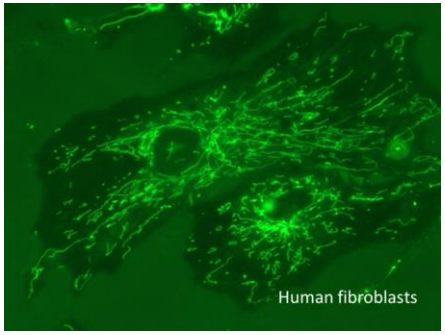
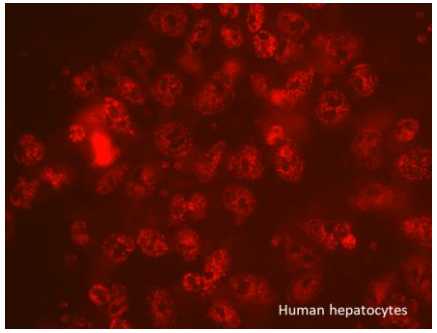
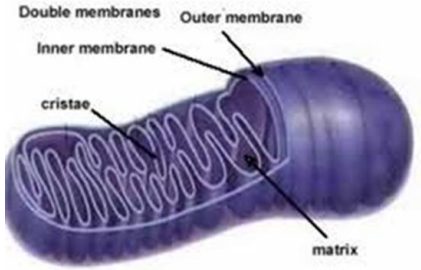
Developments in Understanding the Science Behind ME/CFS

Karl Morten
Nuffield Dept of Women's and Reproductive Health
Auckland Dec 12th 2018







“Who is Dr Karl Morten?”

1991	1996	2001	2003	2005	2018

Mitochondria disease

Leighs disease. Models of mito oxidative stress

mtDNA variants and diabetes

Mouse models of mito oxidative stress (USA)

Mito oxidative stress & diabetes

Group Leader (NDOG)

90 peer reviewed papers and chapters.

2 on ME/CFS

H index 22

BBSRC case award

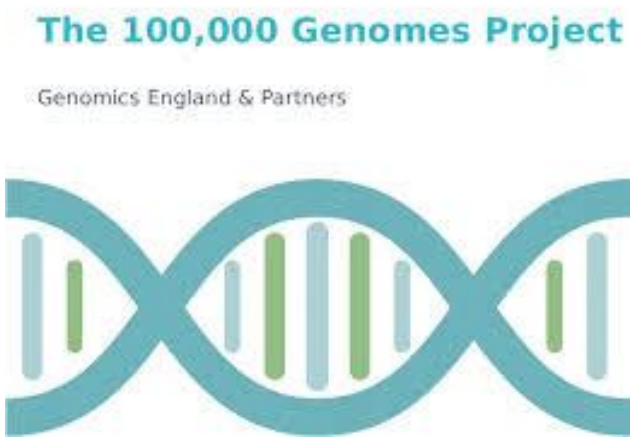
What has changed in research over the last 30 years?

Next generation DNA sequencing

UK Biobank Edinburgh (-80oC freezer)

Disease associated mutations

1990-2003.
13 years, Global project.
Single genome
cost £2 billion(£2000,000,000)



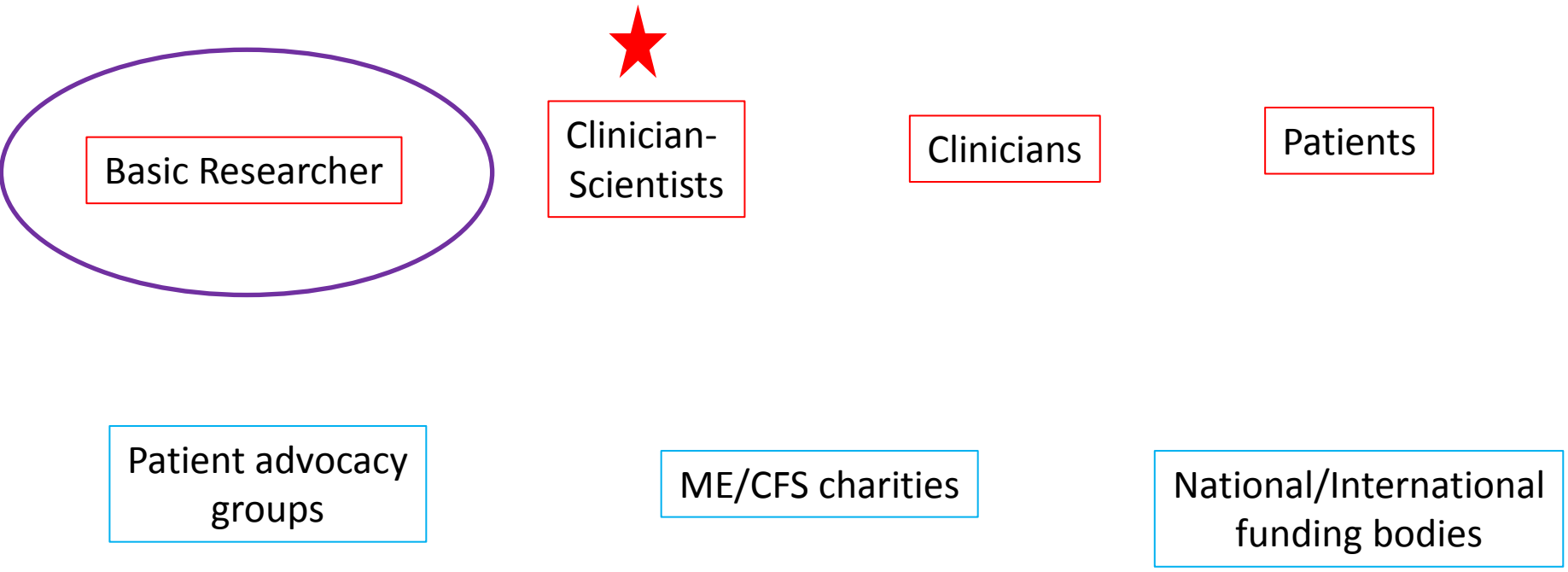
2013-2018
5 years, 100,000 genomes
Genomics England
1 genome sequence takes 30 minutes
and costs £600
1 million sequences in the next 5 years

What does this mean for ME/CFS patients?

