# Do fats fuel the fire?
Insights from metabolic challenges to highlight early markers of metabolic dysregulation

Prof. Helen Roche
    Prof Melissa Morine & Dr Colm O'Grada
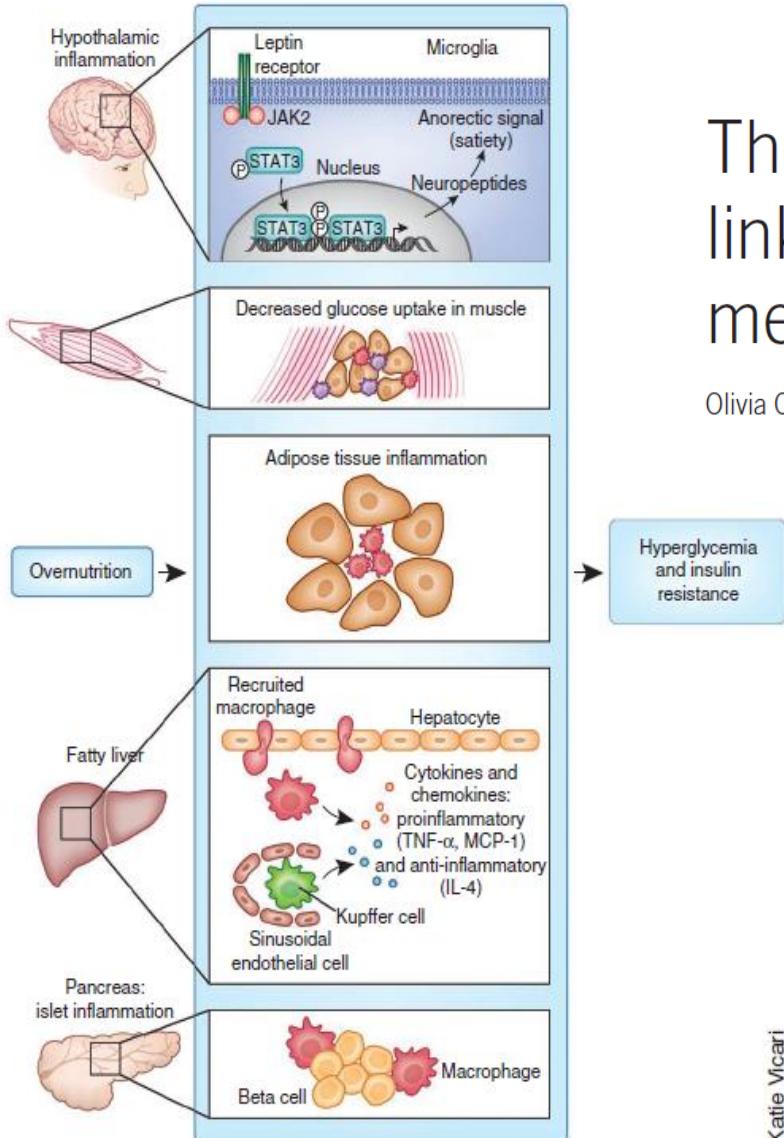
JINGO Symposium 15th November 2013

Obesity is a pro-inflammatory state

Macrophage infiltration into adipose tissue leads to insulin resistance and type 2 diabetes (T2D)

The cellular and signaling networks linking the immune system and metabolism in disease

Olivia Osborn & Jerrold M Olefsky
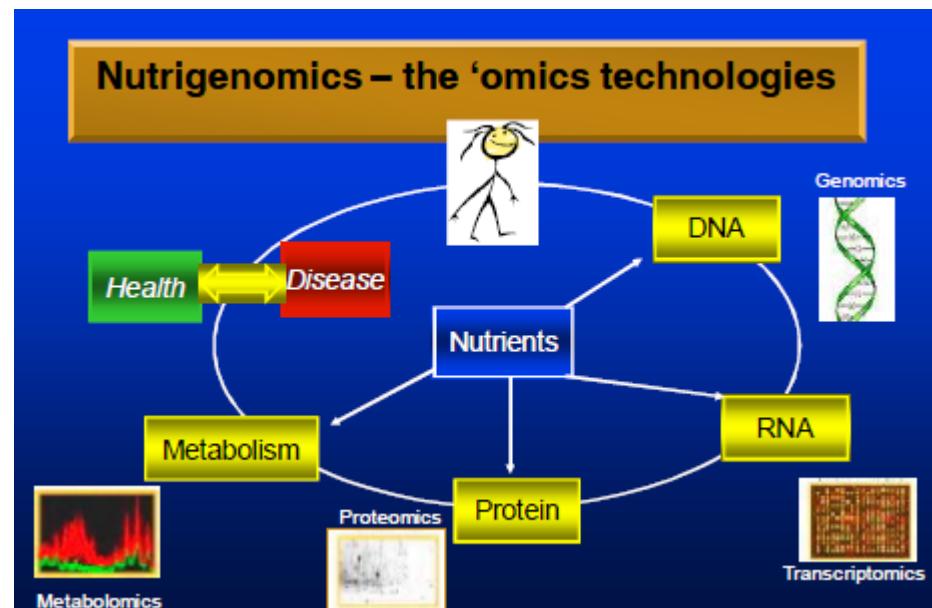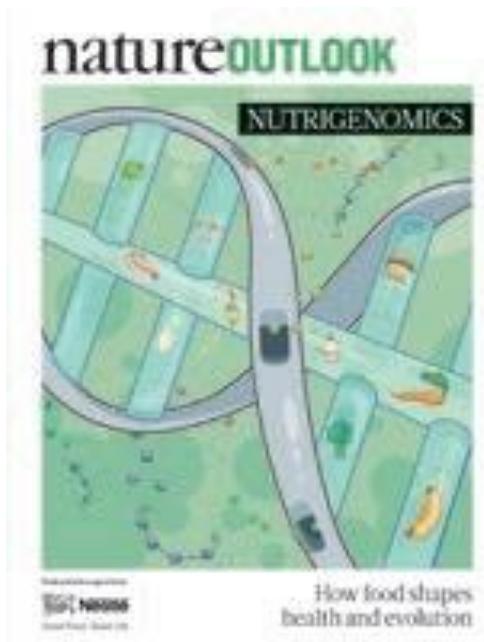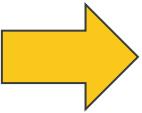
Figure 1 Schematic of integrative physiology. Nutrient overload activates inflammatory responses in adipose tissue, liver, skeletal muscle, pancreas and the hypothalamus, contributing to systemic insulin resistance and glucose intolerance. STAT3, signal transducer and activator of transcription-3; JAK2, Janus kinase-2.

New biomarkers of metabolic health
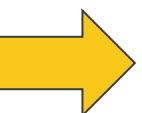
Nutrigenomics approaches
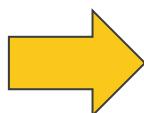
Transcriptomic Workflow

Isolate RNA

Run microarrays

Quantify results

Collect PBMC

Pathway/Network Analysis

Single Gene Analysis

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Overview

Defining new biomarkers of metabolic health.

How PBMC transcriptome reflects that of adipose?

How do 'physiological stressors' (age and BMI) modulate the response to metabolic stress?

Can this response be pulled back.........

Does fitness effect the PBMC transcriptome?

Defining new biomarkers of metabolic health

PBMCs reflect the immune component of the WAT transcriptome—Implications as biomarkers of metabolic health in the postprandial state

Colm M. O'Grada[1,2], Melissa J. Morine[1,3,4], Ciara Morris[2], Miriam Ryan[2], Eugene T. Dillon[1,2], Marianne Walsh[2], Eileen R. Gibney[2], Lorraine Brennan[2], Michael J. Gibney[2] and Helen M. Roche[1,2]

Scope: Food and nutrition studies often require accessing metabolically active tissues, including adipose tissue. This can involve invasive biopsy procedures that can be a limiting factor in study design. In contrast, peripheral blood mononuclear cells (PBMCs) are a population of circulating immune cells that are easily accessible through venipuncture. As transcriptomics is of growing importance in food and metabolism research, understanding the transcriptomic relationship between these tissue types can provide insight into the utility of PBMCs in this field.

