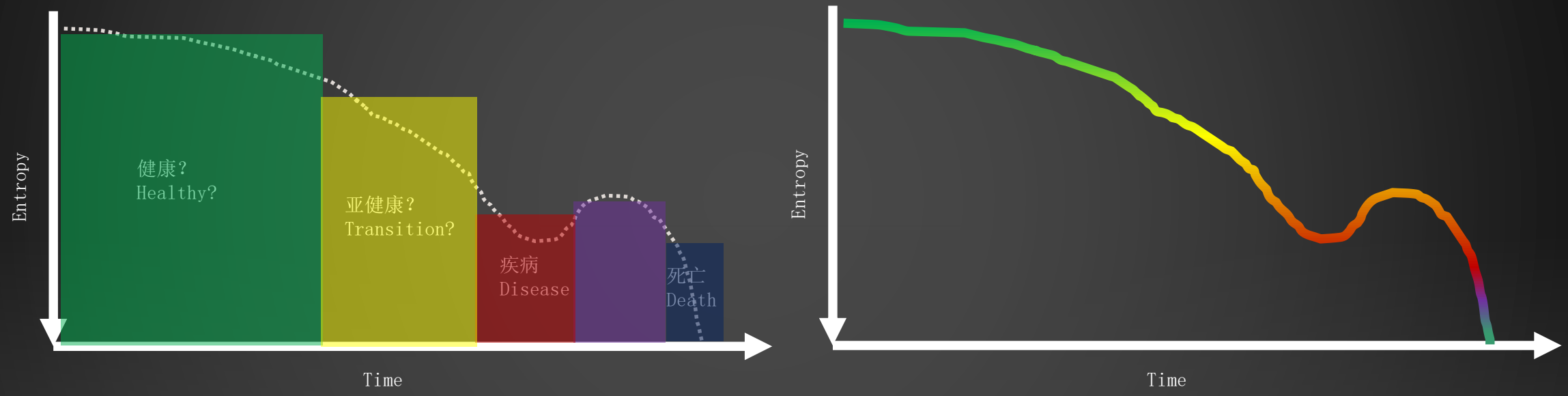
AN INTEGRATIVE MULTI-OMICS VIEW OF MOLECULAR DYNAMICS IN HUMAN SUBJECTS UNDERGOING FASTING-REFEEDING COURSE

AN EXAMPLE OF A META-COMPUTING FRAMEWORK FOR HETEROGENEOUS HIGH-DIMENSIONAL DATA FROM POPULATION

HEALTH MANAGEMENT



- Most concerned condition results from state-transition that could not be formed in one day.
- Diseases should be predicted and prevented instead of being precisely diagnosed and perfectly treated.

BIOINFORMATICS FOR PERSONAL HEALTH MANAGEMENT

- Predict the future progression direction and trend of life
- 预测生命状态的未来演变方向和趋势
- Predict how interventions impact on progression trend of life
- 预测干预措施及其组合方案对生命状态未来演变趋势的影响
- Given a goal status, provide feasible solution maximizing the success rate to fulfill.
- 给定生命状态的未来目标，求极大化该目标实现概率的可行干预方案。

- Technically, this is trying to model human life (to an extent that an action would make sense to significantly change one's future)
- 技术上来说，就是对人类生命活动进行建模，提供可影响人类决策的行为建议

FASTING-REFEEDING COURSE AS A DEMO PROCESS

- Commercially available program
- 7 days of water-only fasting with exercises courses and 21 days of refeeding with nutrition recommendations

CHALLENGES IN BIOINFORMATICS FOR PERSONAL HEALTH MANAGEMENT

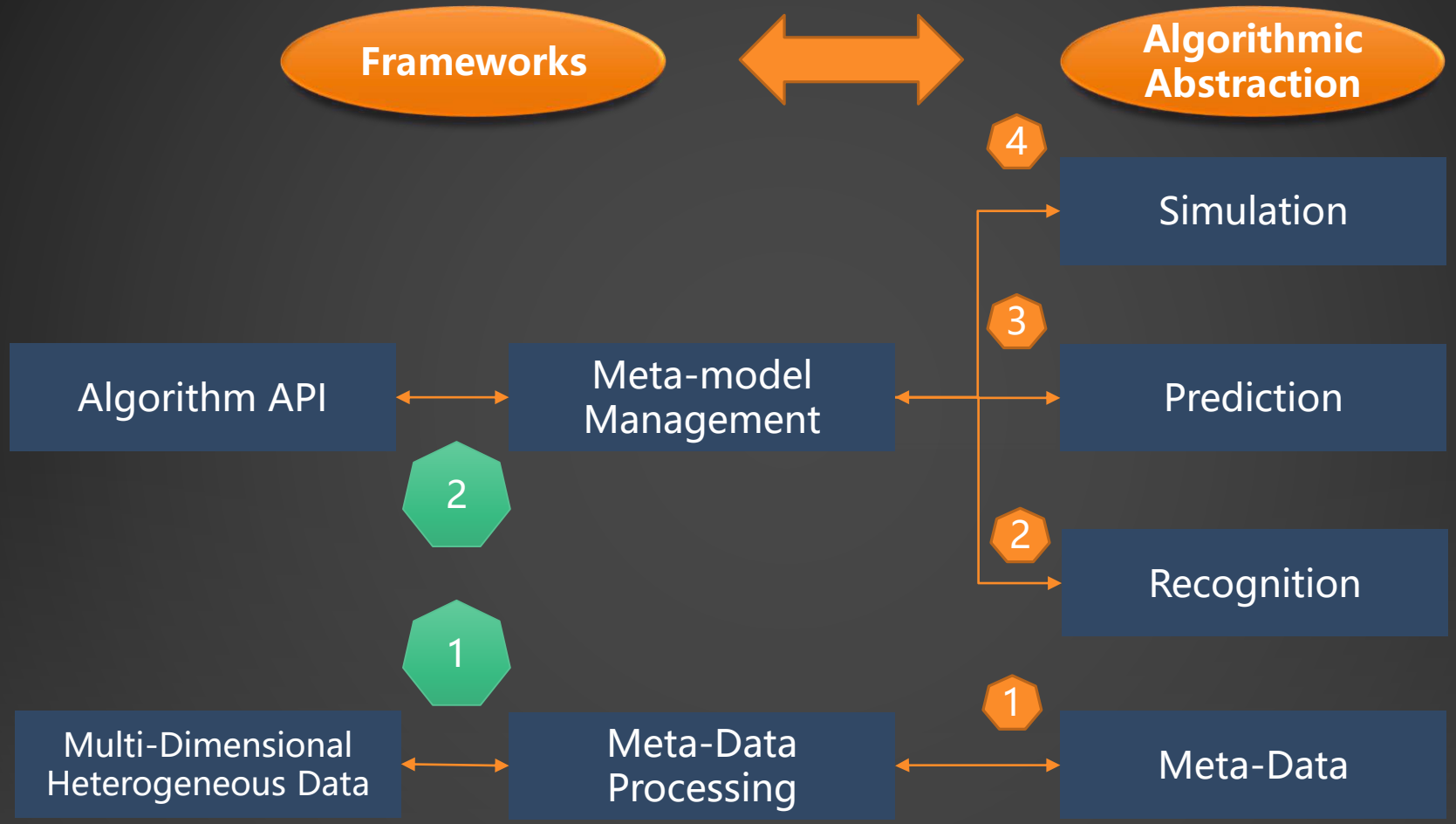
- Heterogeneous multi-dimensional data
- 需观测和分析的数据维度异质
- Sparse and fragmented data sources
- 数据源多而分散，数据流碎片化
- Unexpected mathematical features of data
- 数据特征难以预期
- Open knowledge discovery
- 知识发现方向开放而难以预期
- Time and space dynamics
- 时间、空间的变量进一步复杂化了问题
- Large-scale real-time computing
- 大规模实时在线计算

STUDY DESIGN

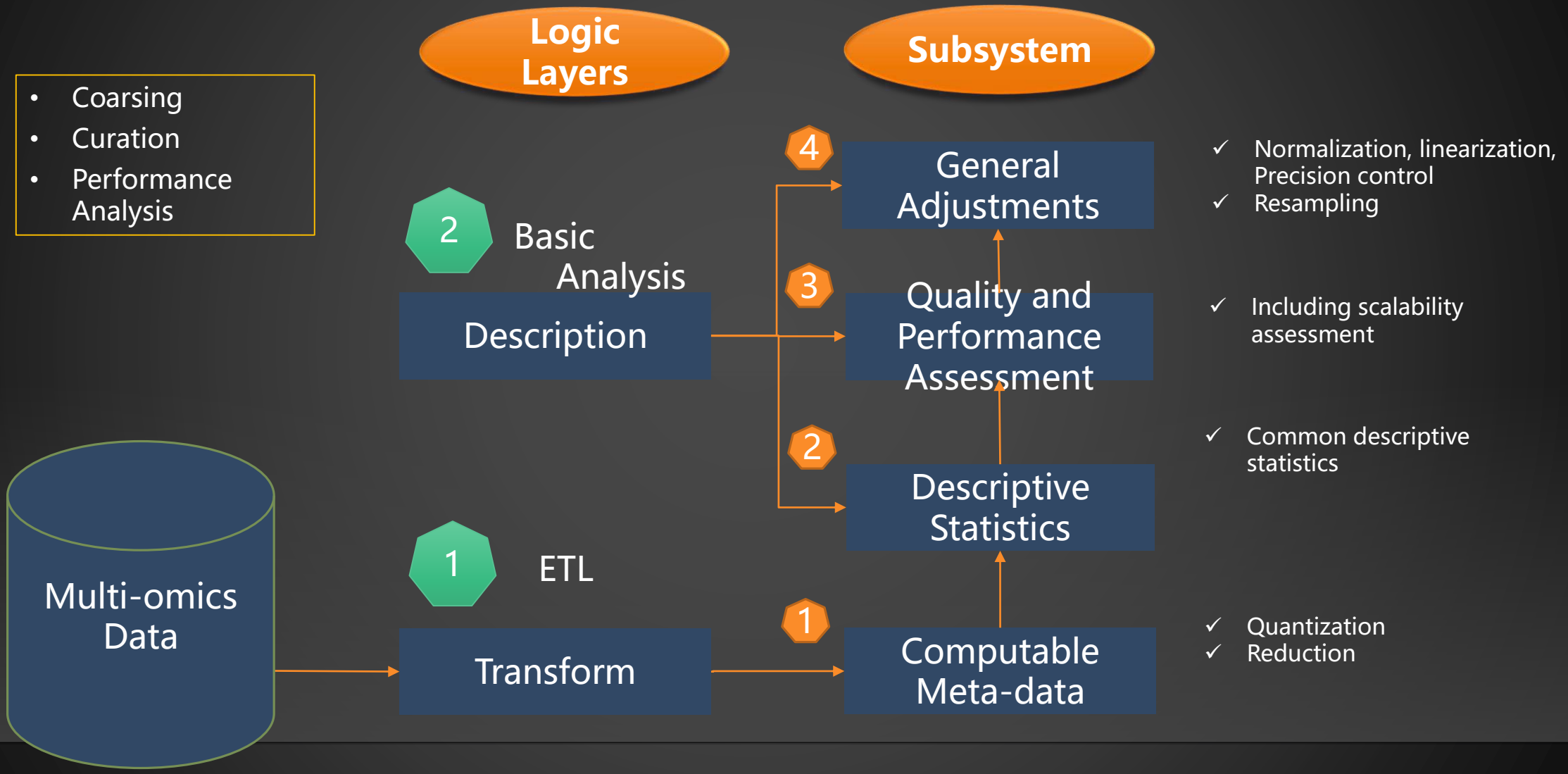
- 13 Data Modalities (features)
  - Blood glucose (1)
  - Blood pressure (3)
  - Blood routine (41)
  - Body fat (17)
  - Fitbit (14)
  - HealthTell (antibody repertoire) (126,000)
  - Blood metabolites (293)
  - Urine metabolites (422)
  - Microbiome (200 unique)
  - MindWave (?)
  - SomaLogic (Circulating protein) (4001)
  - Urine routine (17)
  - DNA
  - RNA
    - mRNA (32357)
    - miRNA (Plasma:1116, PBMC:1693)
    - lncRNA (10002)