- The 100,000 Genomes project focused on cancer and rare genetic disorders

- Next step is to study more complex diseases and we need to get ME/CFS in the mix by increasing our understanding of the illness!!!

Crucial working relationships in medical research



The biggest challenge in ME/CFS is to get everybody to work together!

Development of a new drug

Identify a therapeutic target

Test tool compounds in vitro and in animal models

Manage the condition or cure?

Screen compound libraries & testing (£100 million plus)

Clinical trials £'s?

Disease management

Metformin

Front line treatment for type II diabetes.
439 million people affected by 2030
Glucose lowering drug.

French Lilac

Guanidine

Metformin

"In medieval times, a prescription of *Galega officinalis* was used in folklore medicine to relieve the frequent urination accompanying the disease that is today known as diabetes mellitus.

Establishing cause or effect is an issue for all chronic diseases

Alzheimer's disease

Age of onset

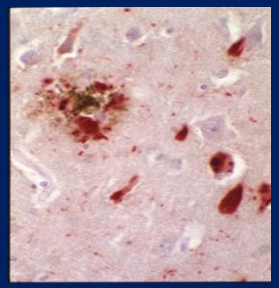
Treatment

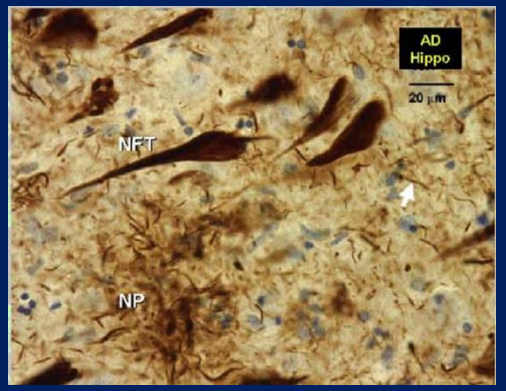
70 80 yrs

Dementia

Amyloid plaques

Tau tangles





Hippocampal pathology

To stop or prevent a disease you need to hit the right target(s) !