Methods and results: We examine this relationship within eight subjects, in two postprandial states (following oral lipid tolerance test and oral glucose tolerance test). Multivariate analysis techniques were used to examine variation between tissues, samples, and subjects in order to define which genes have common/disparate expression profiles associated with highly defined metabolic phenotypes. We demonstrate global similarities in gene expression between PBMCs and white adipose tissue, irrespective of the metabolic challenge type. Closer examination of individual genes revealed this similarity to be strongest in pathways related to immune response/inflammation. Notably, the expression of metabolism-related nuclear receptors, including PPARs, LXR, etc. was discordant between tissues

Conclusion: The PBMC transcriptome may therefore provide a unique insight into the inflammatory component of metabolic health, as opposed to directly reflecting the metabolic component of the adipose tissue transcriptome.

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Table 1. Description of subjects. Subjects ($n = 8$; 4 male, 4 female) from the MECHE cohort that underwent paired adipose biopsy.

	MEAN	SE
Age (years)	38.88	4.46
Weight (kg)	97.66	4.51
BMI (kg/m^2)	34.50	1.91
WHR	0.97	0.05
Fasting glucose (mmol/L)	5.23	0.20
Fasting TAG (mmol/L)	1.18	0.24
Fasting total cholesterol (mmol/L)	4.66	0.32
Post-OGTT glucose (mmol/L)	6.93	1.01
Post-OLTT TAG (mmol/L)	1.96	0.48

Post-OLTT time point is 240 min post-OLTT and post-OGTT time point is 60 min post-OGTT. WHR, waist-to-hip ratio.
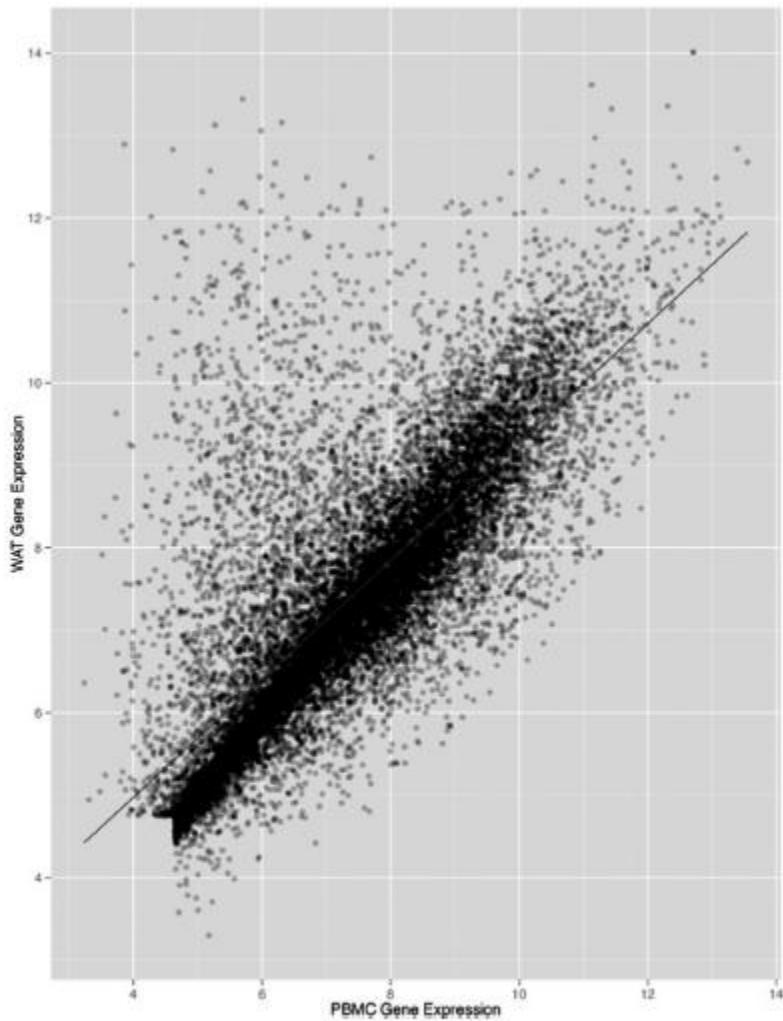
Figure 1. Correlation between WAT and PBMC gene expression across 16 039 filtered genes present on the microarray ($\rho = 0.763$, $p < 2.2 \times 10^{-16}$, $n = 16$ arrays per tissue).

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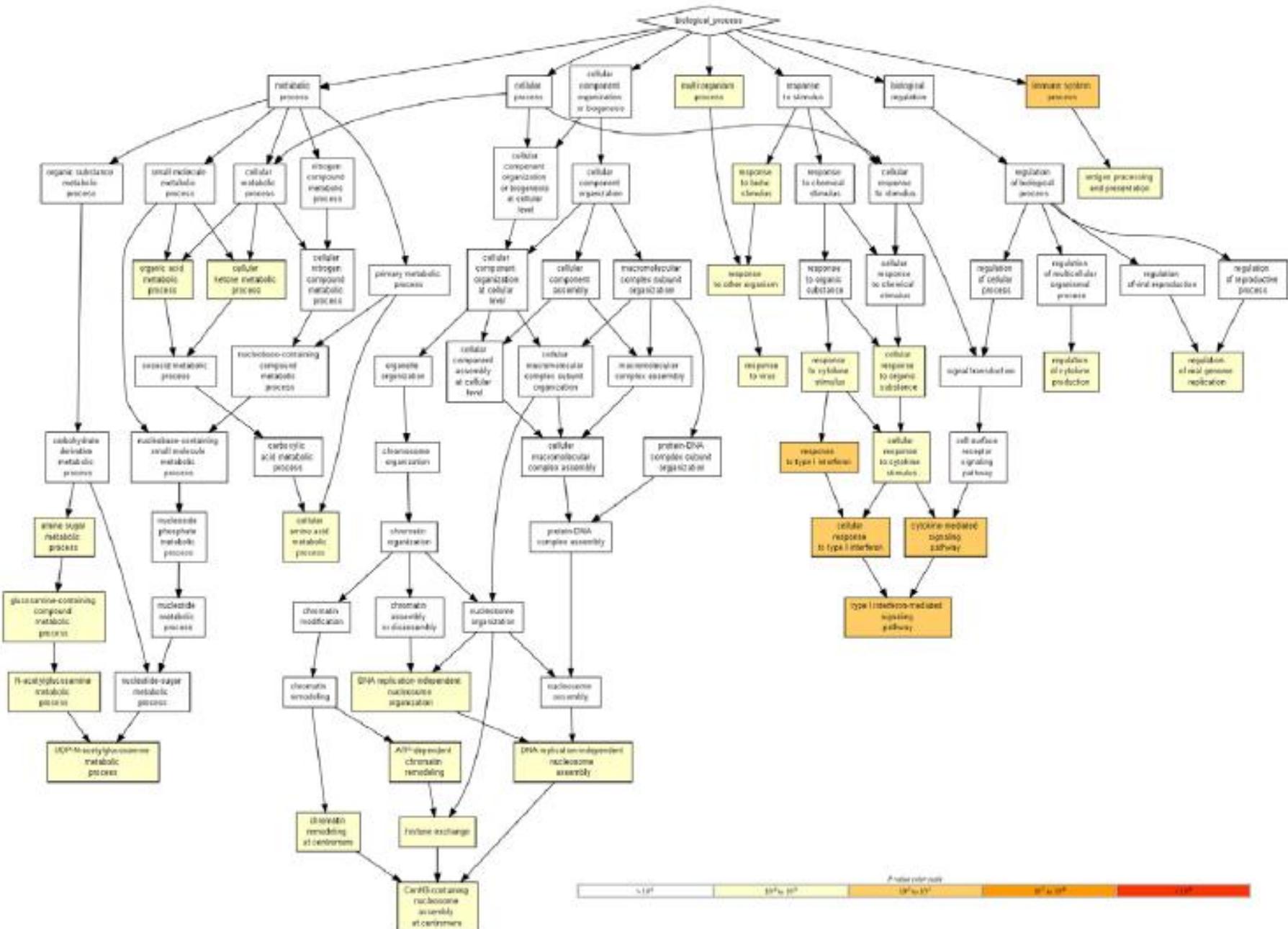
Figure 2. Enriched GO processes present in maximally correlated WAT and PBMC genes. GO biological processes overrepresented in a ranked list of genes, sorted by correlation between PBMC and WAT expression.