Transparent boxes = 1 or more samples per day

Meta-Computing Design

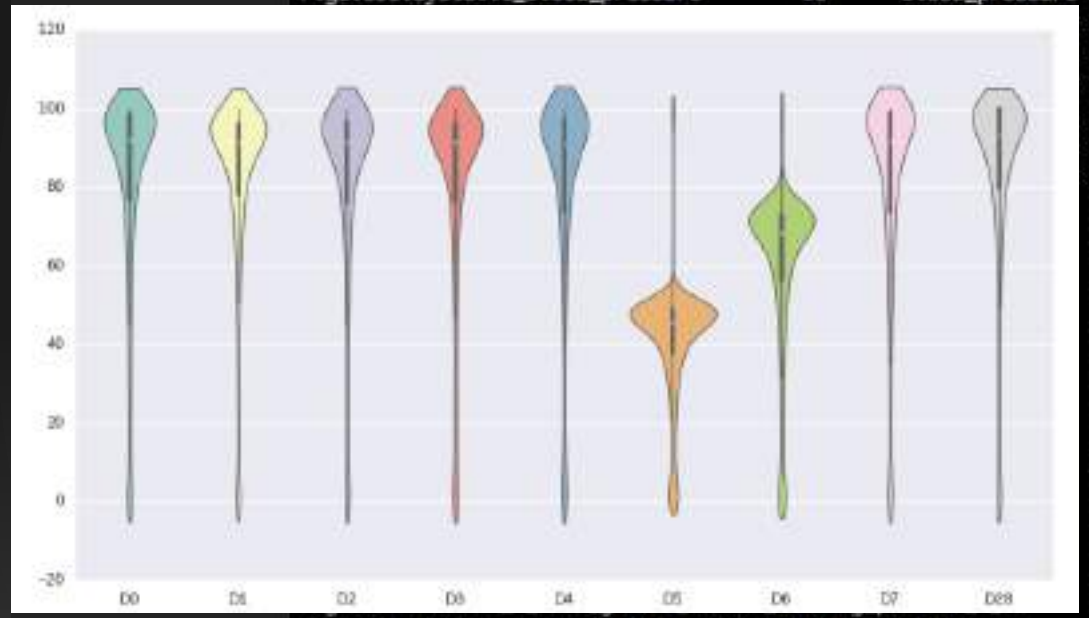


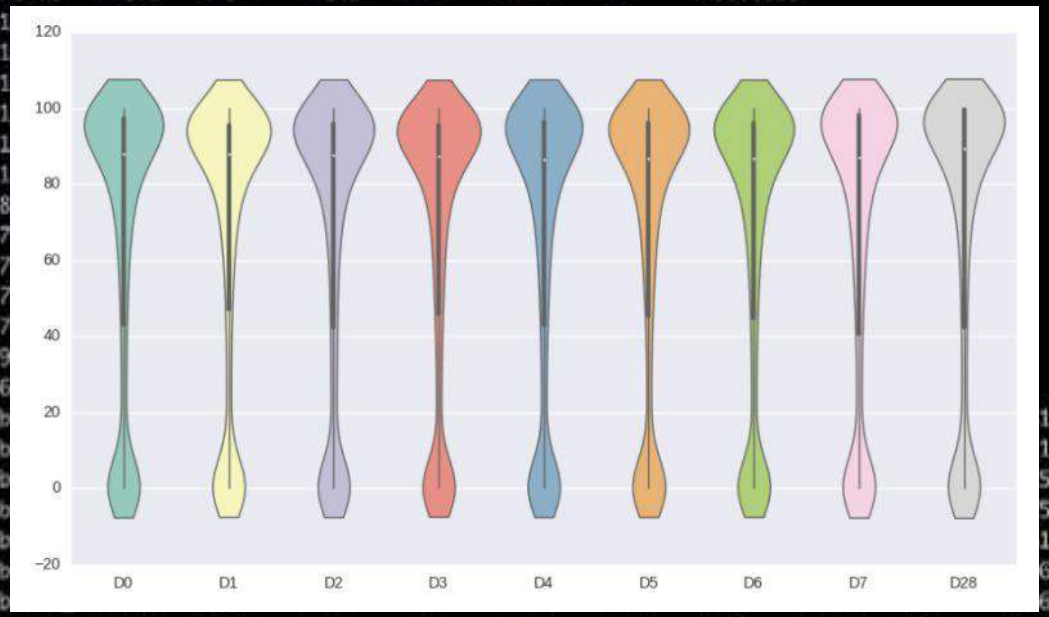
Meta-Data Processing

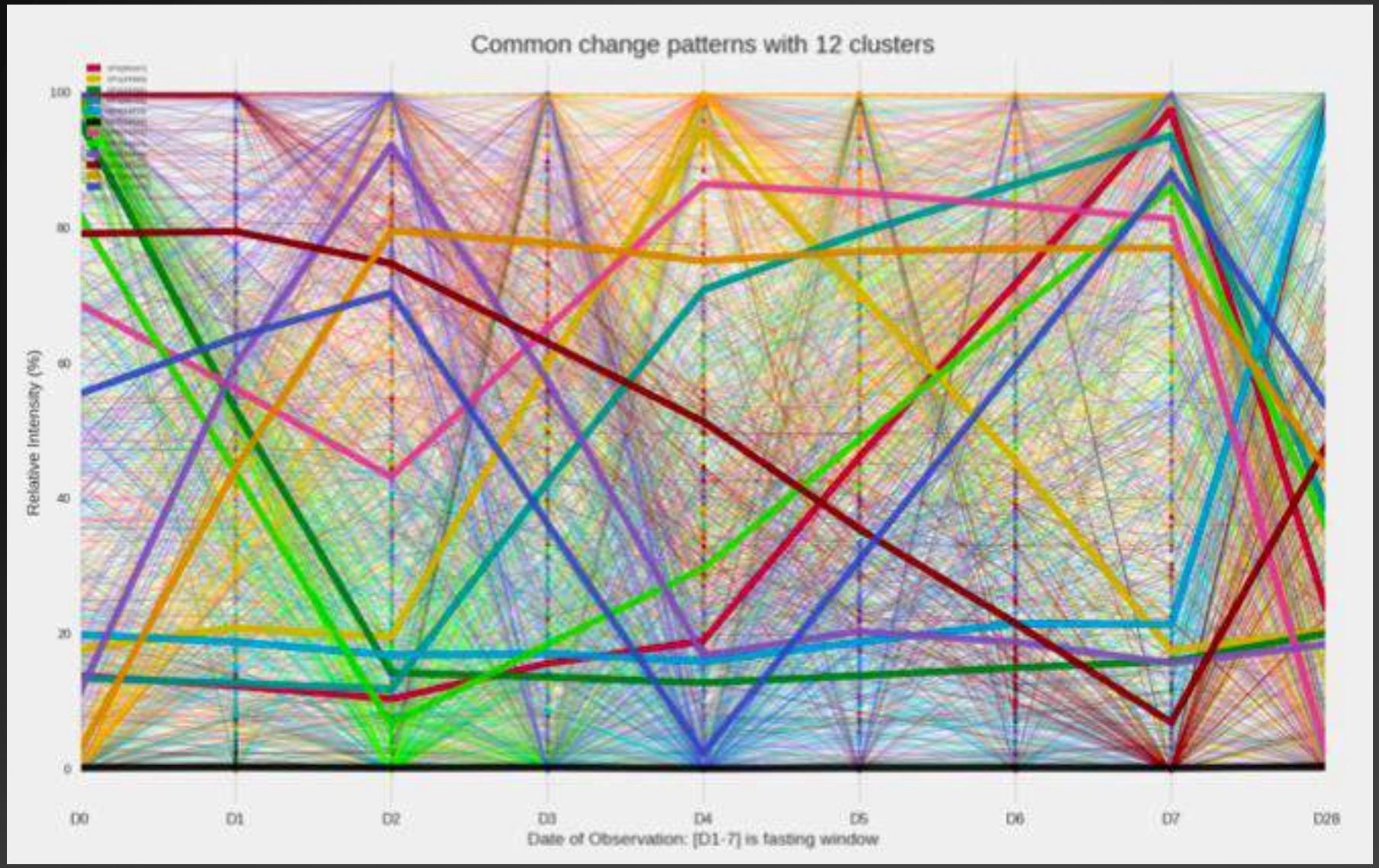


INTEGRATED DATA MATRIX

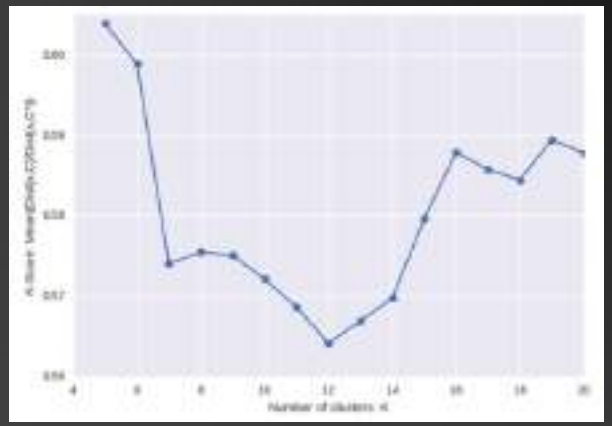
Metric	Sample	DataType	D0	D1	D2	D3	D4	D5	D6	D7	D28				
Pigu:095:Diastolic_blood_pressure			10	blood_pressure	62.5	62.5	68	66.5	67.5	68	68.5	71	62.1666		
Pigu:095:Diastolic_blood_pressure			22	blood_pressure	75	75	72.5	64.5	67	68	74	75	68.25		
Pigu:095:Diastolic_blood_pressure			231	blood_pressure	71	71	65	64	66.5	67	68.5	65	69.75		
Pigu:095:Diastolic_blood_pressure			232	blood_pressure	92.5	92.5	84	82.5	80	73.5	77	79	83.6		
Pigu:095:Diastolic_blood_pressure			233	blood_pressure	57	57	56.5	53.5	54.5	57	56.5	63	55.4285		
Pigu:095:Diastolic_blood_pressure			42	blood_pressure	71	71	67.5	67	69	73.5	72	81	71.7142		
Pigu:095:Diastolic_blood_pressure			48	blood_pressure	73.5	73.5	66	67.5	68.5	70	66.5	68	70.1666		
Pigu:096:Systolic_blood_pressure			10	blood_pressure	102.5	102.5	116.5	113	115.5	112	112	117	106.8333		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			10	blood_routine	4.05	3.505	2.96	3.0099	3.22	3.28	3.34	3.48	2.815		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			22	blood_routine	3.68	3.475	3.27	3.51	3.21	2.0099	3.79	3.6	3.0085		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			231	blood_routine	3.02	2.695	2.37	2.42	2.07	2.73	2.78	3.14	2.354		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			232	blood_routine	2.7	2.58	2.46	2.6	2.91	2.63	2.64	2.65	2.3483		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			233	blood_routine	2.68	2.5949	2.5099	2.6	2.66	2.69	2.71	2.7199	2.4442		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			42	blood_routine	1.78	1.725	1.67	1.62	1.6	3.83	1.88	1.93	1.6385		
Pigu:022:Quantitative_determination_of_immunoglobulin_A			48	blood_routine	3.72	3.37	3.02	3.2599	3.49	4.12	3.62	3.78	3.0528		







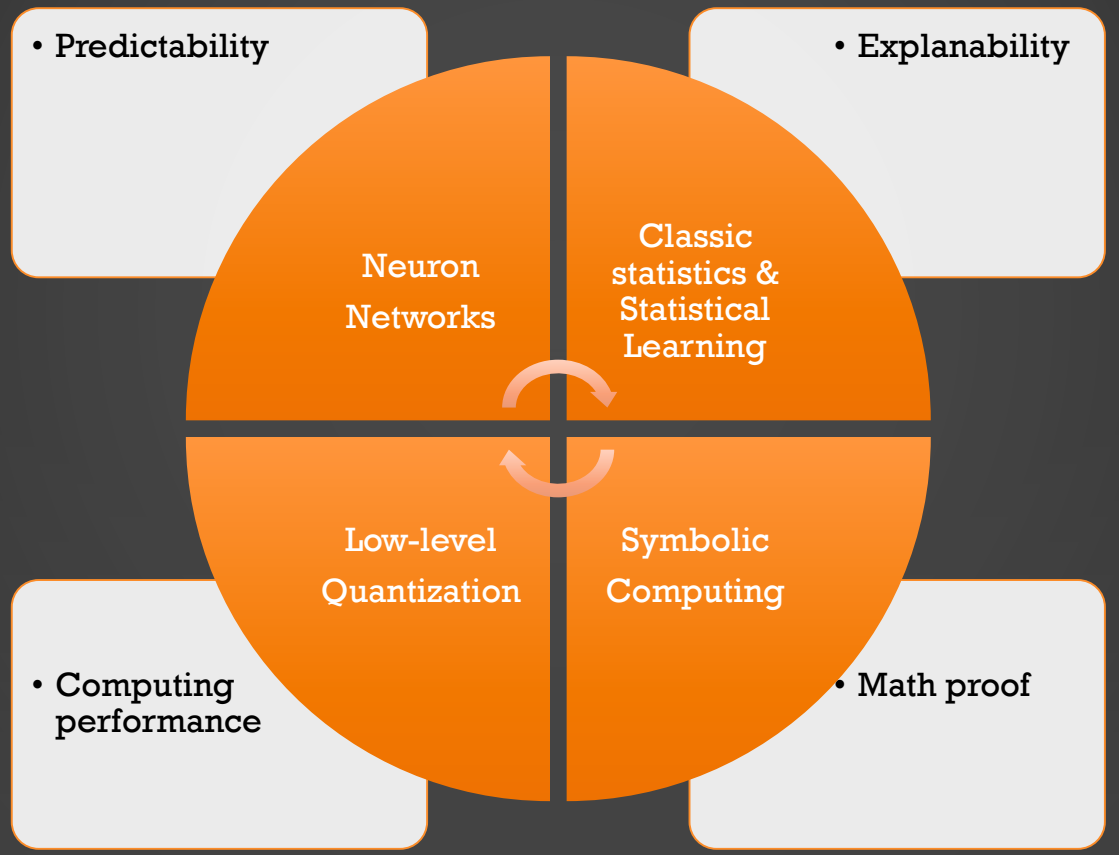
Features with consistency in [4,5,6,7] subjects are [5,807, 2,919, 1,125, 122] respectively