Insomnia

Orthostatic Intolerance

IBS

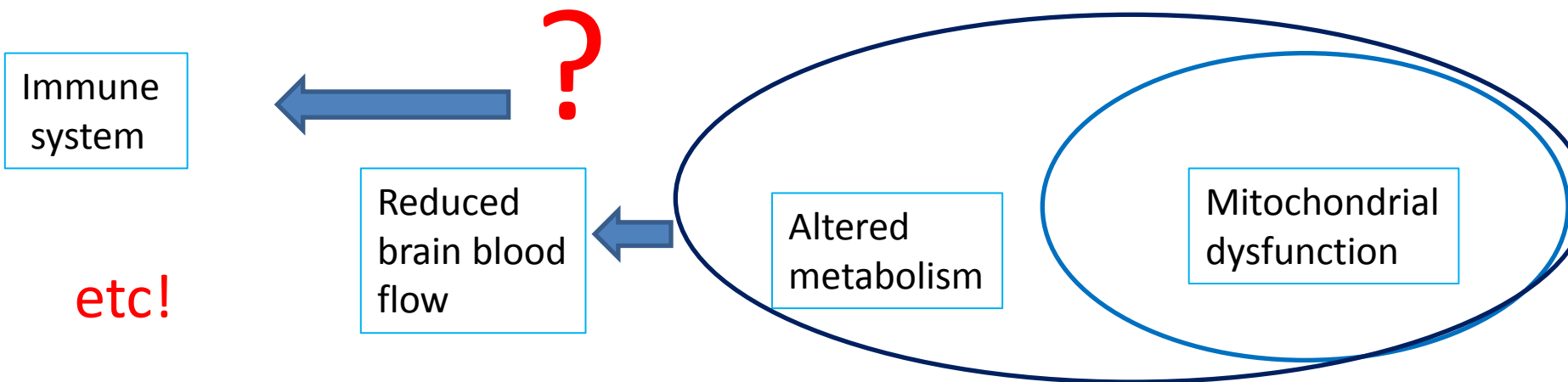
Hypersensitivity

Fatigue

Brain fog

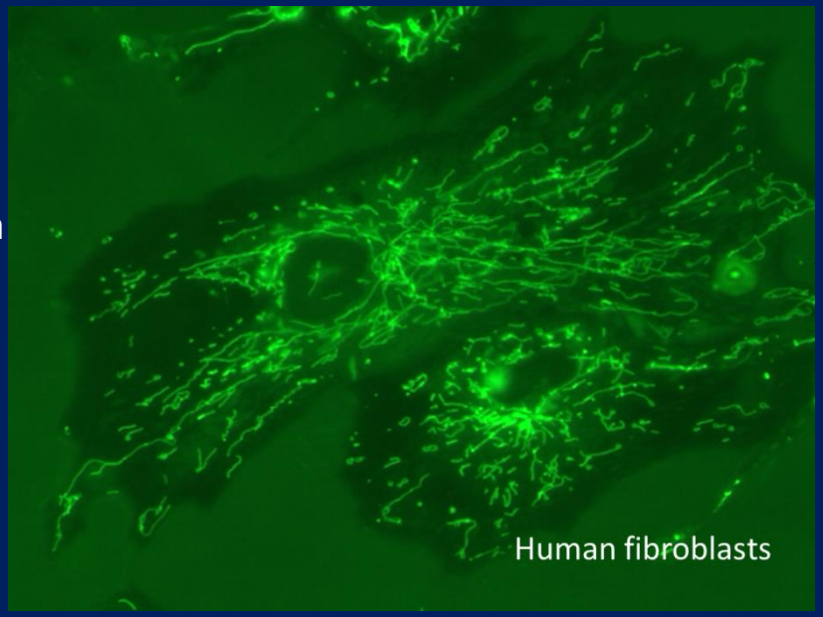
Post-exertional malaise

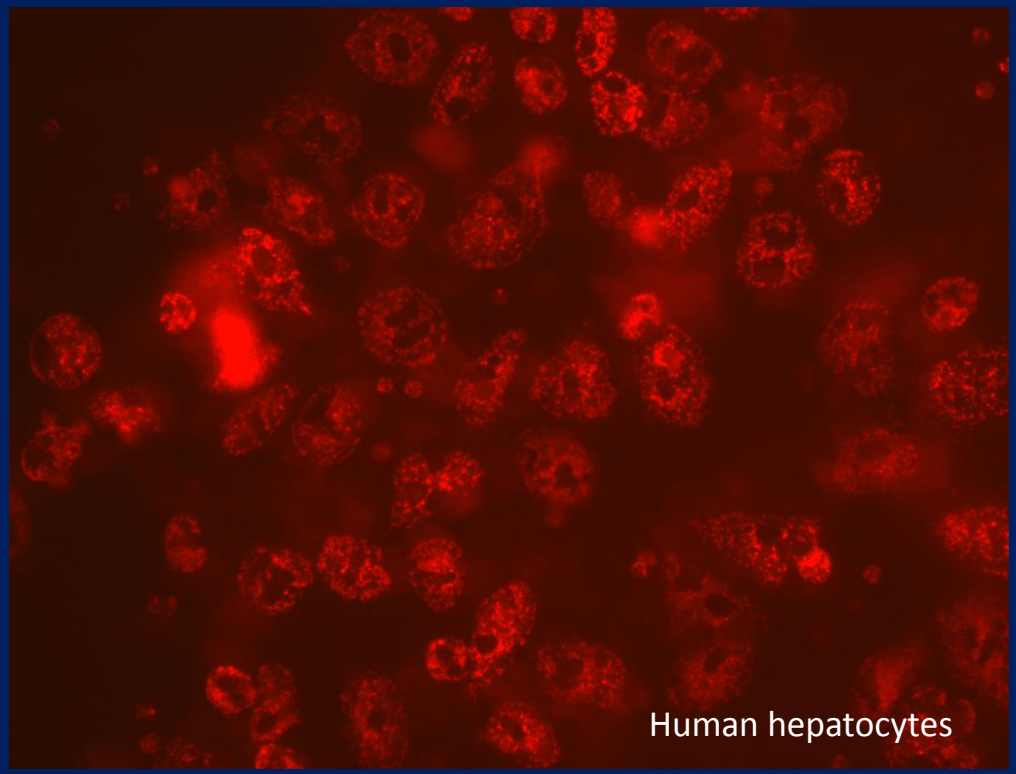
ME/CFS

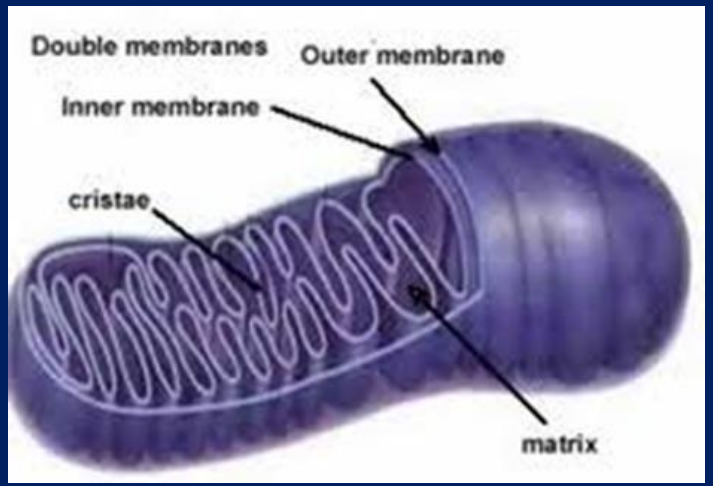


Nucleus

Mitochondria







Mitochondria are not just for energy !

Cellular garbage disposal (Autophagy)

Cellular signalling (ROS)

Immunity

Calcium storage

Cell death /development (Apoptosis)