Table 4. Description of clusters. Differences between clusters as identified by co-inertia analysis.

	Cluster A		Cluster B		
	MEAN	SE	MEAN	SE	*p*
Age (years)	49	3	28.8	3.9	**0.0064**
Weight (kg)	97.24	1.49	98.09	9.63	0.9331
BMI (kg/m^2)	34.82	1.55	34.18	3.81	0.8809
WHR	0.95	0.06	0.99	0.08	0.645
Fasting glucose (mmol/L)	5.55	0.28	4.9	0.17	0.0947
Fasting TAG (mmol/L)	1.69	0.28	0.67	0.13	**0.0174**
Fasting total cholesterol (mmol/L)	5.3	0.41	4.03	0.22	**0.0325**

$n = 4$ per cluster (2 male, 2 female in each). *p* values in bold represent significantly different ($p > 0.05$) values between clusters. WHR, waist-to-hip ratio.
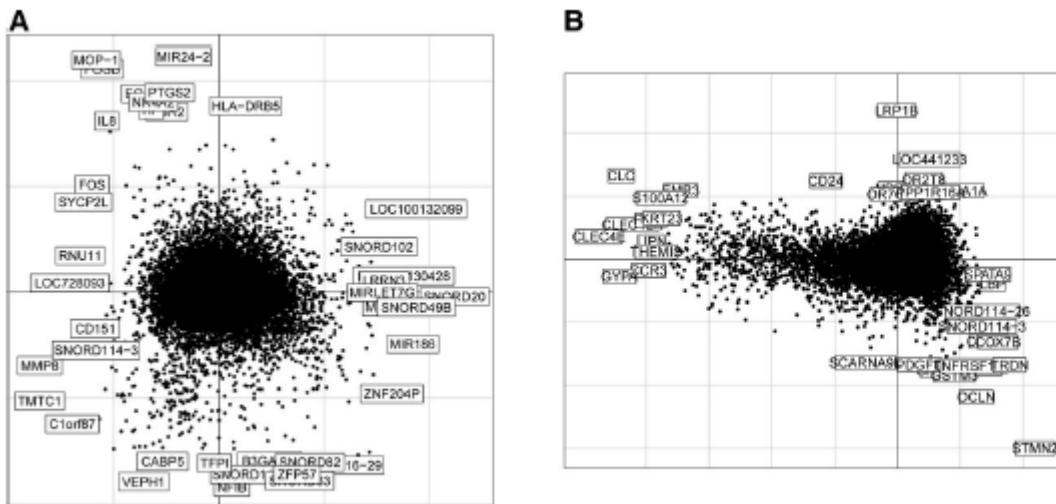
Figure 4. Genes most associated with each axis in each dataset. Labeled genes represent the top ten genes associated with each axis in each direction, in the 2-D NSC space for each dataset following co-inertial analysis; (A) PBMC genes, (B) WAT genes.

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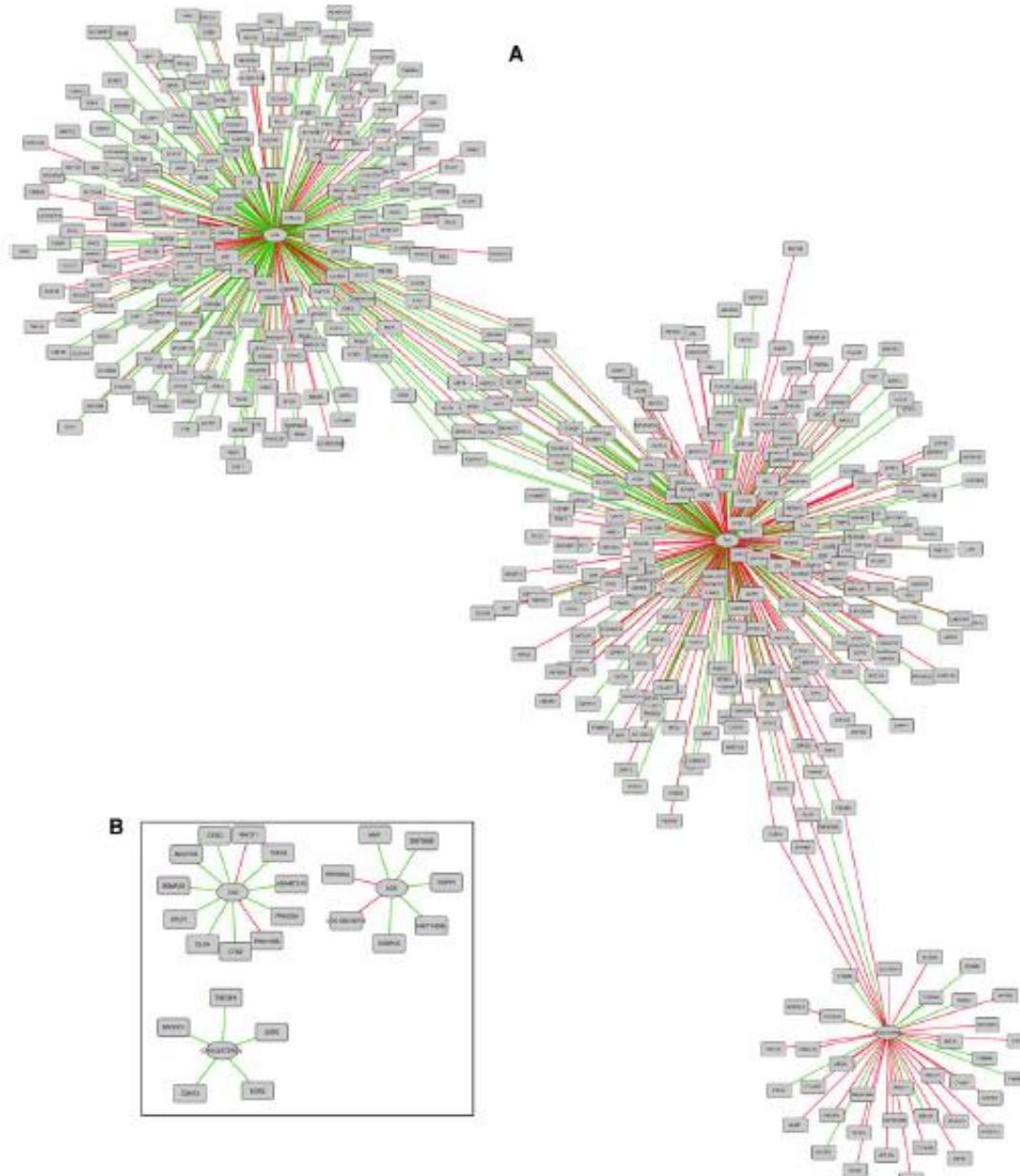
Figure 5. Regularized canonical correlation analysis of PBMC and WAT genes expression with phenotypic variables. (A) PBMC genes with expression associated with age, total cholesterol, and fasting TAG. (B) WAT genes with expression associated with age, total cholesterol, and fasting TAG. Green indicates positive correlation, red indicates an inverse correlation. A cutoff of $r - 0.8$ was used for correlation values in this graph.

Overview

Defining new biomarkers of metabolic health.

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How do 'physiological stressors' (age and BMI) modulate the response to metabolic stress?

Can this response be pulled back.........

Does fitness effect the PBMC transcriptome?