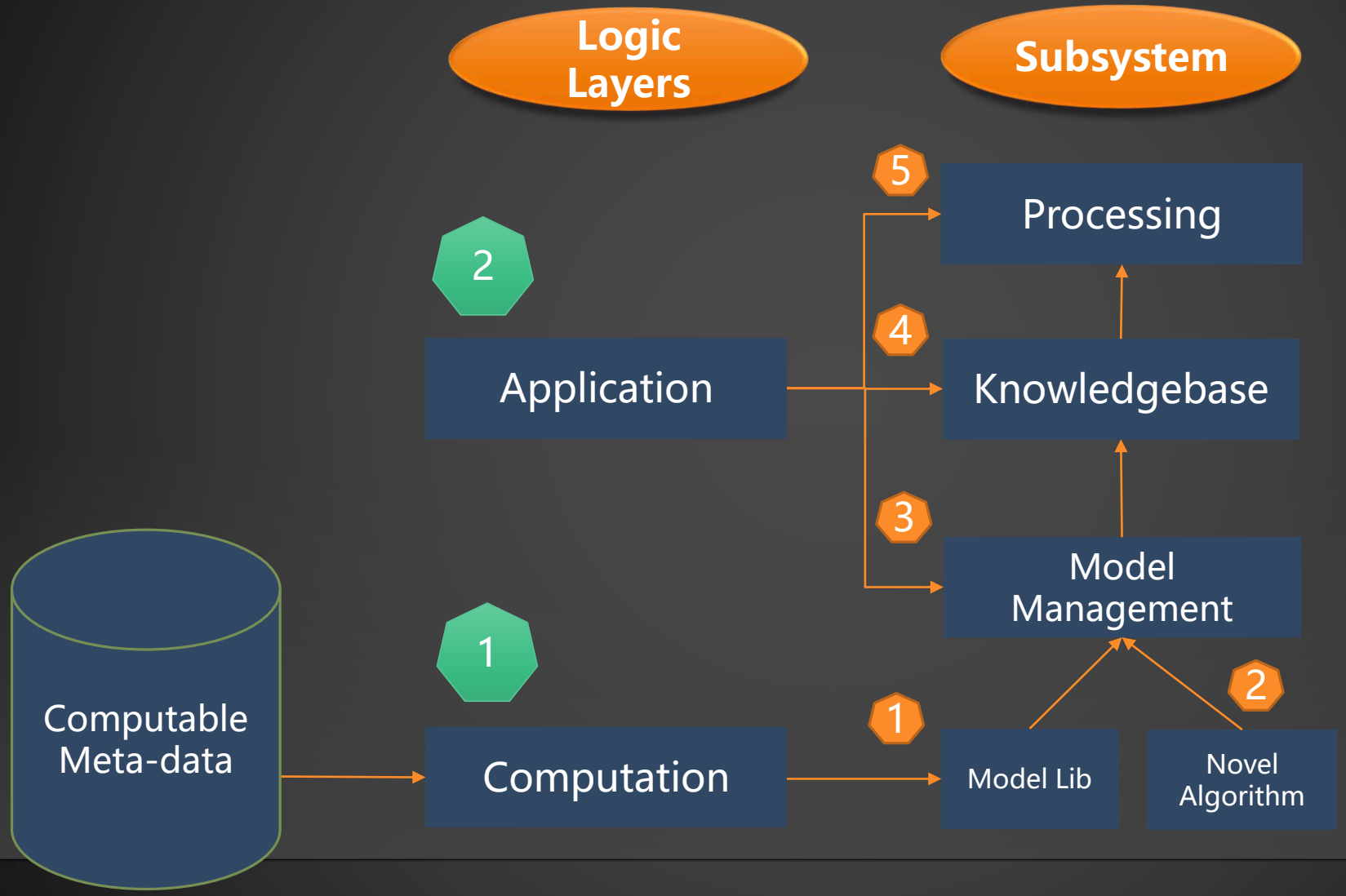
Change Pattern	#Consist Biomole 9973	Changes in Fasting Window	Post-fasting Changes
CP 0	1434	Late Raise	Drop to PFL
CP 1	164	Middle Rase then Drop to PFL	Keep as PFL
CP 2	244	Early Drop then Stay Low	Stay Lower than PFL
CP 3	1066	Middle Rase	Drop to PFL
CP 4	586	Stay Low as PFL	Raise Higher than PFL
CP 5	4903	Stay Still	Stay Still (No changes)
CP 6	418	Stay High as PFL	Drop lower than PFL
CP 7	76	Early Drop then Late Raise	Drop lower than PFL
CP 8	53	Early Raise then Drop to PFL	Keep as PFL
CP 9	385	Late Drop	Raise to PFL
CP 10	268	Early Raise then Stay High	Drop Higher than PFL
CP 11	376	Middle Drop then Raise to PFL	Keep as PFL

PFL = Pre-Fasting Level

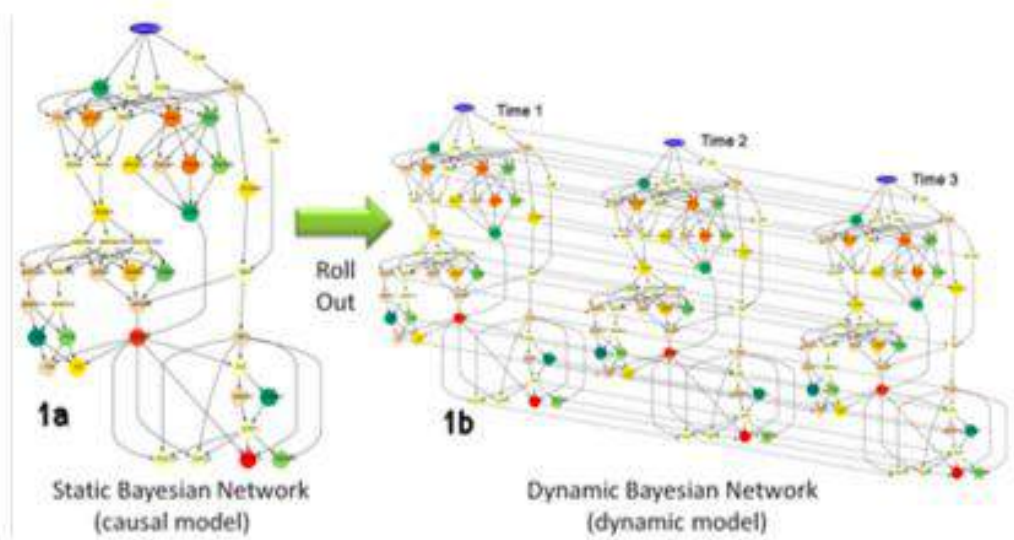
ALGORITHM LIB: STRATIFICATION, ABSTRACTION AND INTERFACING



Meta-modeling Management

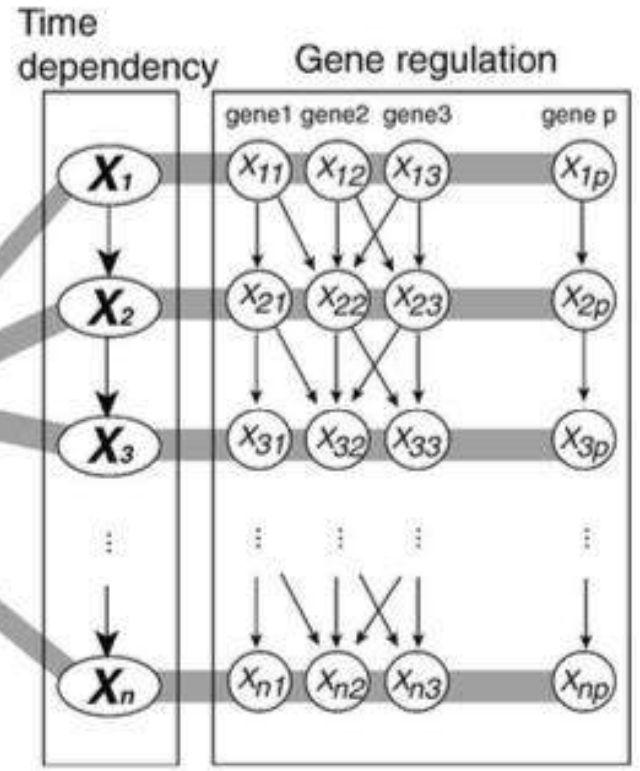


DYNAMIC BAYESIAN NETWORK CONSTRUCTION



Microarray

	gene1	gene2	gene3		gene p
time 1	x_{11}	x_{12}	x_{13}		x_{1p}
time 2	x_{21}	x_{22}	x_{23}		x_{2p}
time 3	x_{31}	x_{32}	x_{33}		x_{3p}
⋮	⋮	⋮	⋮		⋮
time n	x_{n1}	x_{n2}	x_{23}		x_{np}



METHOD 4. Lagged Correlation

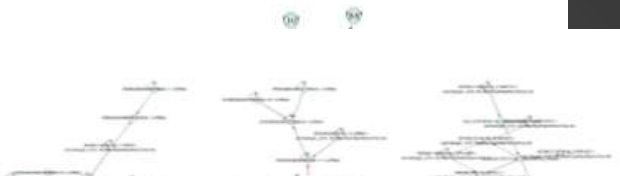
- Correlation test

METHOD 3. ScanBMA

no	id	Sample
1	Pigu:O21:	1
1	Pigu:O21:	2
1	Pigu:O21:	2
1	Pigu:O21:	2
1	Pigu:O21:	2
1	Pigu:O21:	4
1	Pigu:O21:	4
2	Pigu:O22:	1
2	Pigu:O22:	2
2	Pigu:O22:	2
2	Pigu:O22:	2
2	Pigu:O22:	2
2	Pigu:O22:	4
2	Pigu:O22:	4

- Once the p-valu
  then a regulato

- The network inference
  series of variable selec
  which parent nodes (re
  inferred for each targe
  BMA framework accou
  uncertainty in variable
  averaging over the pos
  from multiple models,
  posterior model probal
  a greedy approach to
  space and uses the Oc
  principle to eliminate u
  and efficiently selects
  be averaged over.



METHOD 2. ebdbNet

- **Step 1: Model Selec**

  We construct a block-
  time series bio-featur
  the matrix H equals th

- **Step 2: Estimation**

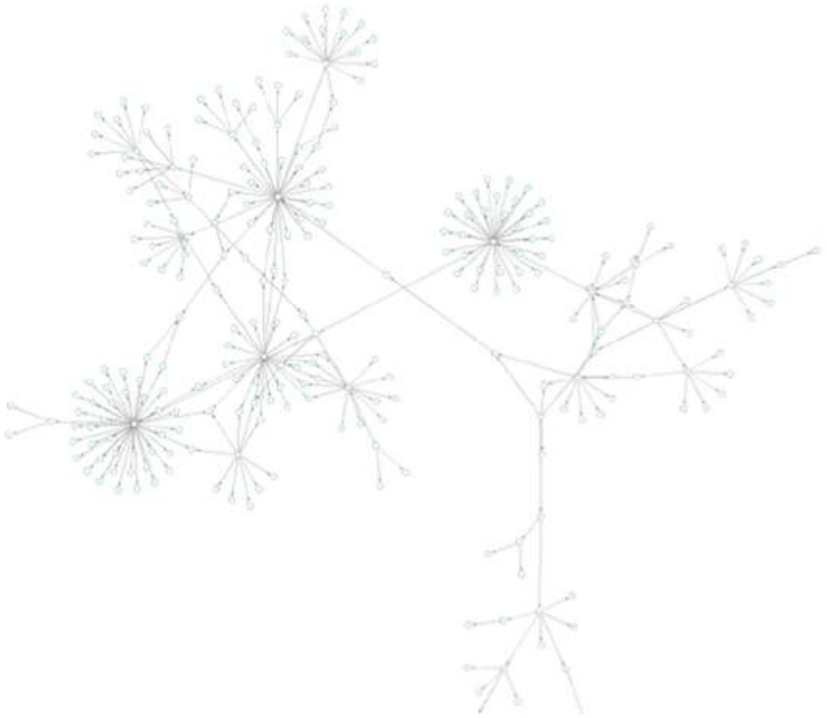
  The Kalman filter and
  values of the hidden s

- **Step 3: Calculation**

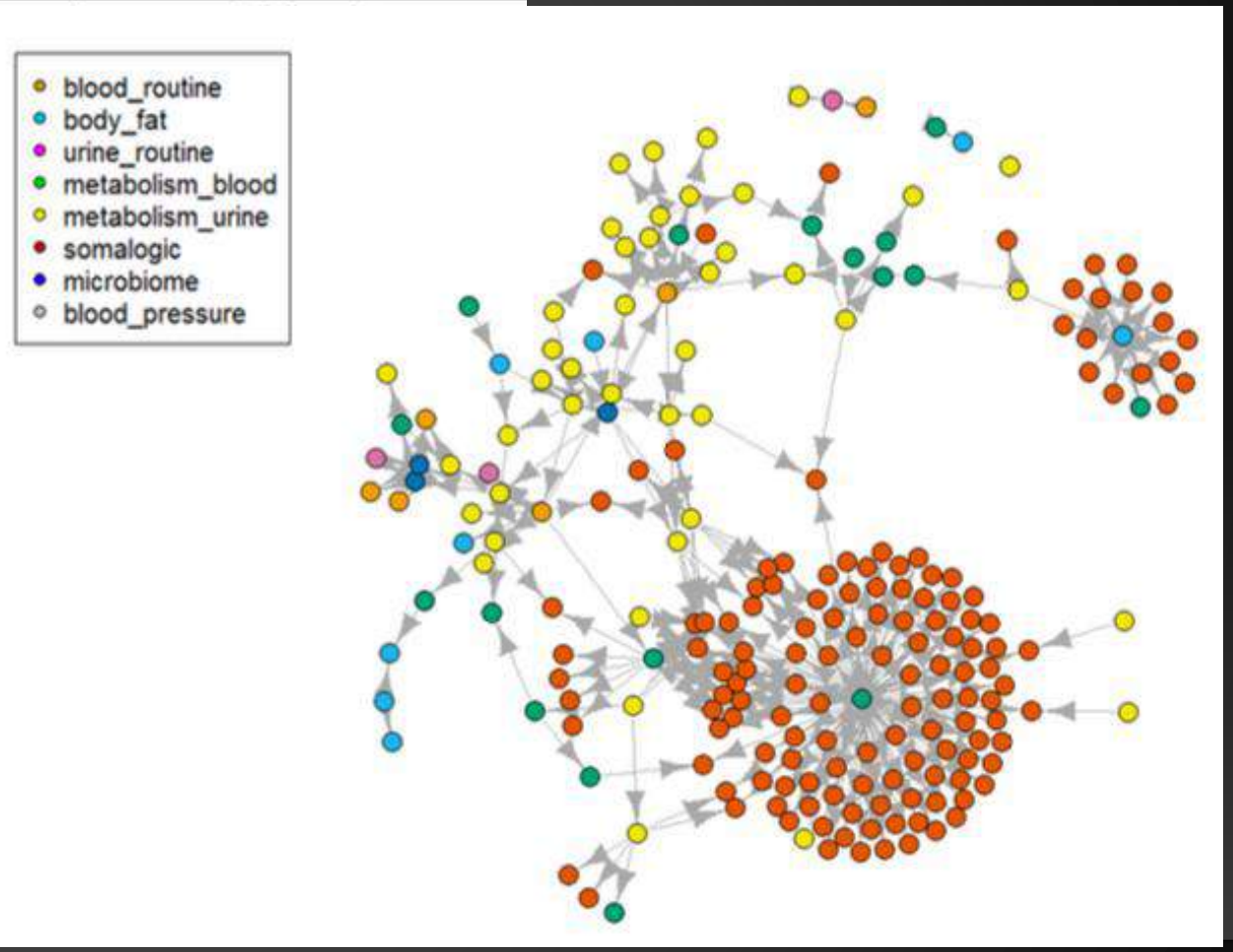
  The posterior means
  model can be calculat
  estimates of the hype