ATP

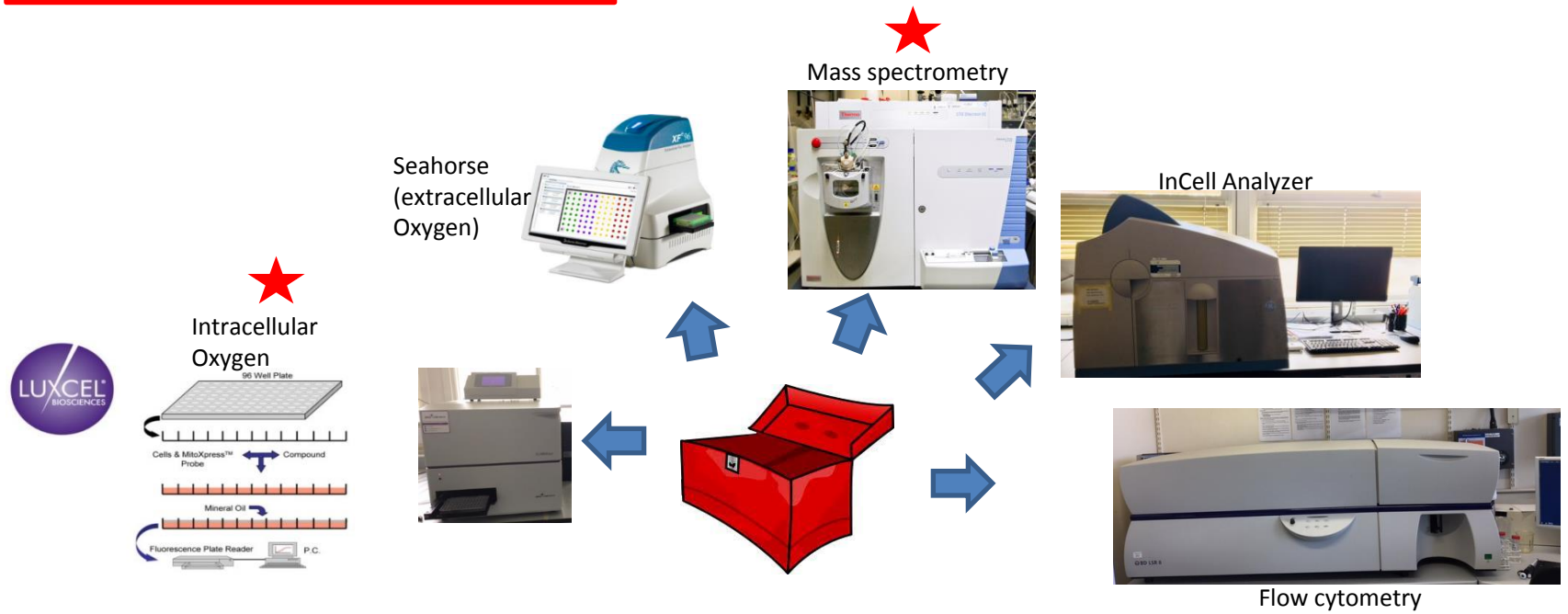
Synthesise the building blocks of the cell (i.e lipid, DNA, enzyme cofactors)

Mitochondrion

"36 ATP molecules are produced from one molecule of glucose with mitochondria working but only 2 ATP are produced if mitochondria completely fail"

Measurements of mitochondrial function

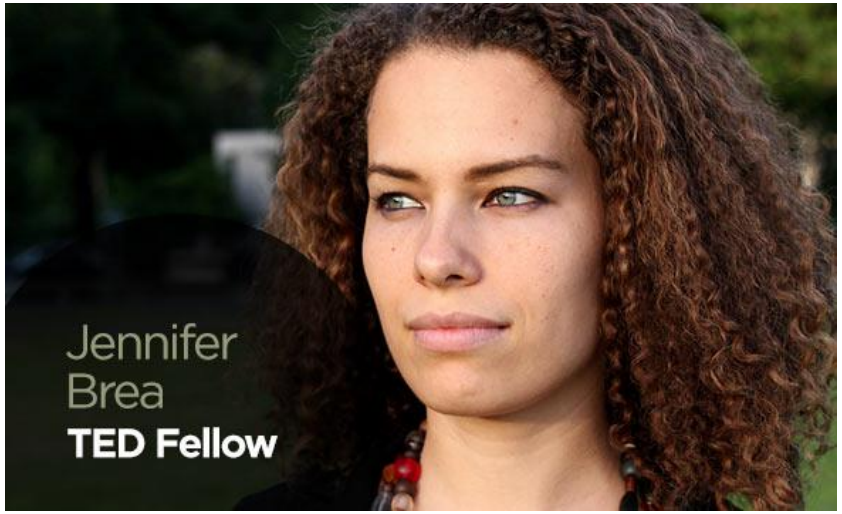
- ATP production/levels
- Mitochondrial membrane potential
- Mitochondrial morphology
- Mitochondrial/whole cell respiration
- Mass spectrometry-metabolomics

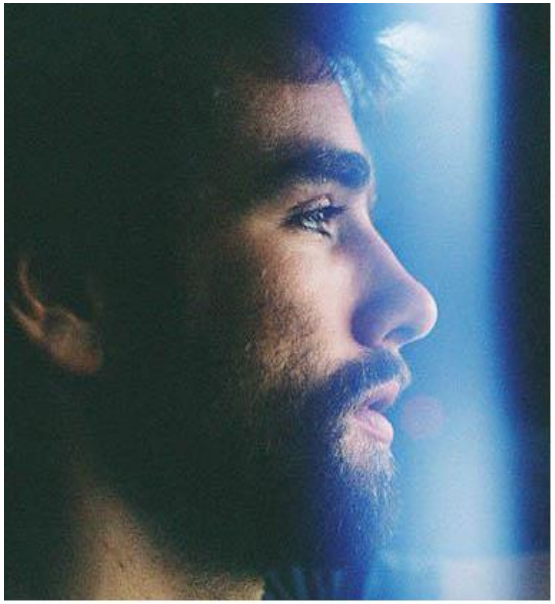


Talk outline

- Introduction to ME/CFS
- Current and future research into ME/CFS in Oxford
  - Biomarker discovery
  - Identification of blood borne drivers
  - Multi-disciplinary clinical studies in Oxford

Jennifer Bray & Witney Defoe





“Unrest”
New Documentary on ME/CFS

- 15 million people affected world wide.
- Cost to the UK economy of £3.3 billion per year.
- Between 85-95% of patients living with ME/CFS do not have a diagnosis.

Human cost: The Strong family

The 5 main symptoms of ME/CFS

Institute of Medicine report , Feb 2015

- Reduction or impairment in ability to carryout normal daily activities, accompanied by profound fatigue.
- Post-exertional malaise (a dramatic worsening of symptoms after physical, cognitive or emotional effort).
- Unrefreshing sleep.
- Cognitive impairment.
- Orthostatic intolerance (symptoms worsen when a person stands upright and improve when a person lies back down)

Problems in ME/CFS

- Clinical diagnosis has been difficult
- No diagnostic test
- End point for treatment trials is subjective based on questionnaires and fatigue scores
- Distrust between patient groups and the medical profession
- Very little research funding

IS CFS/ME just one disease?