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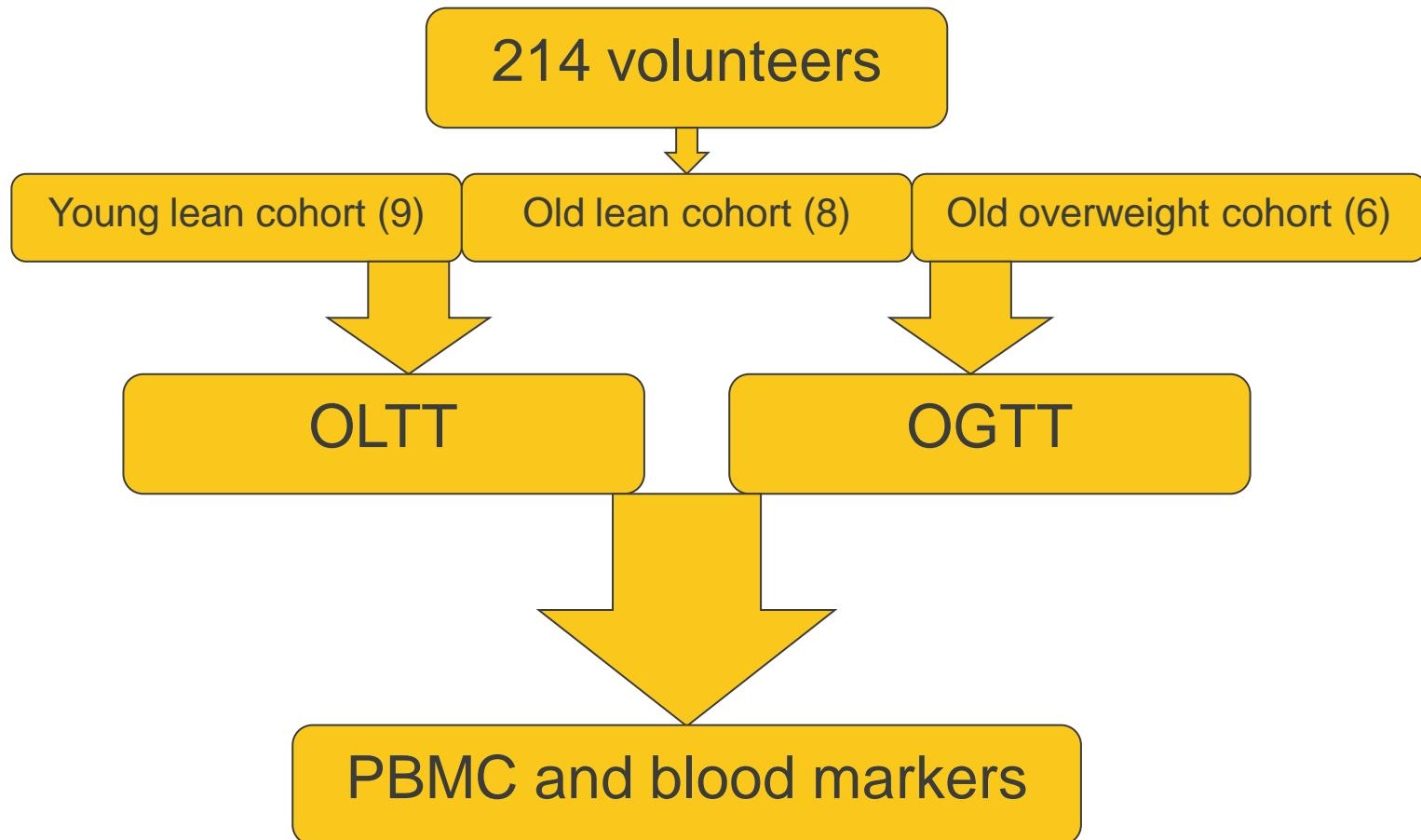
Part Two:
Impact of age & weight on OLTT v OGTT

214 volunteers

Young lean cohort (9) | Old lean cohort (8) | Old overweight cohort (6)

OLTT | **OGTT**

PBMC and blood markers

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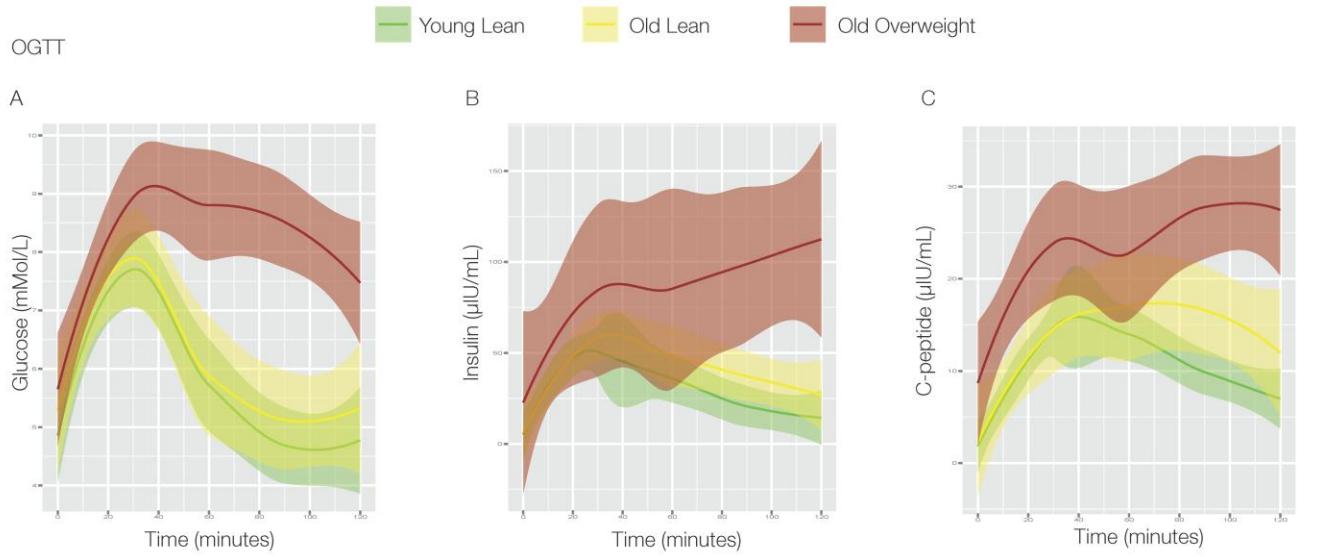
Cohorts

	Young Lean		Old Lean		Old Overweight		P
	MEAN	SE	MEAN	SE	MEAN	SE	
Age (years)	25	1.21	48	3.61	49	2.23	< 0.0001
Weight (kg)	73.79	2.93	72.55	5.09	108	7.17	0.0002
BMI (kg/m^2)	23.45	0.77	24.8	1.3	36.8	2.8	< 0.0001
Body Fat %	13.82	1.25	26.07	3.17	44.72	4.43	< 0.0001
TAG (mmol/L)	1.01	0.12	1.06	0.21	1.77	0.24	0.0364
Glucose (mmol/L)	4.78	0.09	4.85	0.12	5.52	0.18	0.0013
Cholesterol (mmol/L)	3.92	0.19	5.03	0.48	5.4	0.49	0.0267
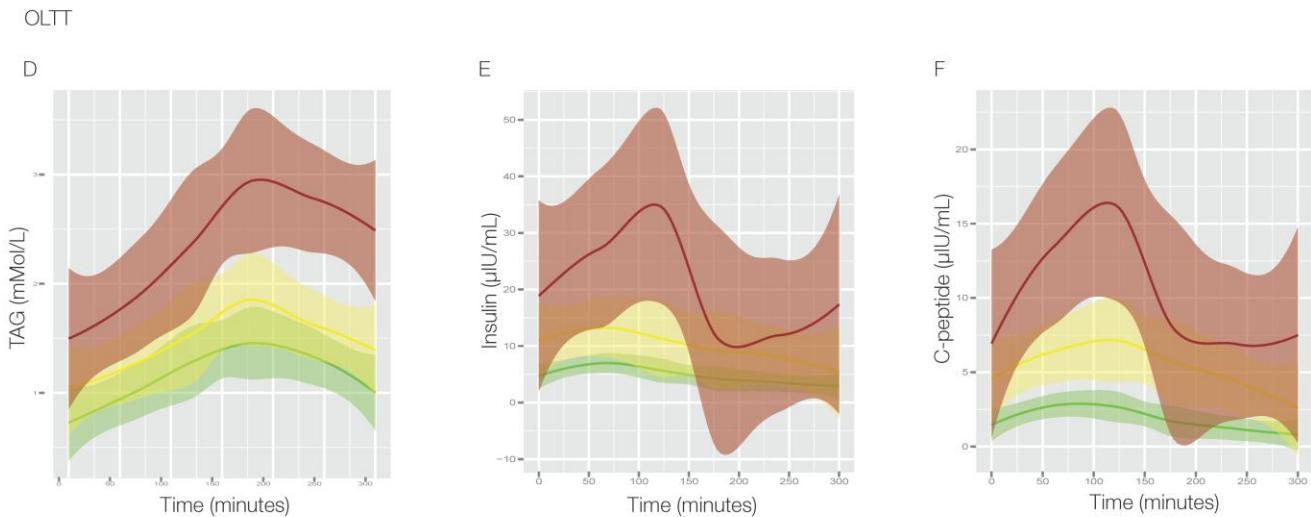
Different metabolic response