METHOD 1. G1DBN

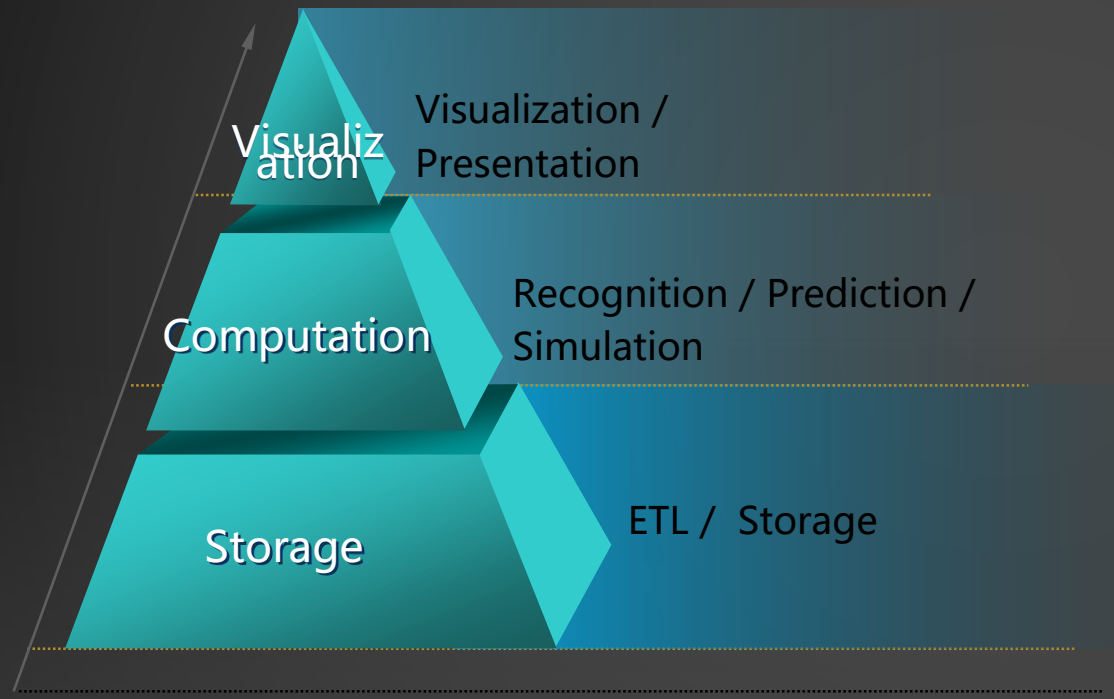
- Two-step dependence (linear relationship) inference
- Step 1: Dimensionality reduction through conditional dependence inference
- Step 2: Multivariate regression



ICX_Pathway	Path
ICX:PW6013:Alpha6_beta4_integrin-ligand_interactions	
ICX:PW7126:Netrin_mediated_repulsion_signals	
ICX:PW7501:Regulation_of_Insulin-like_Growth_Factor_(IGF)_transport_and_uptake_by_Insulin-like_Growth_Factor_Binding_Proteins_(IGFBPs)	
ICX:PW7501:Regulation_of_Insulin-like_Growth_Factor_(IGF)_transport_and_uptake_by_Insulin-like_Growth_Factor_Binding_Proteins_(IGFBPs)	
ICX:PW7244:Intrinsic_Pathway_of_Fibrin_Clot_Formation	
ICX:PW6214:prion_pathway	
ICX:PW3914:Inflammatory_Response_Pathway	
ICX:PW7244:Intrinsic_Pathway_of_Fibrin_Clot_Formation	
ICX:PW7126:Netrin_mediated_repulsion_signals	
ICX:PW7242:Common_Pathway_of_Fibrin_Clot_Formation	
ICX:PW5948:Glypican_1_network	
ICX:PW4115:cbl_mediated_ligand-induced_downregulation_of_egf_receptors_pathway	
ICX:PW7225:Disinhibition_of_SNARE_formation	



Application Architecture



Presentation: Front end for users

Computation: Intense Computing by hardware acceleration, R / Matlab interfacing etc.

ETL / Storage: Multimedia Database on Hadoop / HANA etc.

Fasting Suppressed

- **Extracellular Matrix Organization**
  - Activation of MMPs
  - Collagen formation / degradation
  - Degradation of ECM
  - Assembly of collagen fibrils and other multimeric structures
- **Hemostasis**
  - Platelet degranulation
  - Formation of Fibrin Clot
  - Response to elevated platelet cytosolic Ca2+
- **Metabolism of Proteins**
  - Regulation of IGF transport and uptake by IGFBPs
  - Post-translational protein phosphorylation
  - Peptide hormone biosynthesis
- **Other**
  - Neutrophil degranulation
  - Antagonism of Activin by Follistatin
  - EPH-ephrin mediated repulsion

Late Risers

- **Immune System**
  - Regulation of IFNA
  - TRAF6 mediated IRF7 activation
- **Developmental Biology**
  - Keratinization

Transient and Post Increased

- **Immune System**
  - Signaling by interleukins
    - Interleukins 4, 13, 6
  - Regulation of IFNA
- **Cell-Cell Communication**
  - SIRP family interactions
- **Signal Transduction**
  - PTK6 Regulates RTKs

Long-term Suppressed

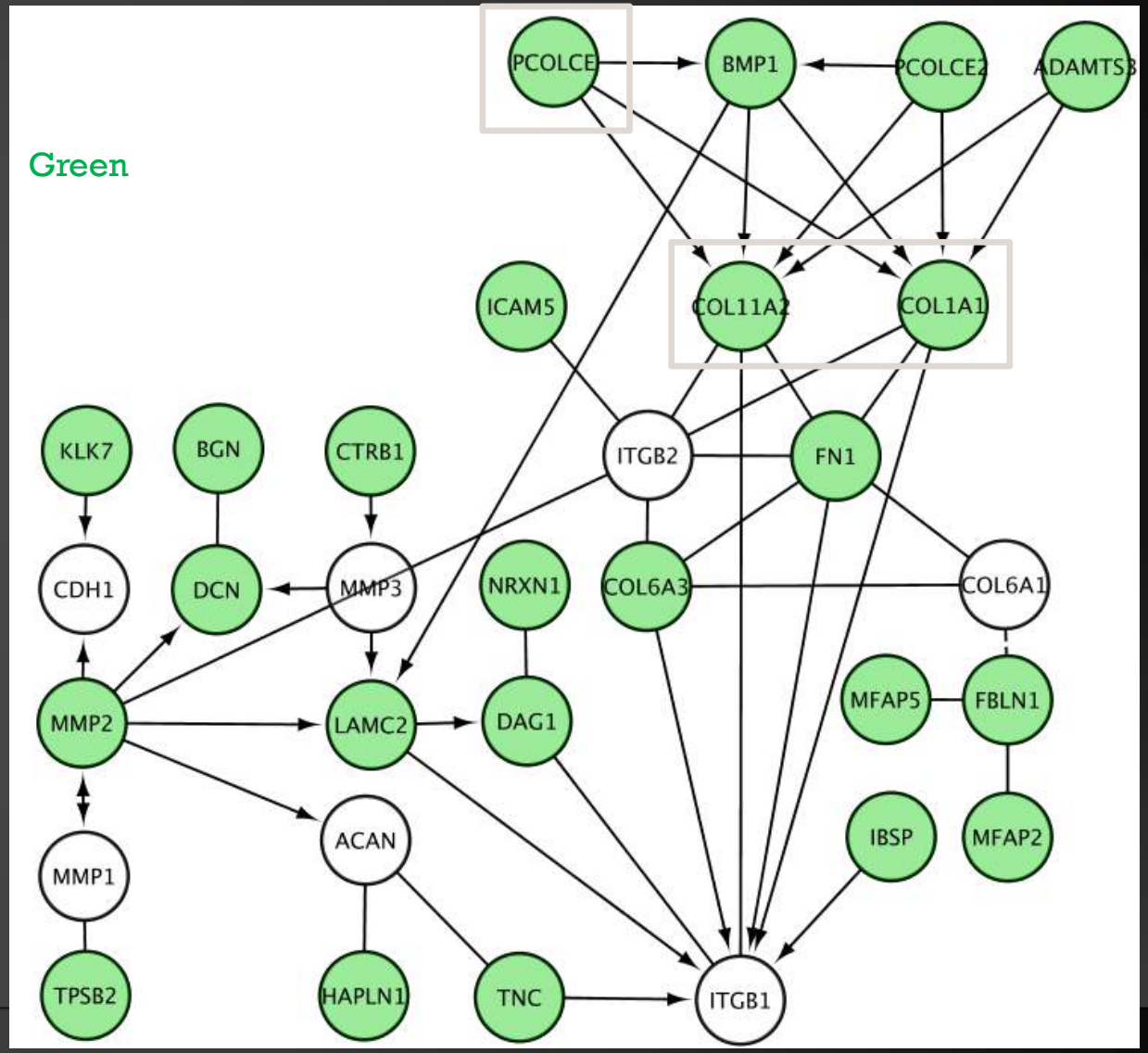
- **Immune System**
  - Complement cascade
  - Neutrophil degranulation
  - Innate immune system
- **Hemostasis**
  - Platelet activation, signaling, aggregation, degranulation
- **Metabolism of proteins**
  - Post-translational protein phosphorylation

Early Risers

- **Immune System**
  - Innate immune, Adaptive immune, TLR4 cascade, Cytokine signaling, SCF-KIT, B-cell receptor, CD28-CTLA, DAP12 signaling, etc.
- **Metabolism of RNA**
  - Deadenylation-dependent mRNA decay, Regulation of mRNA stability by proteins
- **Hemostasis**
  - Platelet activation, signaling, aggregation; Response to elevated platelet cytosolic Ca2+, GPVI-mediated activation cascade
- **Signal Transduction**
  - Growth Factors Signaling (IGF), Signaling by Wnt, PI3K/AKT activation,
- **Neuronal System**
  - Trafficking of AMPA receptors

DECREASED EXTRACELLULAR MATRIX (ECM) PROTEINS DURING FASTING

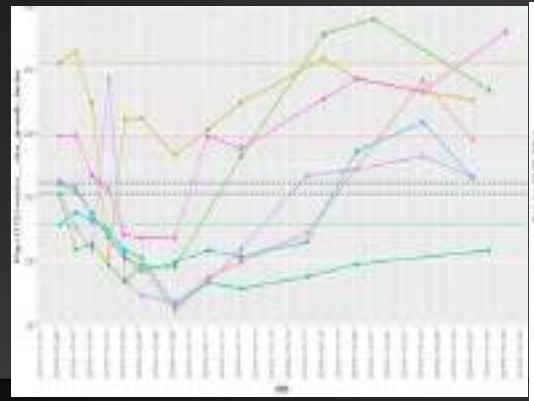
- All proteins are decreased during fasting, suggesting that ECM and collagen are being degraded
  - Decreased collagen biosynthesis due to fasting (PMID: 12670795, 15543950, 3980462)
  - Serum collagen type 1 decreased during weight loss in individuals (PMID: 8574277, 19937152)
  - Decreased bone formation (PCOLCE, marker of formation) in women after fasting for four days (PMID:8530611)
- ECM is an important regulator of insulin action
  - Collagen and ECM proteins are increased in diet-induced obesity in muscle and liver (PMID: 26059707)



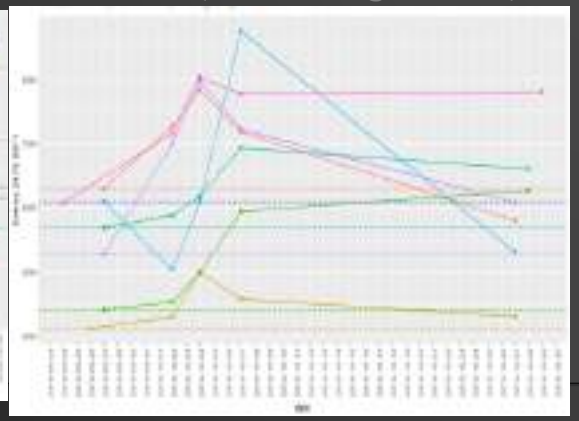
EARLY RISERS: INSULIN GROWTH FACTOR SIGNALING DURING FASTING

- Short-term fasting (5 days) has been shown to decrease IGF1 by more than 60% and increase IGF-1 inhibiting proteins by more than 5 fold (PMID: 24440038)
  - Circulating IGF1 (as per Somalogic) are increased during fasting (inconsistent with literature), however, IGF levels from blood routine are decreased during fasting
- IGFBP1
  - IGFBP1 increased during fasting in healthy obese and nonobese but unchanged in diabetic patients (PMID: 7512573)
  - IGFBP1 increased during fasting in children (PMID: 9851789)
  - Serum levels of IGFBP1 during fasting can vary depending on the individual glucose tolerance (PMID: 19463807)
    - Two participants (231, 233) have a sharper spike during fasting for both IGFBP1 somamer features than the rest of the participants
- IGFBP2
  - Increased IGFBP2 expression during fasting (PMID: 25695641)
  - Increased IGFBP2 after weight loss in obese adolescent girls (PMID: 8968852)

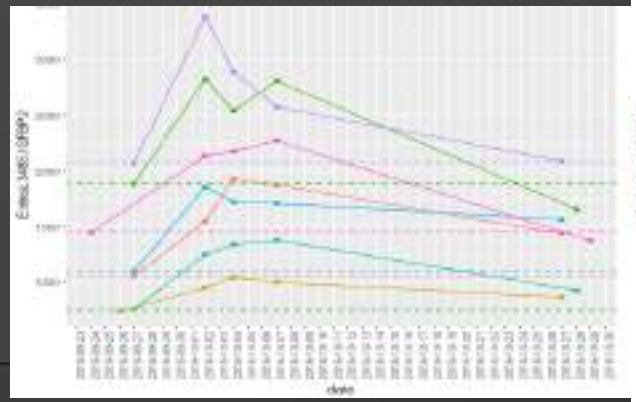
IGF1 (Blood Routine)



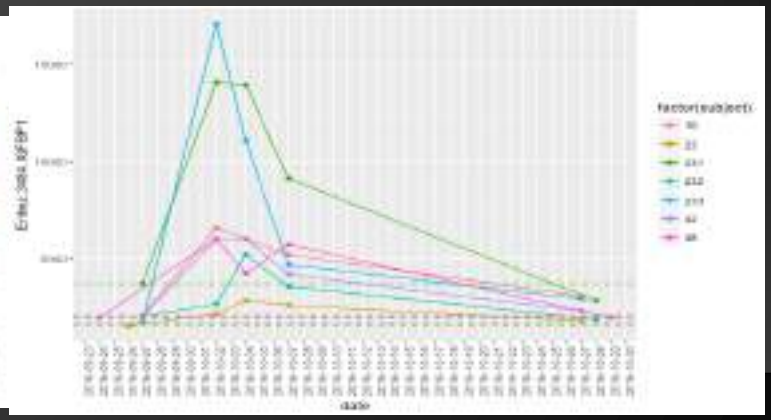
IGF1 (Circulating Protein)