Natural history of CFS/ME

Present to GP with Fatigue (10-15% of visits)

Fail to improve

Referred to hospital

Diagnosis of CFS/ME

Mild- Moderate

Put on managed programme

Some "recovery"

Group C

"Recover"

Group D

Too sick to attend

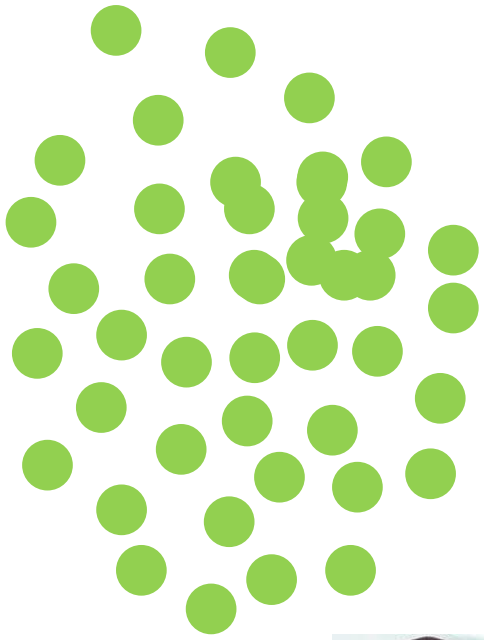
Fail to recover

Group B

Group A

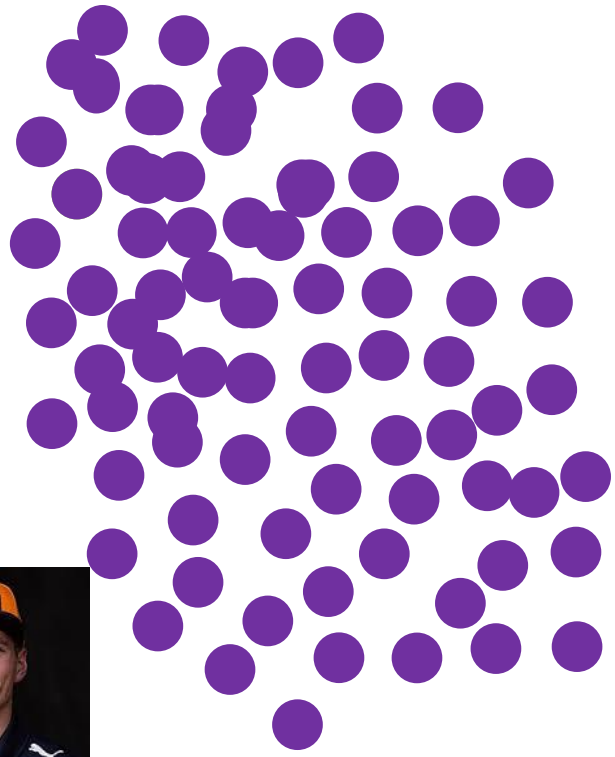
We need to identify the key drivers of ME/CFS!

Control Group



Unknown

ME/CFS patients

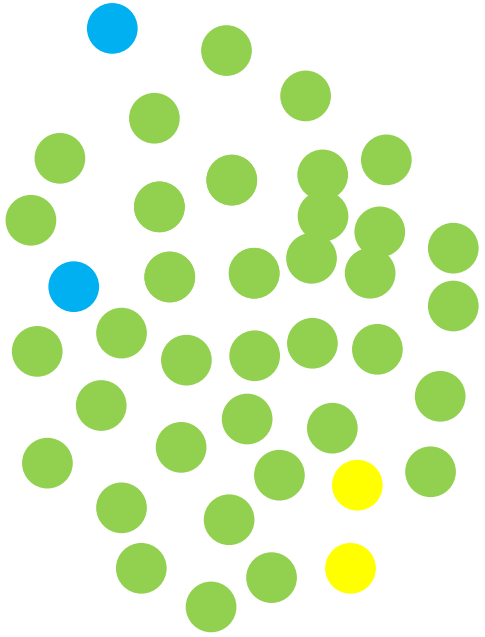


Known

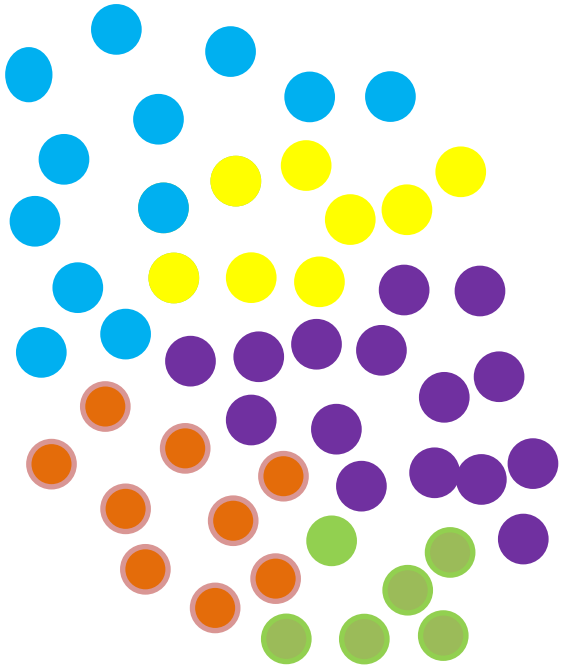
Are all ME patients the same with the same causes and symptoms?

Defining the ME/CFS cohort with biomarkers

Control



ME/CFS



A new Rituximab study failed to show efficacy in a larger drug/placebo trial: have we not selected the right group of patients?