OGTT
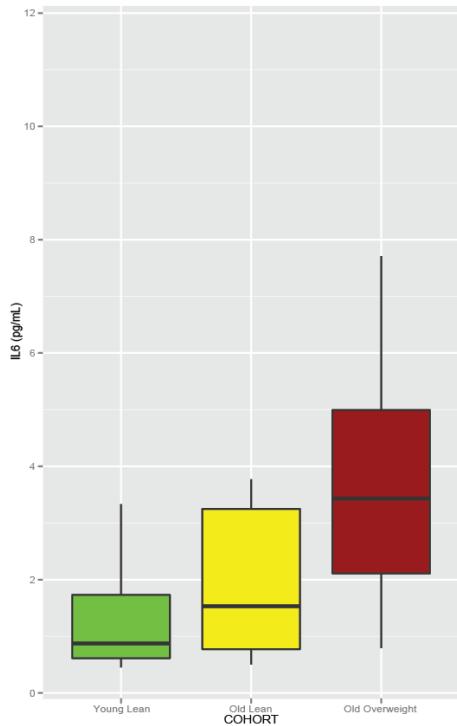
OLTT
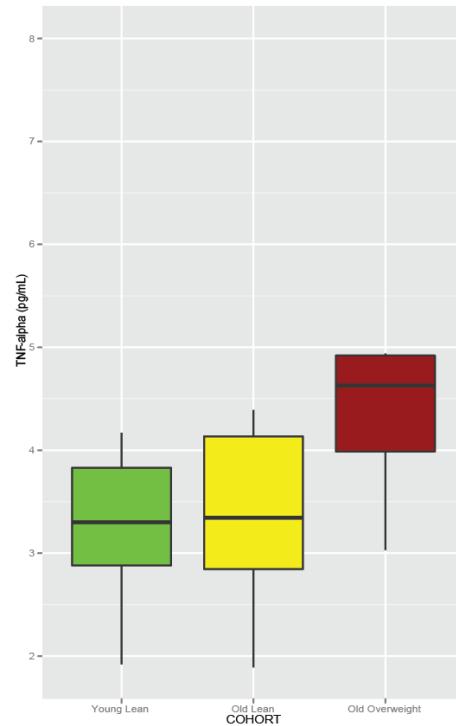
Inflammatory responses

Fasting IL-6   Fasting TNFα   Fasting IL-10
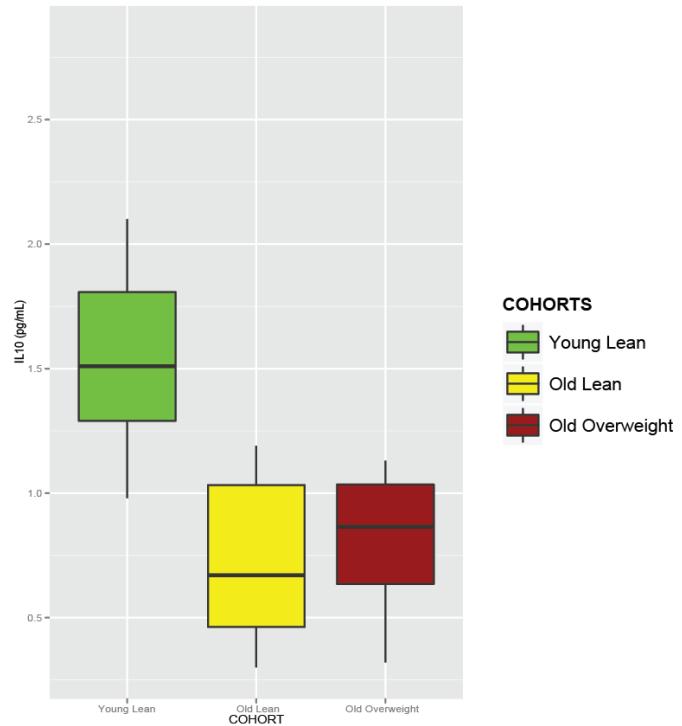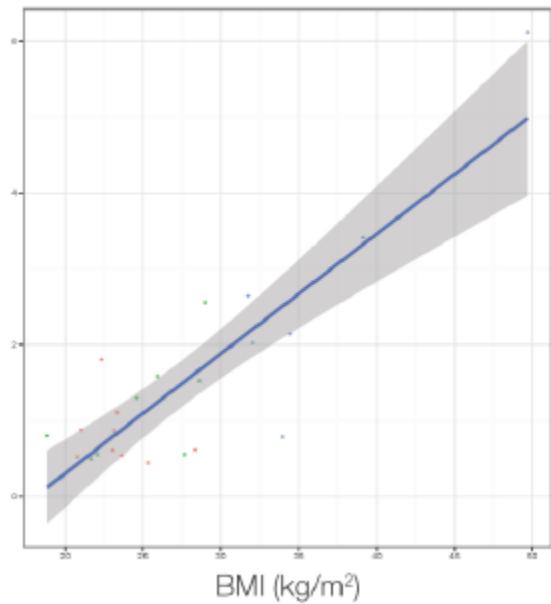
$P = 0.000874$ $P = 0.0443$ $P = 0.00724$
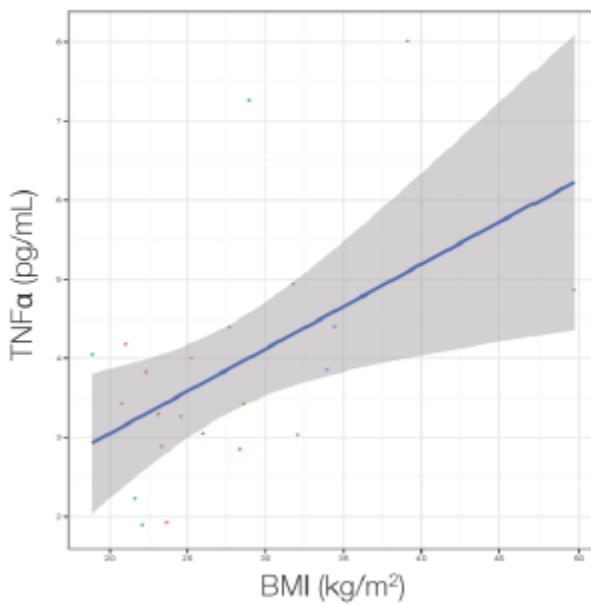
Inflammatory responses – according to BMI and age
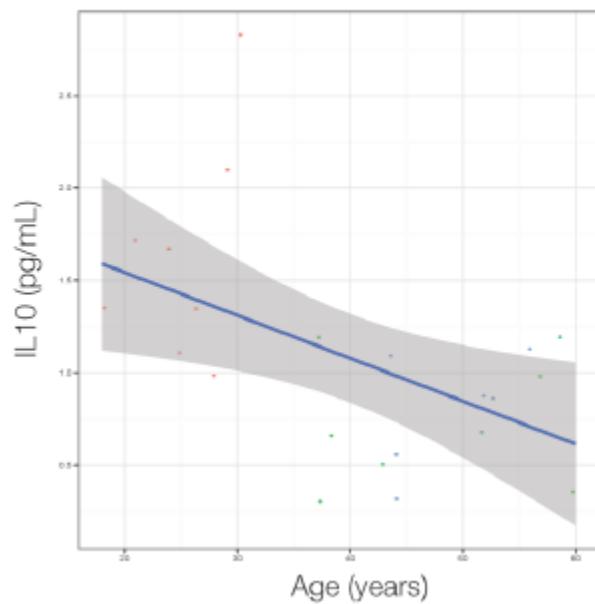
A
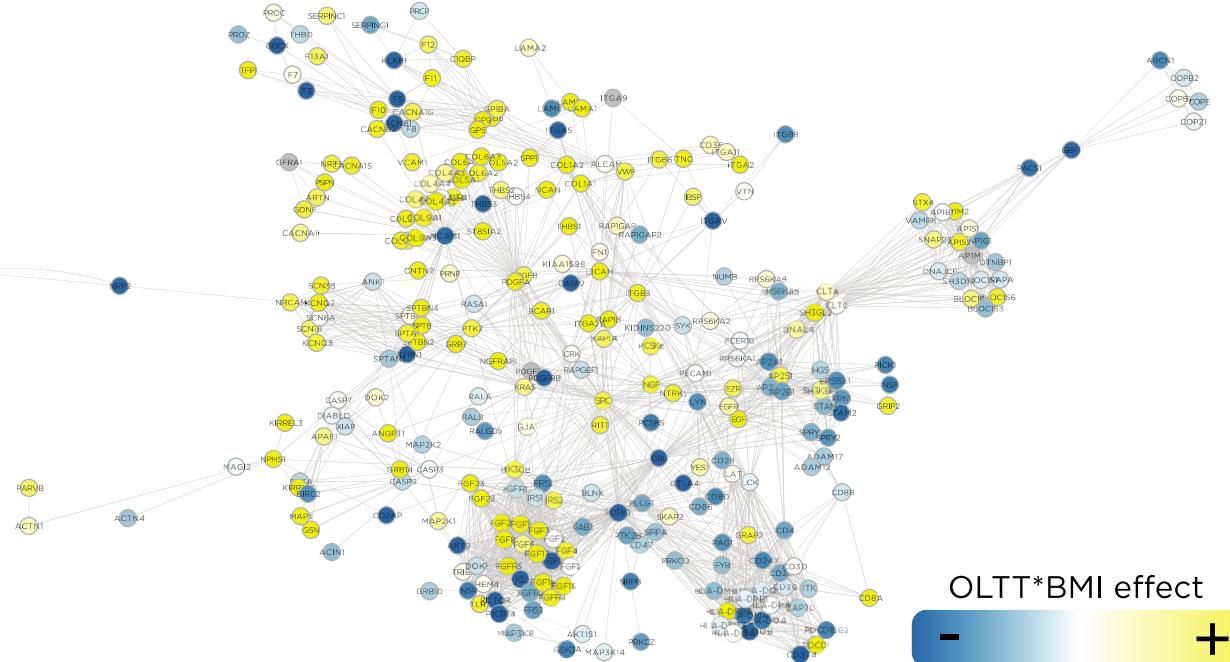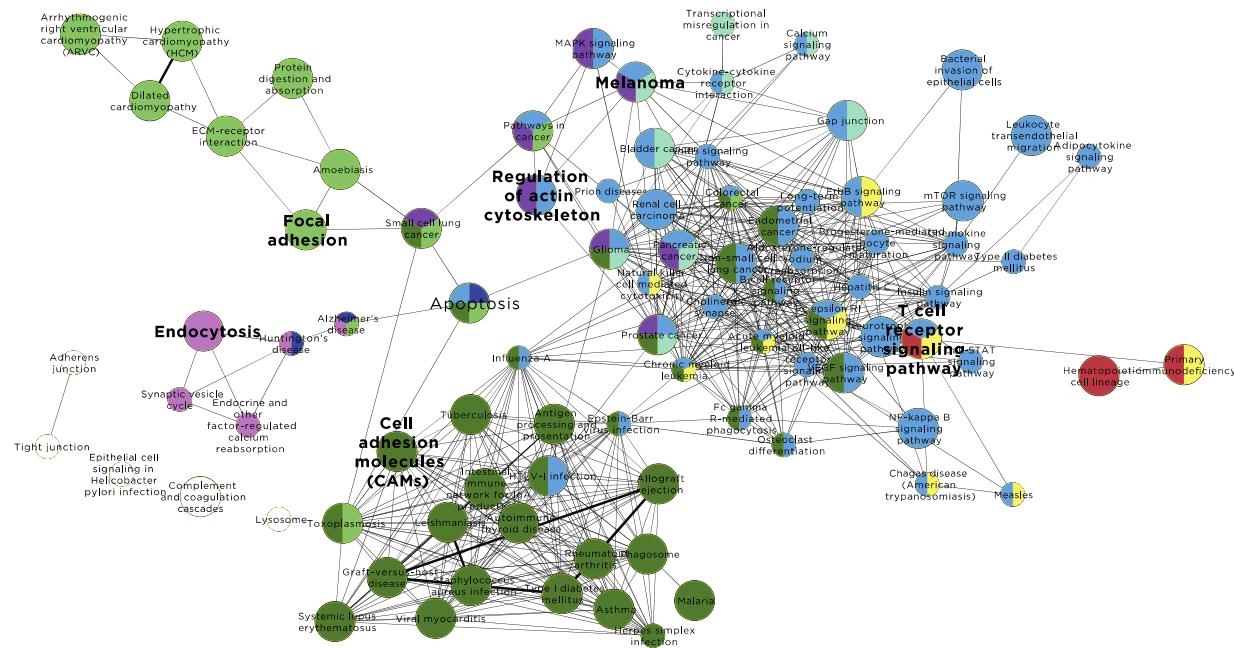
OLTT*BMI effect