IGFBP2 (Circulating Protein)

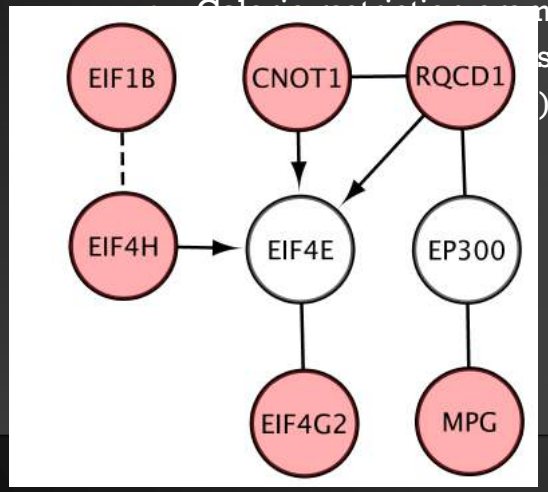


IGFBP1 (Circulating Protein)

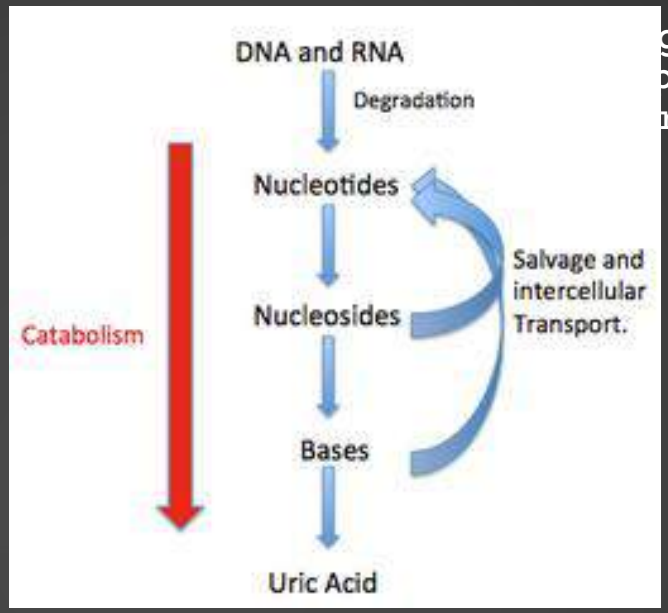


EARLY RISERS: METABOLISM OF RNA

- Fasting may affect genomic stability and protein synthesis
  - CNOT1, RQCD1 (CNOT9): Part of CCR-NOT complex that is mRNA deadenylation and degradation
    - Stress stimulates recruitment of this complex and trigger mRNA degradation (PMID: 22416820)
  - EIF4H, EIF4G2, EIF1B: Translation initiation factors
  - MPG: Initiate base excision repair pathway
    - Galactic acetylation promotes genomic stability by specifically base excision)



Red = Increased during fasting
White = Not in clusters / not measured



gradation of DNA and RNA may be linked urine catabolism since uric acid is reased in blood

IMPDH1 (inosine monophosphate dehydrogenase 1) catalyzes conversion of IMP to XMP which is the rate-limiting step in guanine nucleotide synthesis

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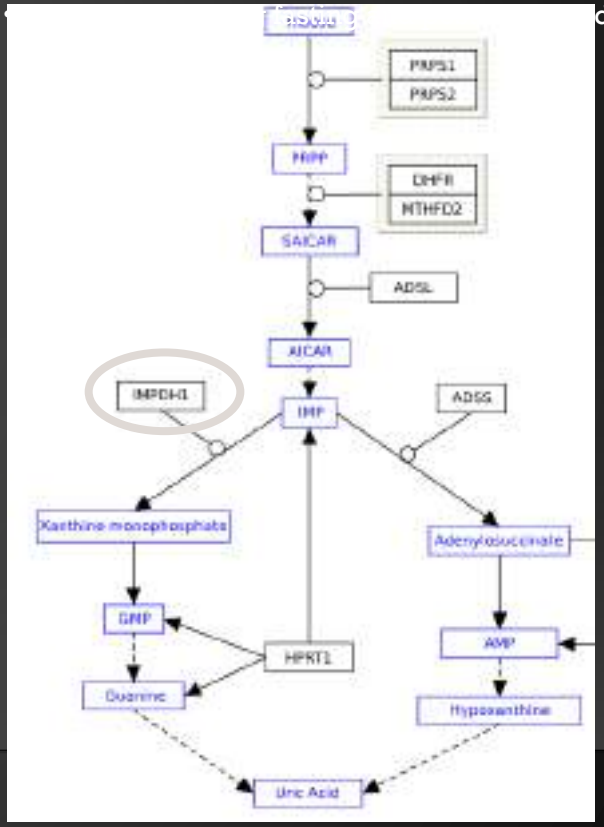
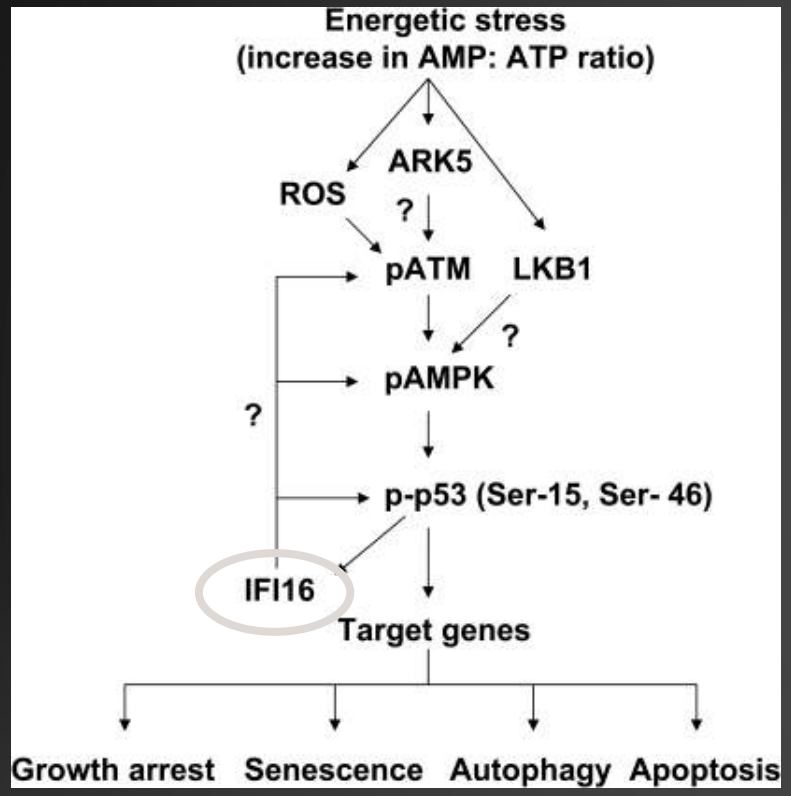


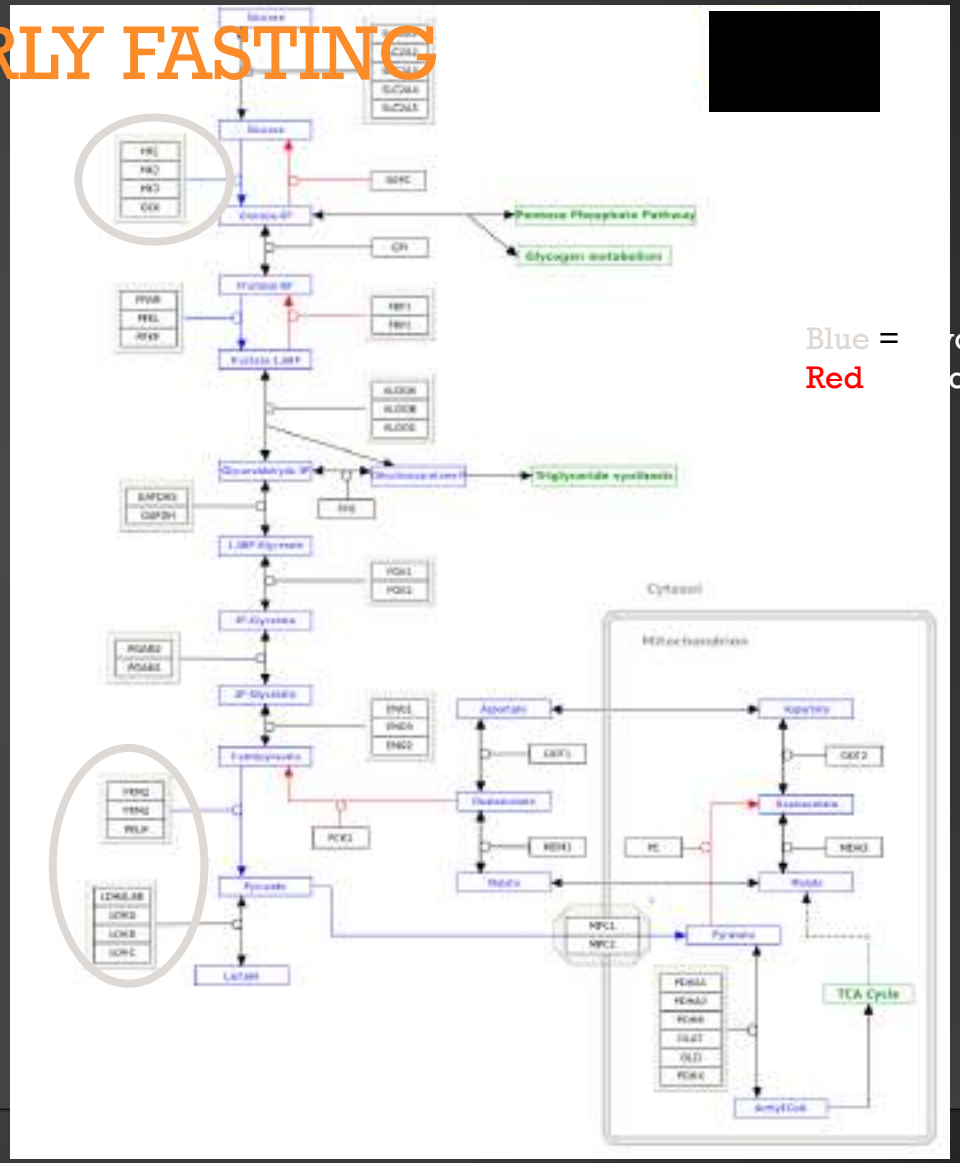
TRANSIENT AND POST-INCREASED: IFI16 MAY CONTRIBUTE TO AUTOPHAGY INDUCTION



- IFI16 drives the assembly of NLR-independent inflammasomes which has been shown to have crosstalk with autophagy (PMID: 23276949)
  - Autophagy induction may depend on the the presence of specific inflammasome sensors, and inflammasomes are degraded during autophagy (PMID: 24213677
- Fasting has been shown to induce autophagy in mice (PMID: 20534972, 21471734)
- Liver autophagy contributes to maintenance of blood glucose and amino acid levels in mice
- Glucose restriction in human fibroblasts increase IFI16 protein levels which is associated with energetic stress-induced autophagy (PMID: 21573174)

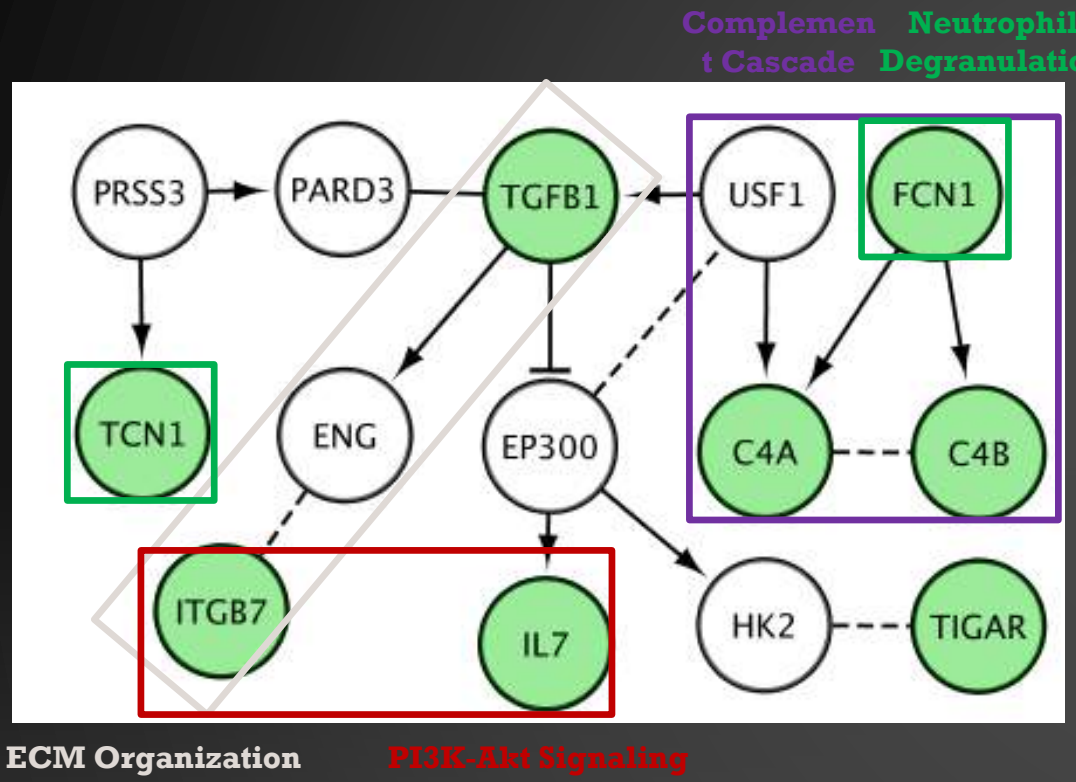
TRANSIENT AND POST-INCREASED: GLYCOLYTIC ENZYMES MAY DRIVE GLUCONEOGENESIS DURING EARLY FASTING

- Amino acid breakdown into energy and glucose/ketones occurs during fasting as evident by the metabolites observed in the blood
- HK2, PKM2, and LDHB (enzymes in glycolysis pathway) may be a downstream effect of the amino acid breakdown and eventually leads to increased pyruvate and gluconeogenesis
- During fasting, pyruvate is produced through glycolysis (PMID: 24692138)