B-Lymphocyte Depletion in Myalgic Encephalopathy/ Chronic Fatigue Syndrome. An Open-Label Phase II Study with Rituximab Maintenance Treatment

Øystein Fluge, Kristin Risa, Sigrid Lunde, Kine Alme, Ingrid Gurvin Rekeland, Dipak Sapkota, Einar Kleboe Kristoffersen, Kari Sørland, Ove Bruland, Olav Dahl, Olav Mella

Published: July 1, 2015 • https://doi.org/10.1371/journal.pone.0129898

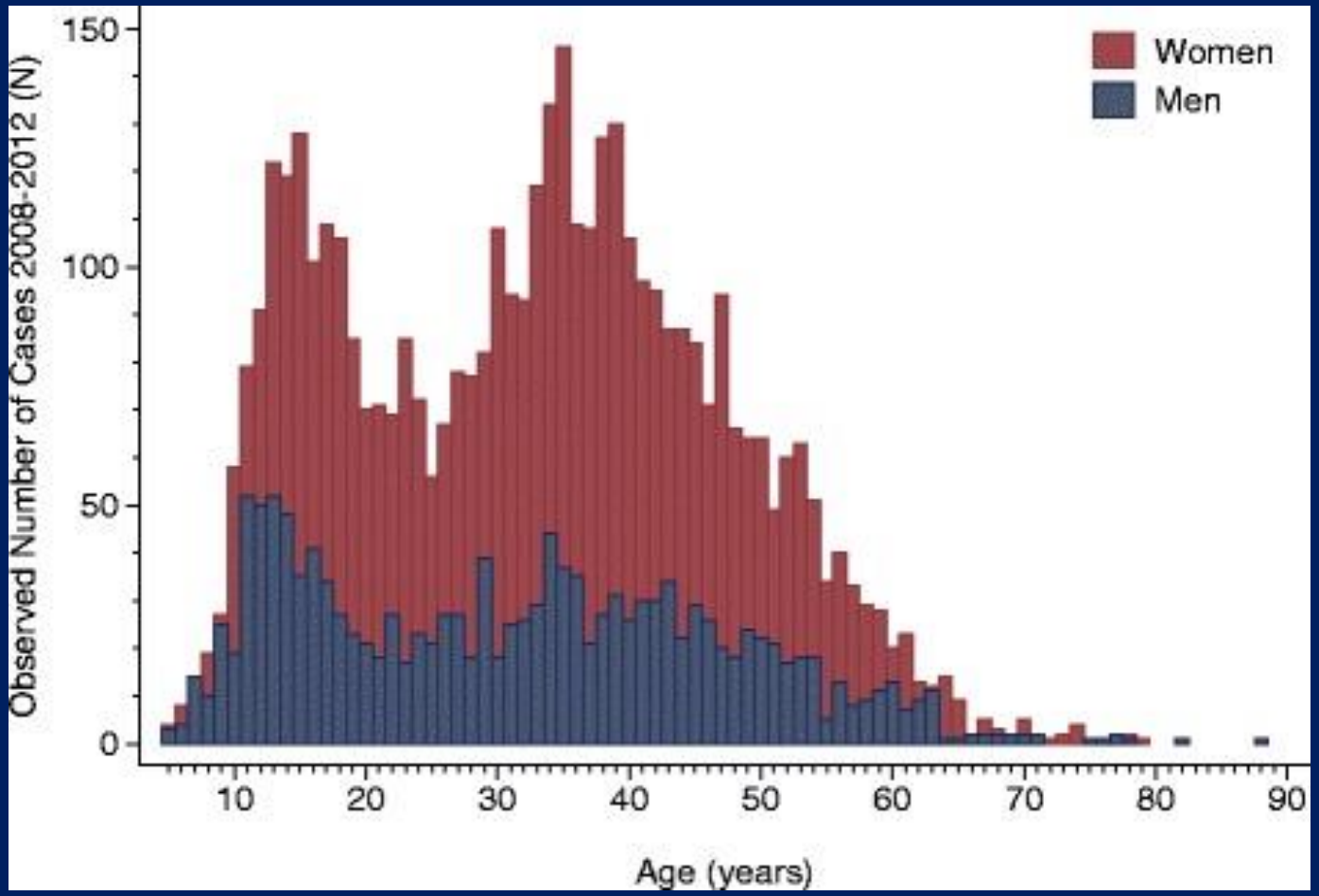
Early smaller trial showed a positive response in 68% patients. Not a blinded trial

3x $9.4 million grants for ME/CFS research from the NIH

Maureen Hanson, the Liberty Hyde Bailey Professor in the Department of Molecular Biology and Genetics, and Andrew Grimson, associate professor of molecular biology and genetics, inspect a high-throughput DNA sequencer in the Biotechnology Resource Center.