− +

B

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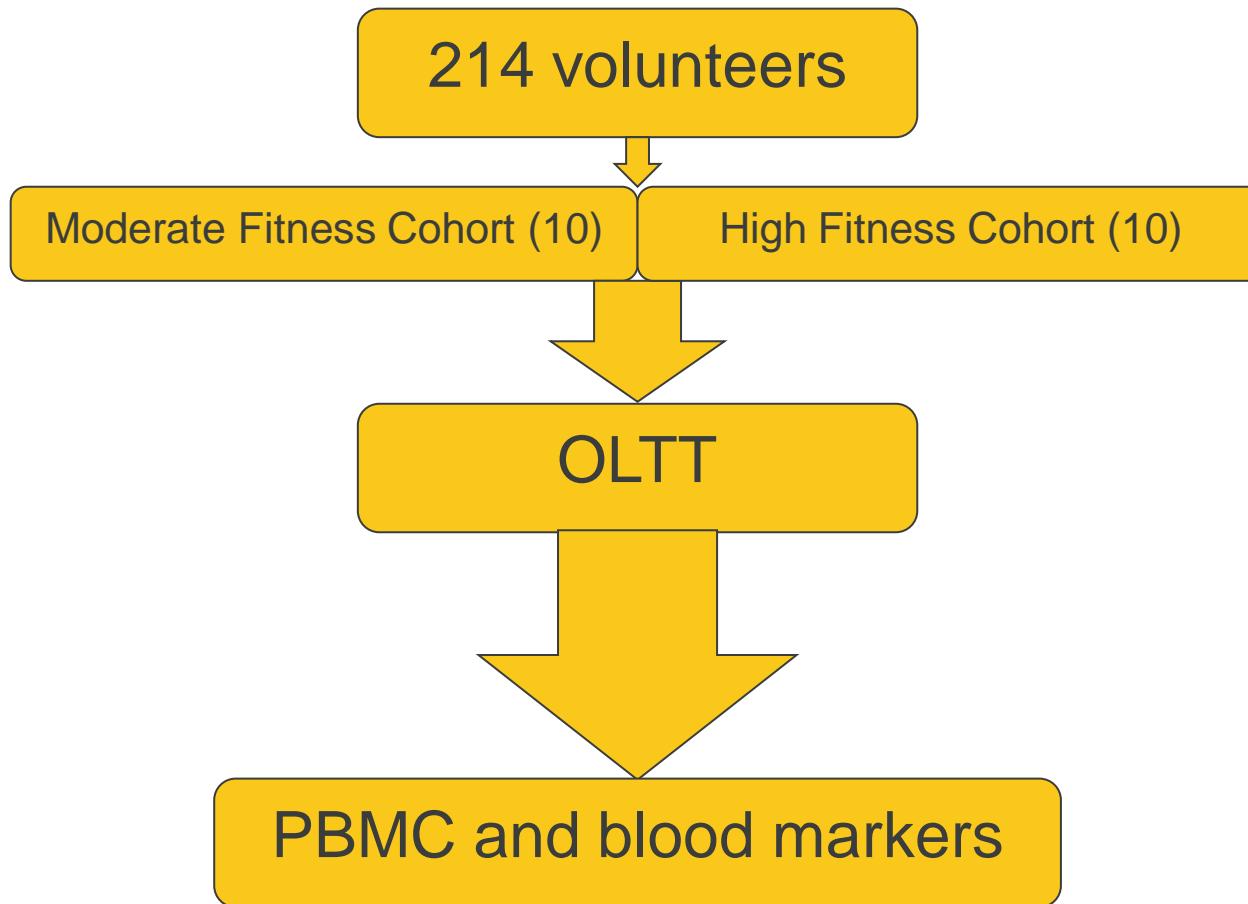
Part Three:

Fitness levels, OLTT and the PBMC transcriptome

214 volunteers

Moderate Fitness Cohort (10)   High Fitness Cohort (10)

OLTT

PBMC and blood markers
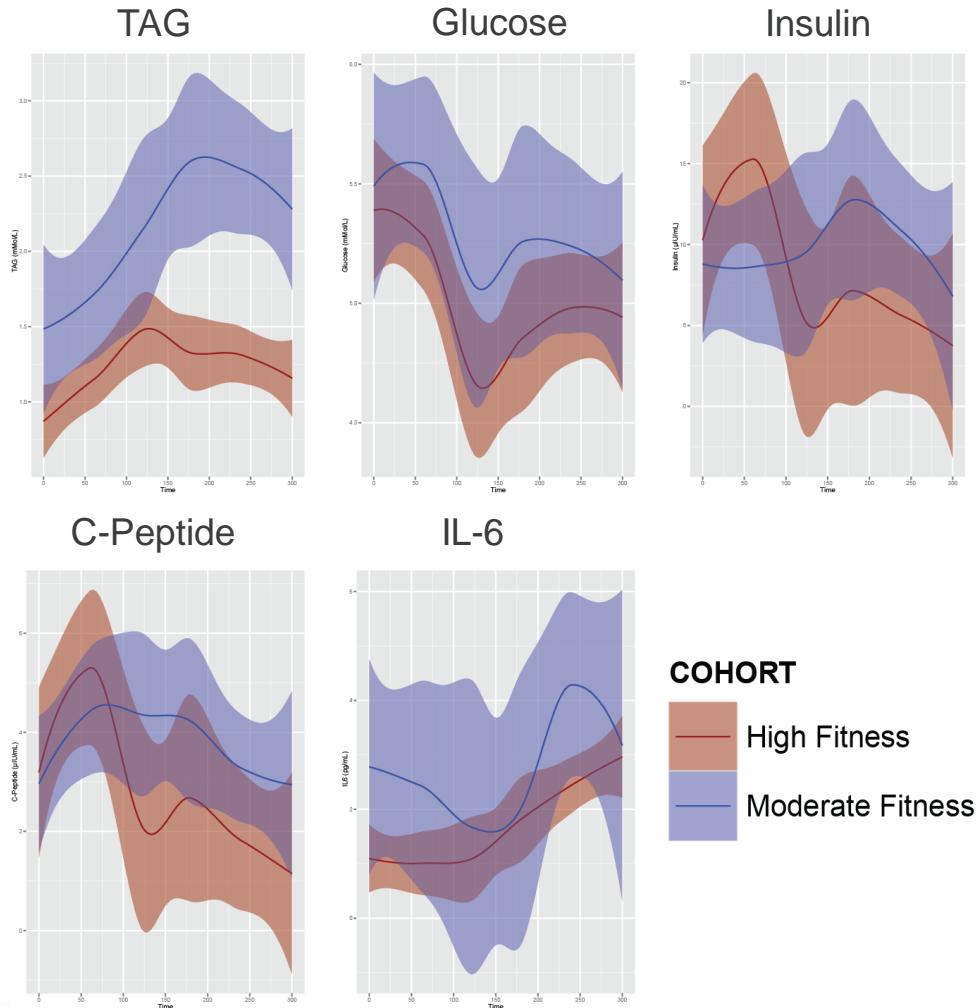
Fitness cohorts

	Moderate Fitness		High Fitness		P
	MEAN	SE	MEAN	SE	
VO$_2$ Max/FFM	42.49	3.14	72.04	1.03	< 0.0001
Age (years)	41.51	4.57	32.70	3.17	0.216 (ns)
Weight (kg)	91.82	5.6	81.84	3.74	0.165 (ns)
BMI (kg/m^2)	29.00	1.78	25.77	0.99	0.154 (ns)
Body Fat %	24.78	3.43	17.46	2.59	0.138 (ns)
TAG (mmol/L)	1.39	0.23	0.87	0.12	0.067 (ns)
Glucose (mmol/L)	4.97	0.21	4.85	0.16	0.673 (ns)
Cholesterol (mmol/L)	5.25	0.35	4.54	0.36	0.165 (ns)

Fitness related metabolic response

	COHORT	TIME	INTERACTION
TAG	0.02	0.005	0.29 (ns)
Glucose	0.47 (ns)	0.032	0.54 (ns)
Insulin	0.43 (ns)	0.0005	0.006
C-Peptide	0.83 (ns)	0.0001	0.014
IL6	0.017	0.0002	0.058 (ns)