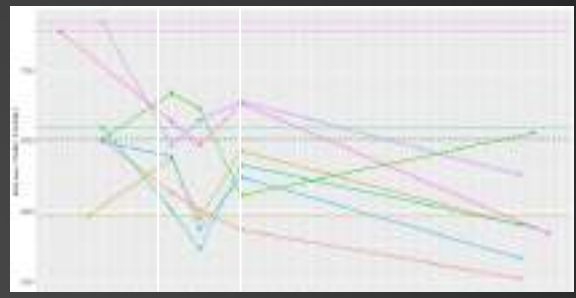
Pathway Diagrams downloaded from WikiPathways

MOST OF LONG-TERM SUPPRESSED CIRCULATING PROTEINS ARE INTERCONNECTED*

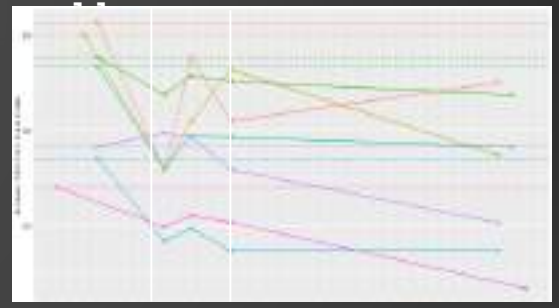


*Using Reactome FI Cytoscape plug-in for protein relationships
Green = Decreased during fasting
White = Not in clusters / not measured
Box= Significantly enriched pathway using Reactome FI

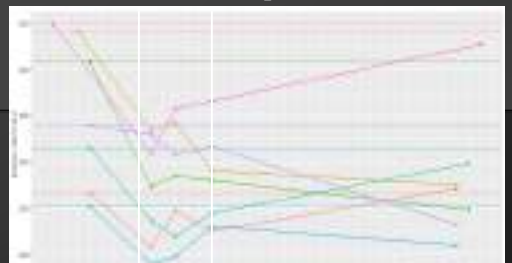
- IL7 has been shown to be involved in body weight and food intake regulation

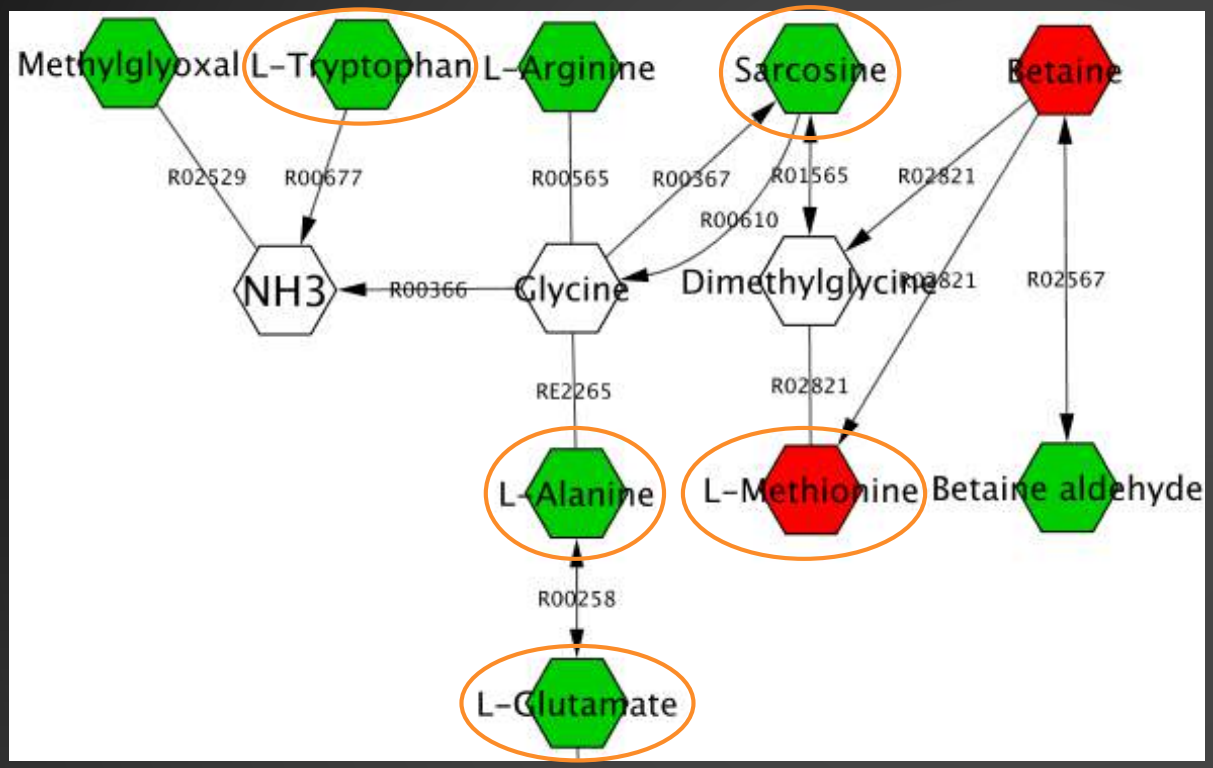


- Dietary restriction and fasting downregulates complement activity in rodents

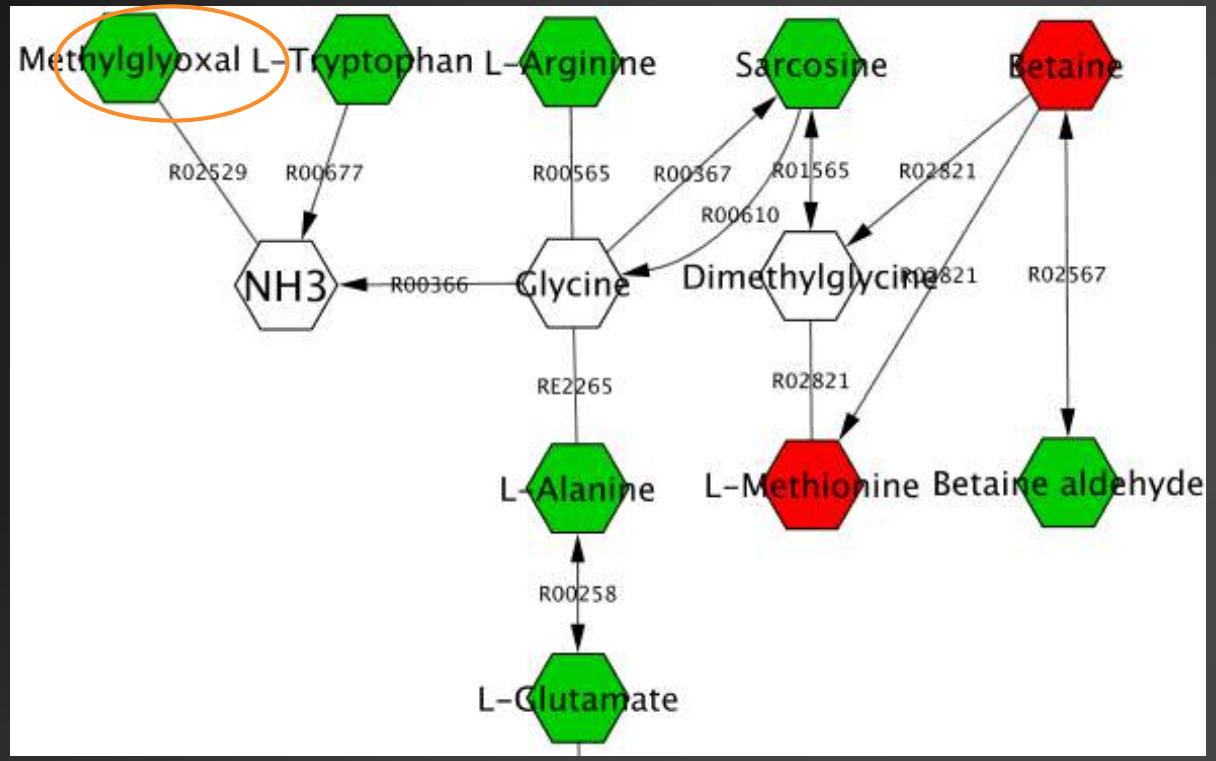


- Serum TGFB1 found to be increased in type II diabetic patients, so its concentration may be affected by glucose levels





Red = Increased during fasting (dark = late rise)
Green = Decreased during fasting
Grey = Increased and decreased during fasting
White = Not measured / not in a cluster



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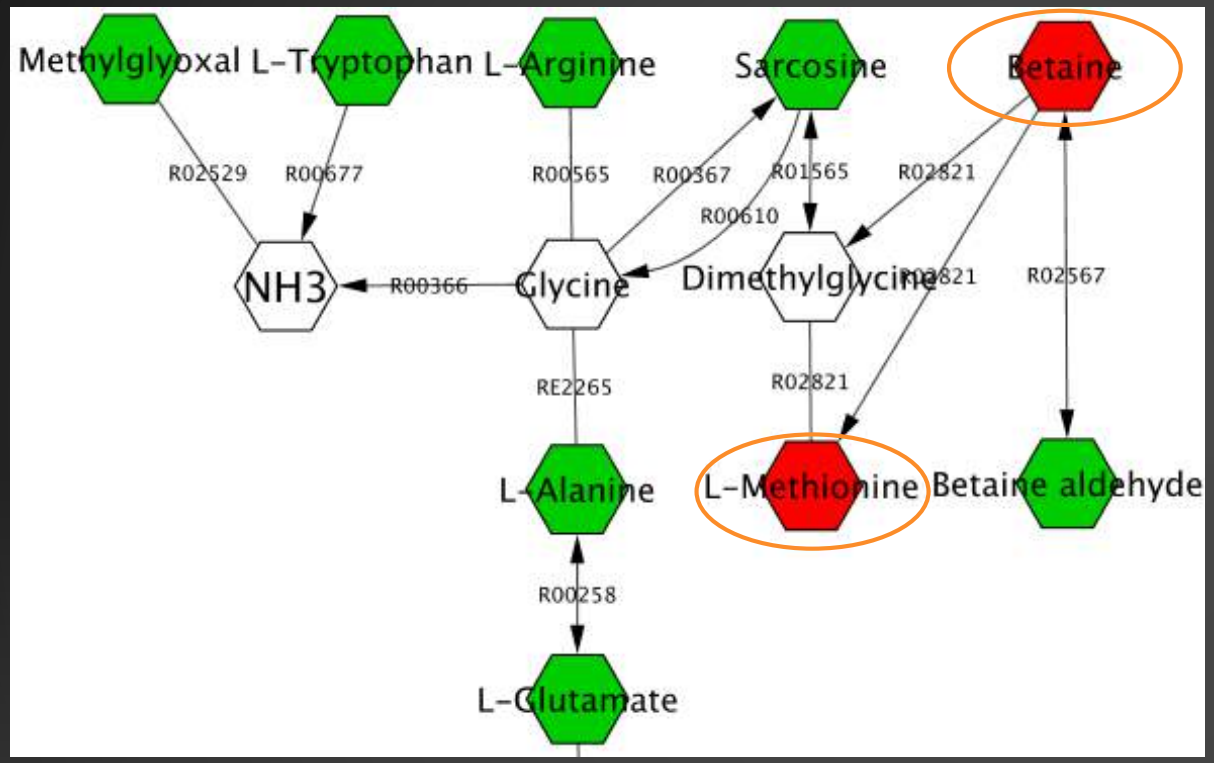

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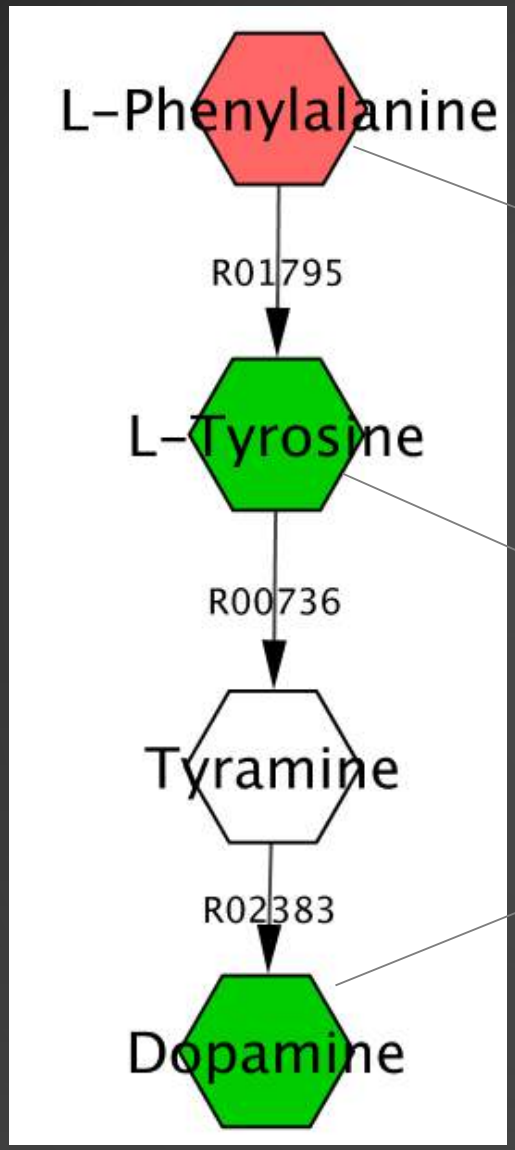
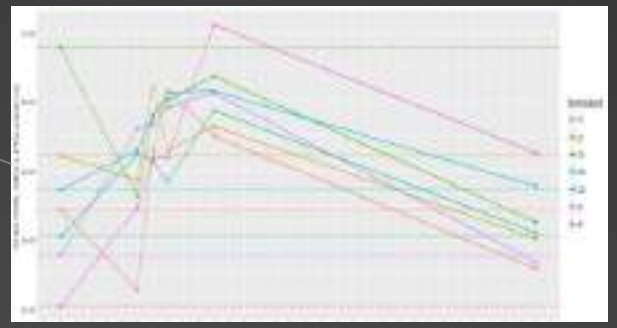
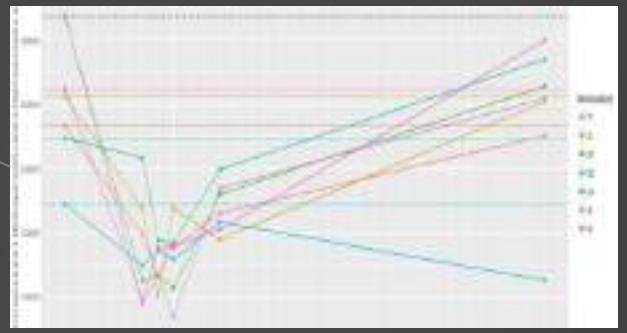
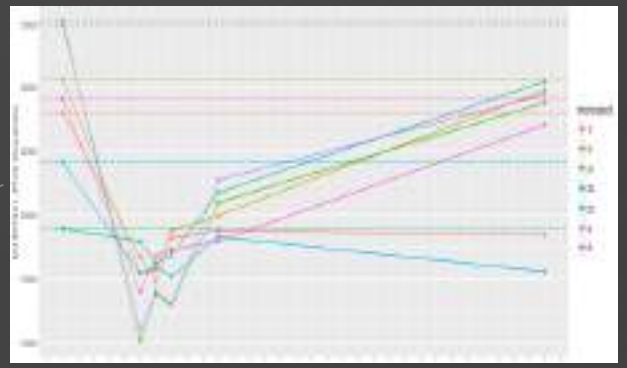