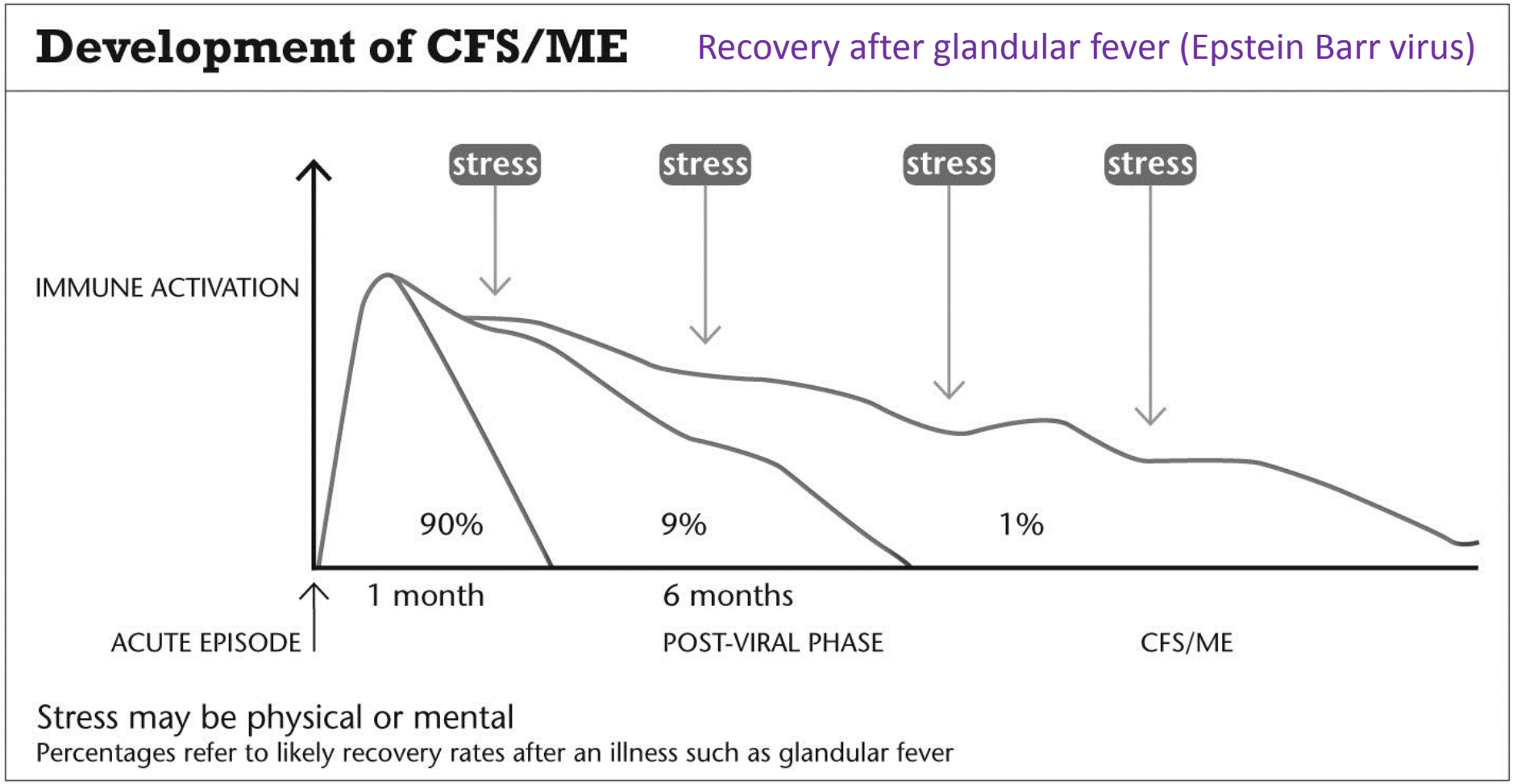
$9.4M NIH grant funds chronic fatigue syndrome center

Two age peaks in CFS/ME: do they the have the same cause?



Bakken (2014)BMC Med. 12: 167.

CFS/ME: a response to infection & subsequent stress ?



Ross Vallings

Figure 1: Rates of GP consultations, tests, prescriptions and referrals from 15 years before until 10 years after a first recorded diagnosis of CFS/ME in adult cases compared with controls

Adult group

Trigger point?