Fitness PBMC Transcriptome – OLTT Response
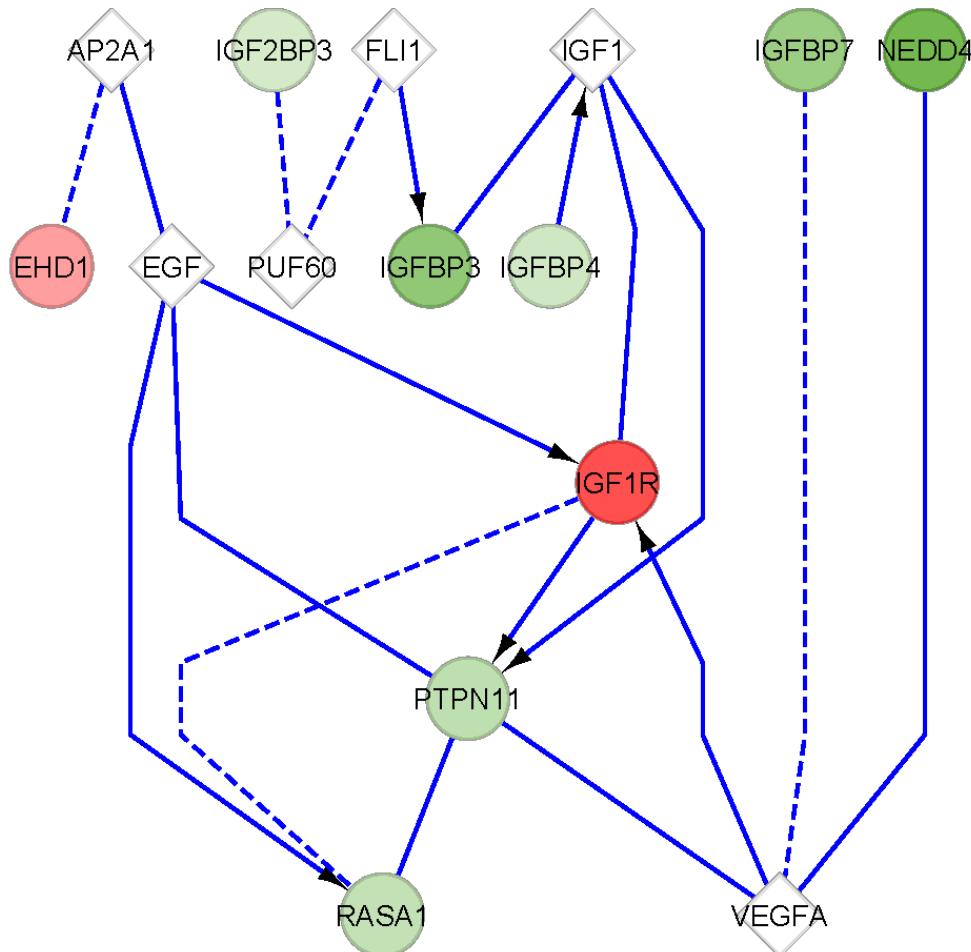
Moderate Fitness	High Fitness
KLF9	FKBP5
CCNG2	ZBTB16
C1orf220	TSC22D3
ZBTB16	C11orf44
C9orf106	OPN1SW
SAP30	PTRF
IRS2	ZNF763
LOC149837	SMAP2
FKBP5	NEUROD1
TBC1D29	NXF3
ZBTB34	CDC42EP2
SERPINA6	AREG
TPST1	AWAT1
ALAS1	PER1
DEFB105A	ZBTB8B
OR2Y1	TMEM82
HSP90AA6P	COMMD3
DDIT4	KLF9
PHC2	LONRF1
PER1	PFDN5
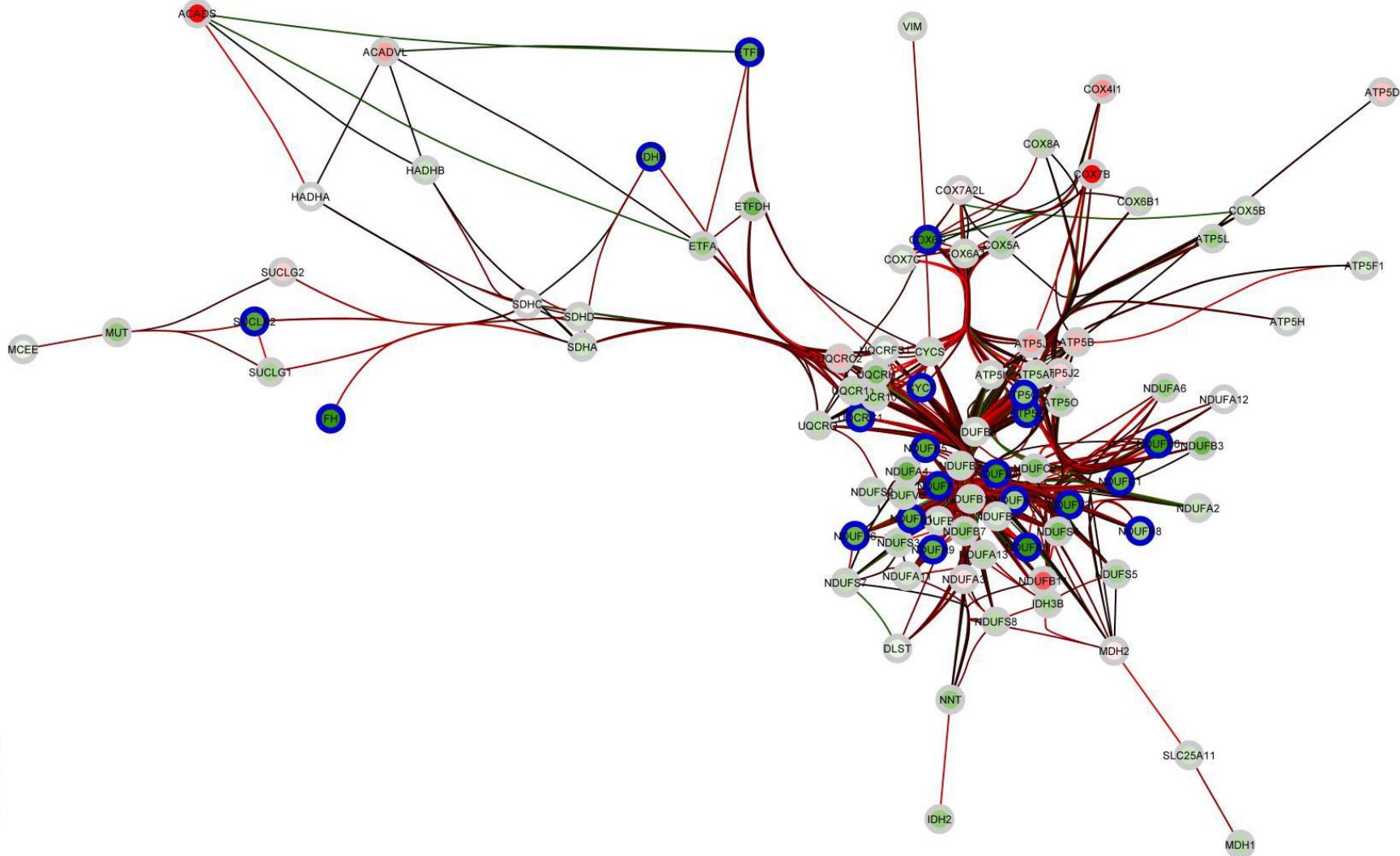
IGF-1 related gene expression down with fitness
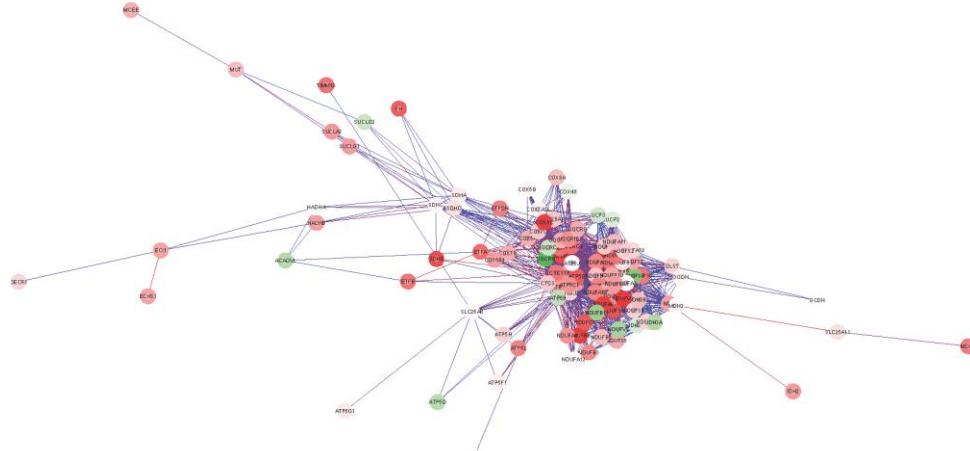
Bi-directional network analysis
- down-regulation of ATP associated genes with fitness

Network analysis between fitness groups

| GO Biological Process	FDR P-value
generation of precursor metabolites and energy	1.11E-120
cellular respiration	1.64E-94
electron transport chain	2.55E-84
energy derivation by oxidation of organic compounds	3.82E-84
oxidative phosphorylation	1.04E-81
respiratory electron transport chain	1.95E-66
ATP synthesis coupled electron transport	9.26E-65
mitochondrial ATP synthesis coupled electron transport	9.26E-65
oxidation reduction	8.73E-63
mitochondrial electron transport, NADH to ubiquinone	1.03E-58
phosphorylation	2.49E-31
aerobic respiration	4.08E-29
phosphate metabolic process	2.89E-27
phosphorus metabolic process	2.89E-27
acetyl-CoA catabolic process	3.22E-24
tricarboxylic acid cycle	3.22E-24
coenzyme catabolic process	4.98E-23
cofactor catabolic process	1.88E-21
acetyl-CoA metabolic process	1.88E-21

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Summary....
Future Perspectives

- OLTT induces a more rigorous metabolic challenge, PBMC transcriptome is affected to the greatest extent which extends plasma biomarkers

- PBMC transcriptome accurate biomarker of adipose inflammatory biology

- Both age and BMI amplify the metabolic response to OGTT and OLTT
  - PBMC transcriptome shows differential regulation of genes related to metabolism, inflammation and circadian rhythm

- Modulate the PBMC transcriptome with different fitness levels
  - Display a variation in metabolic and inflammatory response to OLTT
  - Fasting PBMC transcriptome markedly different in relation to metabolism and energy regulation

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Summary....
Future Perspectives

- Limited by numbers............

- Gender imbalance..........
  - Extension within the context of FIRM 2013

- Joint Programming Initiative – Healthy Life Healthy Diet

- Molecular Nutrition Food Research Review

Nutritional aspects of metabolic inflammation in relation to health – insights from transcriptomic biomarkers in PBMC of fatty acids and polyphenols.
Lydia Afman, Dragan Milenkovic, Helen M Roche
Wageningen University, The Netherlands & INRA Clermont-Ferrand, France

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Mr Eugene Dillon	Ms Tracey Claxton
Dr. Marianne Walsh	Ms Clare Horan