L-Phenylalanine
R01795
L-Tyrosine
R00736
Tyramine
R02383
Dopamine

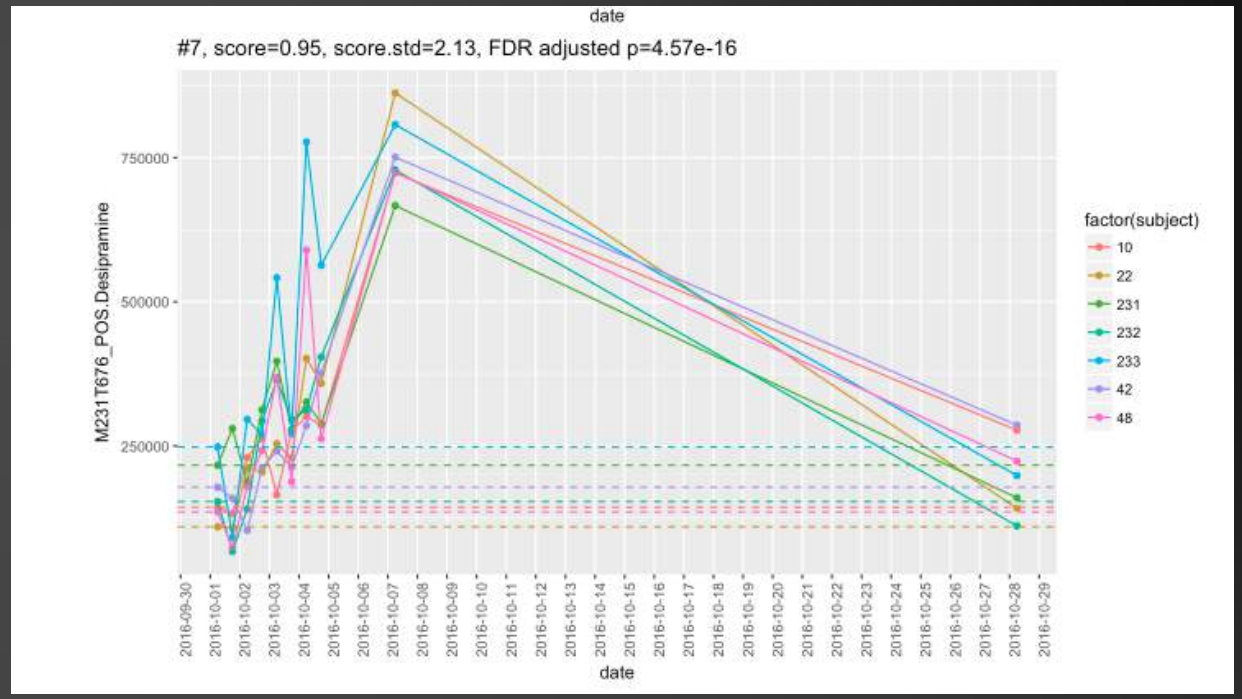






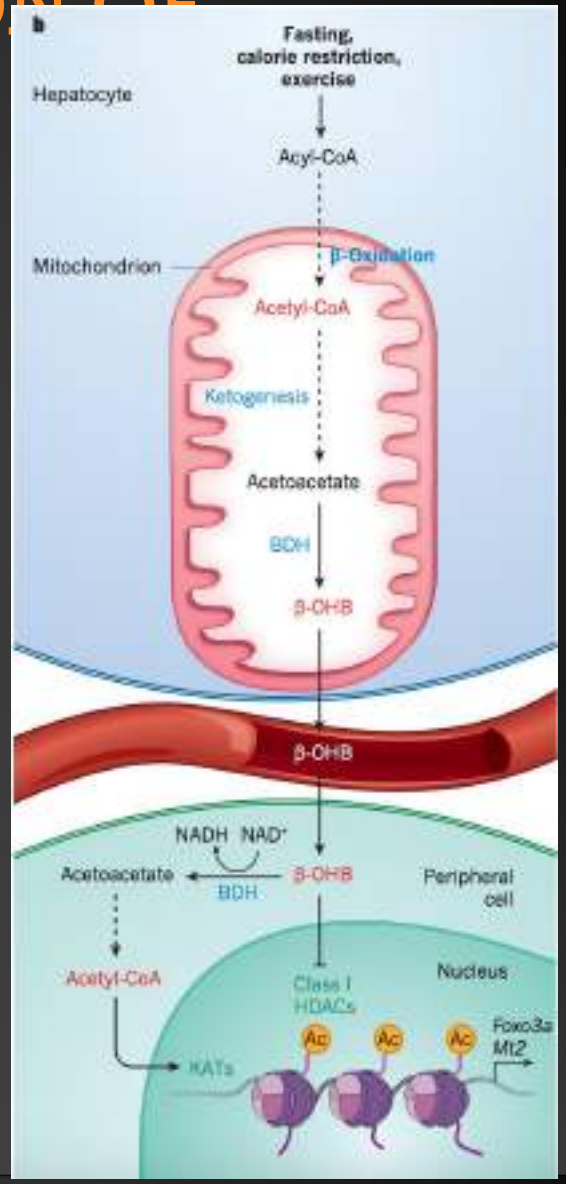
FASTING CAUSED INCREASED LEVELS OF TCA-TYPE MOLECULE IN URINE

- Increase of desipramine in the urine, peaked at day 7 of fasting
  - Desipramine, the active metabolite of imipramine, is a tricyclic antidepressant
  - It inhibits reuptake of norepinephrine and to a minor extent serotonin
  - It's primarily used in depression
    - Other uses include: attention deficit hyperactivity disorder, post herpetic neuralgia
- Supporting evidence for MDD and neuralgic pain
  - Some clinical studies have supported anti-depressive effect of calorie restriction, while the opposite effect had been reported in long-term calorie restriction (PMID: 26412073)
- What is the source? What could this represent?



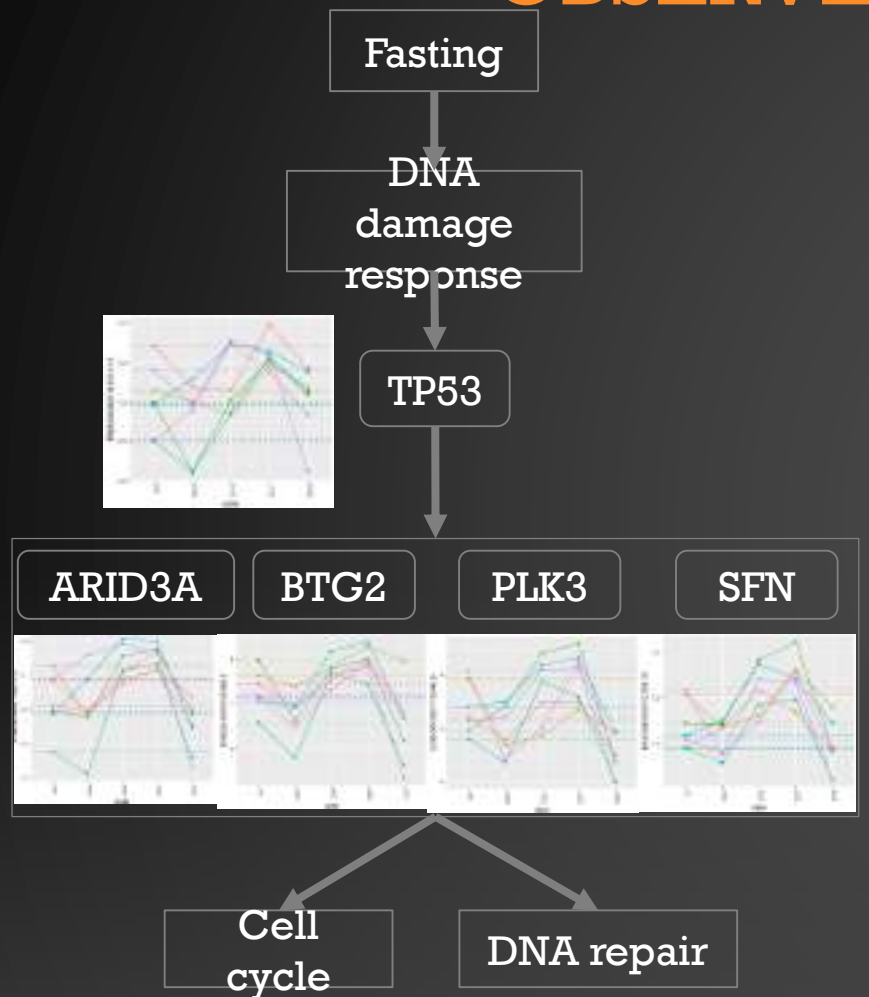
FASTING INDUCES GENE EXPRESSION OF HISTONES

- 83 significant enriched pathways were found using Reactome (FDR $p<0.05$) and can be grouped into 10 high-level processes
    - Gene expression (transcription), cell cycle, signal transduction, disease, DNA repair and replication, chromatin organization, cellular responses to external stimuli, metabolism, metabolism of proteins, and developmental biology
- The majority of enriched pathways are driven by a group of 10 histone genes
    - These core histones (H2A, H2B, H3, H4) and linker histones (H1) package DNA into nucleosomes, which are the structural units of chromatin
    - Histones play a role in transcription regulation, DNA repair and replication, chromatin remodeling and chromosomal stability (reflected in the enriched pathways)
- Many studies have reported elevated circulating histones and nucleosomes in multiple pathophysiological processes (i.e. severe trauma, sepsis, cancer) as well as healthy controls, potentially due to stress (PMID: 25118930, 22783398, 24706102)
    - However, evidence also points to histones functioning as host defense with anti-microbial, anti-bacterial, and anti-viral activity (26939619); thus it is less clear whether histones play a protective part in innate immune response in the above disease studies
- Post-translation modifications of histones shown to be linked to metabolism and fasting (PMID: 25562692, 25773162, 26520657, 24153302)
    - Changes in metabolite concentrations (due to fasting or dietary restriction) can influence histone modification and downstream gene expression
    - Fasting elevates ketone body β-OHB which can inhibit histone deacetylases or increase nuclear acetyl-CoA; acetyl-CoA increases histone acetylation leading to transcription of genes



PMID: 24153302

‘MID RISERS’ CLUSTER: P53 SIGNALING OBSERVED IN RESPONSE TO FASTING

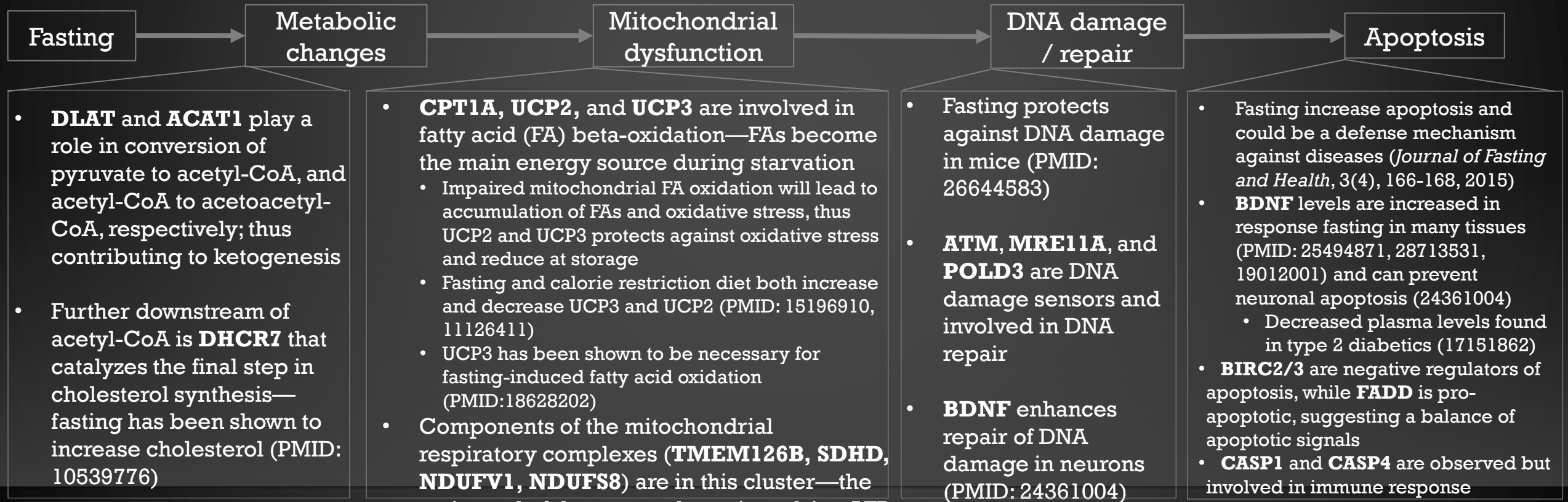


Schema of p53 signaling in ‘Mid Risers’

