selective proteins and pathways

Individual Susceptibility

- VC mice were more sensitive to HFD intake as compared to LFD mice, which partially explained the individual risk of mouse to HFD consumption (16 vs 10)

- The susceptibility to the metabolic dysfunctions and stresses induced by HFD intake was increased by VC exposure via the selective proteins and pathways

- It is reasonable to postulate that in human exposure, VC-exposed occupational workers could be more sensitive to HFD consumption and HFD could potentially render the VC-exposed workers more susceptible to metabolic stresses and syndromes

- This study suggested the importance of differing health risks of each environmental factor toward different population groups in human as well, especially occupational exposure to VC

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