- Evidence shows that p53 signaling mediates the response to fasting and regulates metabolic pathways such as lipid metabolism (PMID:24191950, 23954639, 27811061)
  - Many p53 target genes are reported to be regulated by fasting, which we observed in our data (although not the same genes)
    - PLK3, ARID3A, PTG2, SFN are transcriptionally targets of p53 which are implicated in DNA damage response leading to cell cycle and DNA repair processes
- ARID3A is upregulated by fasting in a mouse model of AML (PMID:27941793) and regulates cell cycle genes (22172947, 23659165)
  - ARID3A knockdown downregulated E2F genes and CDKN1A
- BTG2 has anti-proliferative properties that is p53-dependent and plays a role in DNA damage pathway (PMID:8944033,10657898)
  - Its gene expression is induced by fasting in liver of mice and ectopic BTG2 expression induces gluconeogenic genes, suggesting a role in metabolic regulation (PMID: 24647738)
  - Alternatively, BTG2 is associated with CCR-NOT complex—Stress stimulates complex recruitment and triggers mRNA degradation (PMID: 22416820)
    - Possible connection to the circulating protein ‘Early Risers’ cluster which supports the hypothesis of genomic stability due to fasting

‘SUPPRESSED THEN RISE’ GENES APPEAR TO HAVE PROTECTIVE PROPERTIES

- Although the ‘Suppressed then Rise’ cluster does not have any significant enrichment, a closer look at the genes points to several processes—apoptosis, DNA damage/repair, immune response, metabolism and mitochondrial signaling
  - A schema of potential relationships between these processes (PMID: 25909219)



SUMMARY OF RESULTS

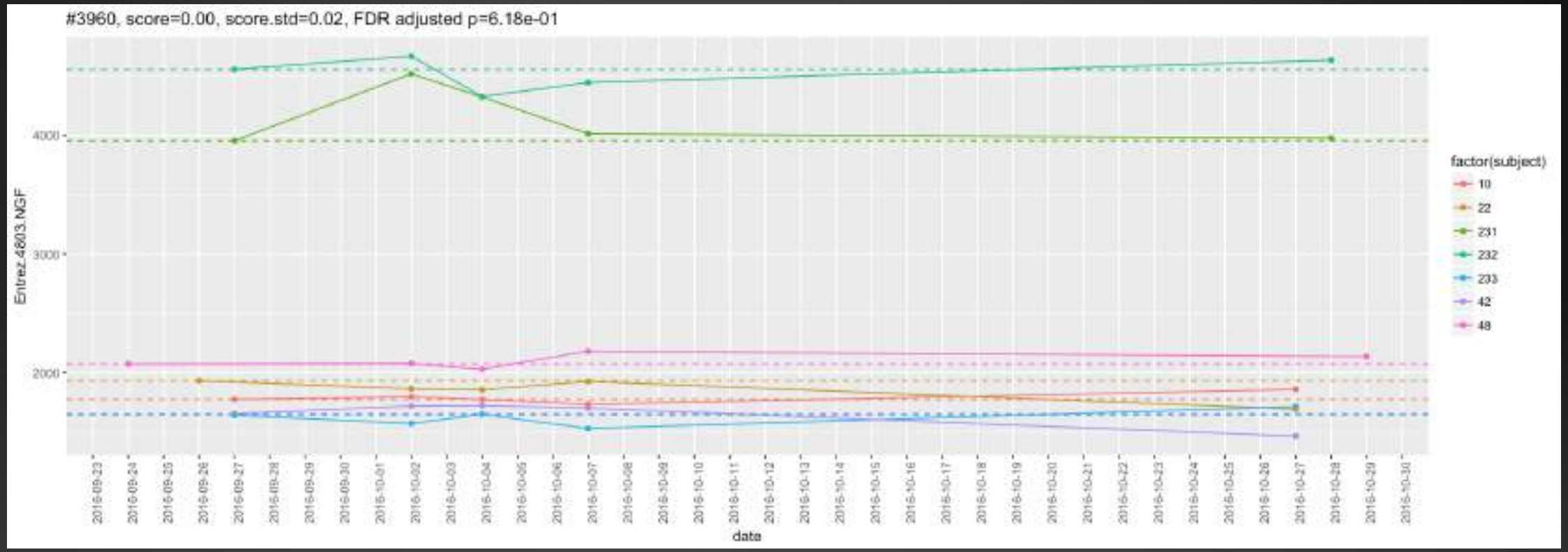
- Common fasting effects on the population fit currently understood models/aspects of fasting/starvation
  - Many vignettes of biology that are corroborated in the literature
- Subjects can be individually characterized based on baseline features that are outliers to the rest of the population
  - Individual subject circulating protein signatures are observed
  - Circulating protein uniqueness can be potentially be linked to DNA alterations and clinical phenotypes
- Observations of anecdotal individual differences in responses to fasting

Idealized Goal of Individual Characterization:

SUMMARY OF RESULTS, CONTINUED

- Based on common responses to fasting, potential fasting health implications identified (intervention potential and contra-indications; hypothetical)
  - Potentially indicated for patients suffering from:
    - Autoimmune disease
      - Fasting leads to sustained reduced systemic inflammation by reducing neutrophil, platelet and complement activation pathways and reducing levels of complement components C3 and C4 in blood routine
    - MDD, neuralgic pain
      - Fasting caused increased levels of TCA-type molecule (desipramine) in urine
    - High risk for CVD
      - Low levels of methylyglyoxal and increased levels of betaine upon fasting
  - Potentially increases capacity for DNA repair
  - Probably contraindicated for patients suffering from:
    - Gout and kidney disorders
      - Increased blood uric acid was observed in all 7, with 5 participants reaching levels above 8.5 mg/dL
    - Cholestasis (any condition in which substances normally excreted into bile are retained)
      - Signs of slowing bile flow: taurolithocholic acid and bilirubin were increased by fasting in all subjects, raising above normal upper limits in 5 of them
    - Hemophilia A (classic hemophilia) and von Willebrand
      - Fasting caused a significant drop on Factor VIII levels

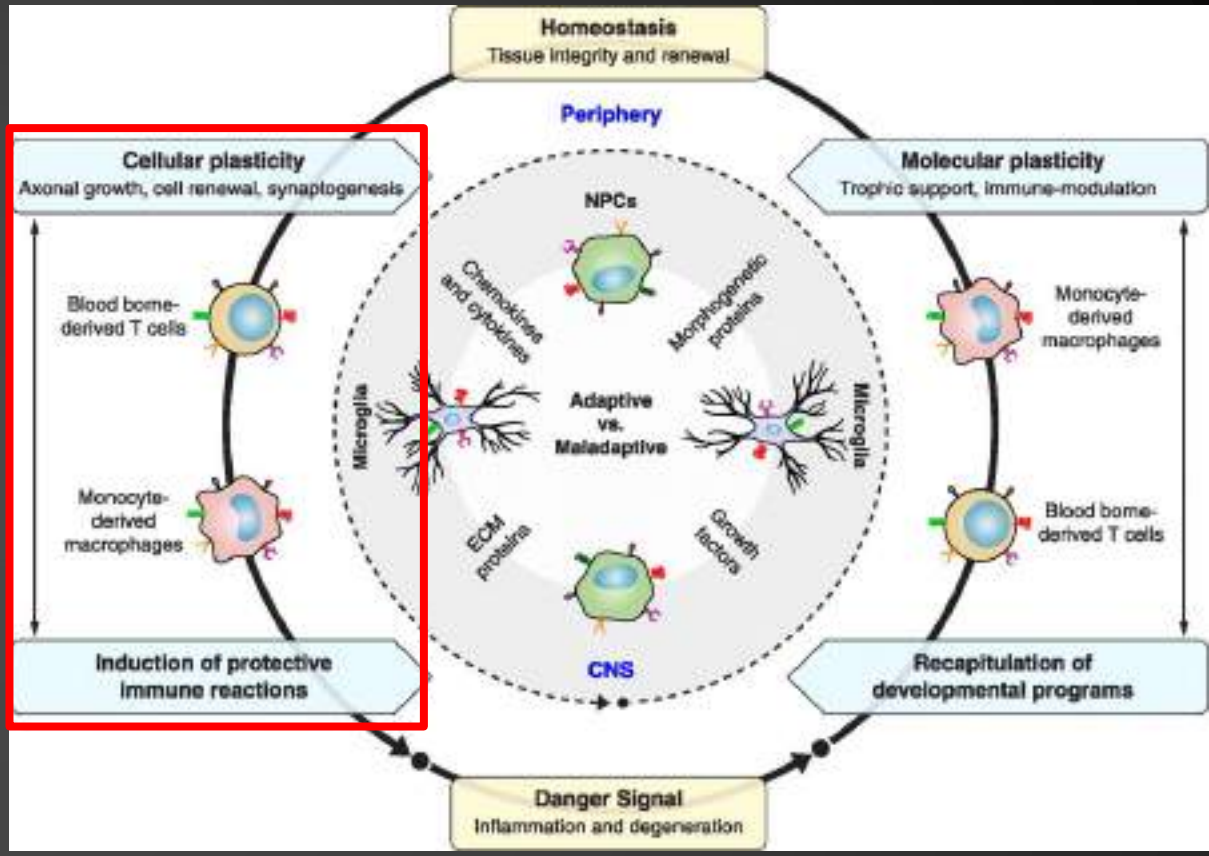
BASELINE (NOT NECESSARILY FASTING RELATED) OUTLIERS CAN BE ASSESSED



NGF for participant 231 indeed exhibits SNPs in their DNA for NGF and shares the same two SNPs with 232. However, participant 10 also has these.

PARTICIPANT #231 MAY DISPLAY A NEUROIMMUNE SIGNAL

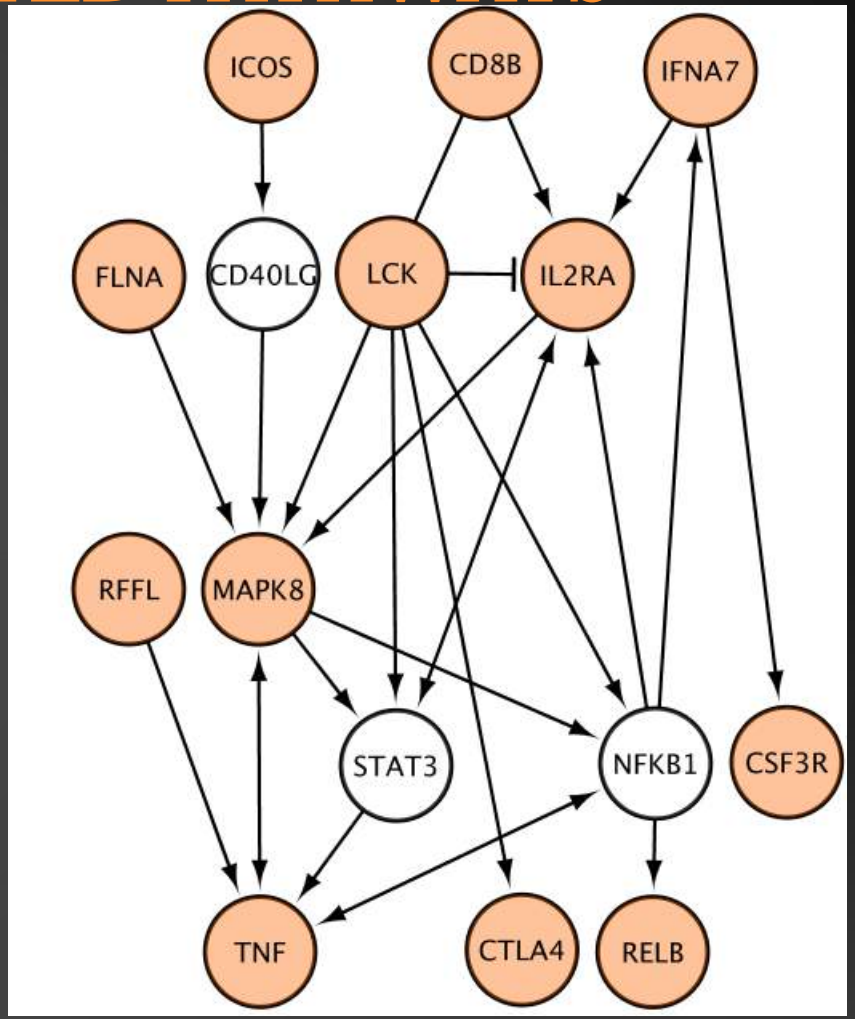
Data Modality	Strongest Outliers	Possible Meaning
Blood Metabolites	Cyclohexyl-amine	• A chemical intermediate in organic synthesis • an artificial sweetener cyclamate is derived from this • Used to manufacture synthetic chemicals such as corrosion inhibitors, insecticides, and plasticizers • Possible link to cardiovascular effects (increased blood pressure and heart rate)
Blood Circulating Proteins	147 proteins	• Enriched for axon guidance, cytokine-cytokine receptor interaction pathway, and infectious disease
Urine Metabolites	1-aminocyclopropane-carboxylic acid	• Synthesized from methionine; partial agonist to NDMA receptor that reduces neuronal damage (9016943, 9424023) • Treatment of this may have anti-depressant and procognitive (memory, recognition) properties (8982680, 25260339)
	L-Proline | D-Proline	• Synthesized from glutamate and converted to hydroxyproline (an indicator of collagen breakdown) (PMID:5765022, 10582130; 14975218) • Stress and anxiety correlated with urinary hydroxyproline and proline concentrations. (21483218)



- **Hypothesis:** Based on the circulating blood protein enrichment and urine metabolites, this participant could have been experiencing stress and anxiety, and his body was responding to this by increasing neuronal protection
  - Stress could trigger a neuroimmune response in which there are interactions between immune and nervous system to protect neurons (PMID: 26176590); neuroimmune signaling has been linked to depression (PMID: 26404713)

ENRICHMENT OBSERVED FOR IMMUNE DISEASES AND IMMUNE-RELATED PATHWAYS*

- T cell receptor signaling (ICOS, CTLA4, CD8B, LCK, TNF)
  - **ICOS**: Functions similarly to CD28 by enhancing T-cell responses to foreign antigens such as proliferation, lymphokine secretion, cell-cell interactions, and antibody secretion by B-cells (PMID: 9930702)
  - **CTLA4**: While CD28 and ICOS are co-stimulatory molecules that stimulate T-cell responses, CTLA4 mediates inhibition of T-cell function
- Other pathways and diseases includes osteoclast differentiation, cytokine-cytokine receptor interaction, systemic lupus erythematosus (SLE), and juvenile rheumatoid arthritis
  - High levels of circulating TNF in serum of rheumatoid arthritis patients compared to controls (PMID: 8457224)
    - However, healthy individuals can have different variations of serum circulating TNF levels (PMID: 21860543)
  - Increased ICOS expression in peripheral blood T cells of SLE patients (PMID: 15987711)



Orange = Extreme outlier
White = Not measured / not extreme outlier

*not all significant by FDR p<0.05

DISCUSSIONS