Consultations per Patient per year

Tests per patient per year

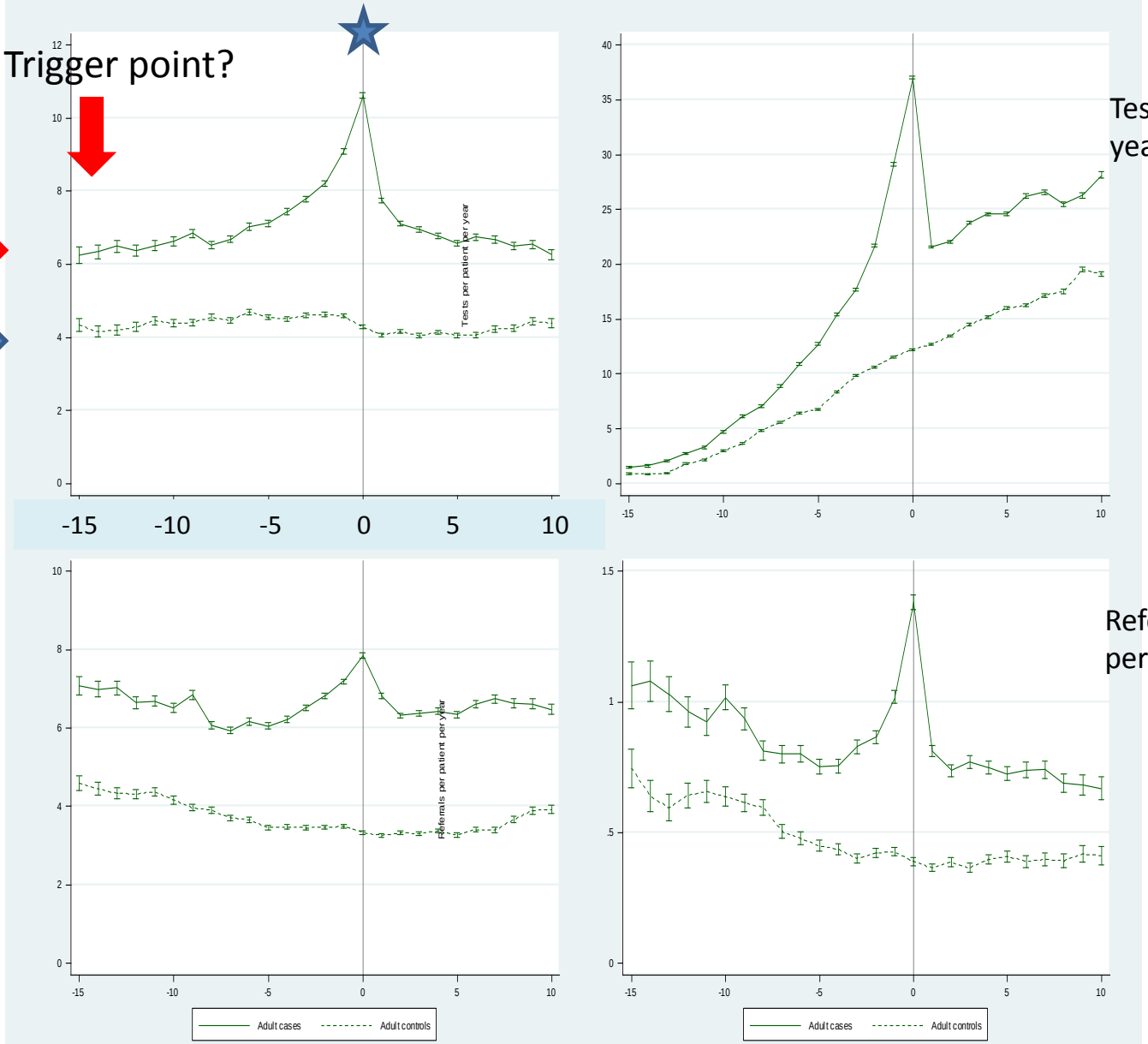
ME/CFS

Control

Time years

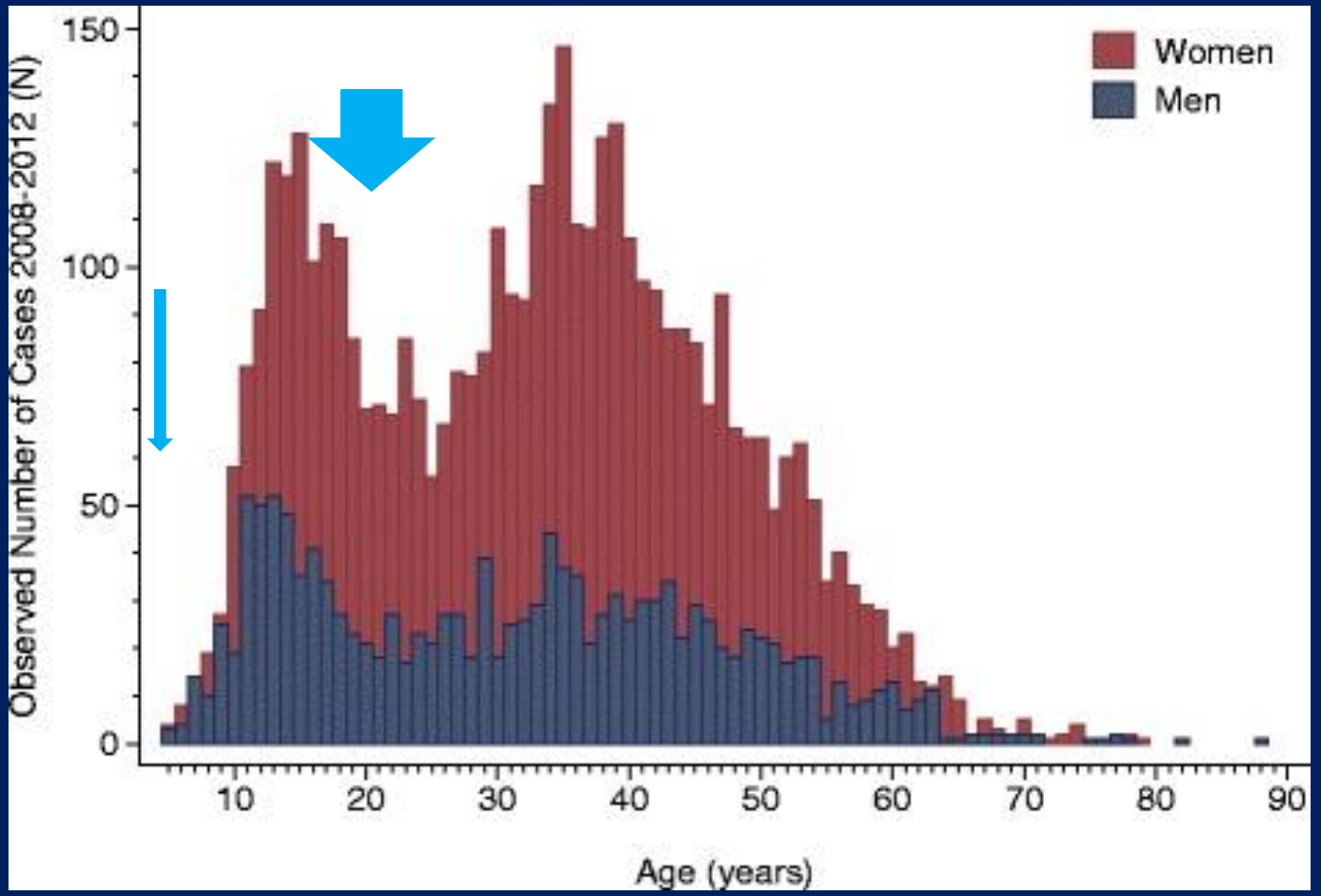
Prescriptions per Patient per year

Referals per patient per year



Simon Collin, Bristol, ALSPAC cohort BMC Fam Pract. 2017 18(1):60.

Are there two trigger points in ME/CFS?



Bakken (2014)BMC Med. 12: 167.

How did we get interested in ME/CFS research?

Norman Booth

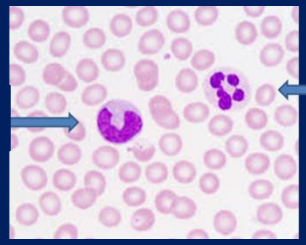
Charles Shepherd

Jamie Strong
NDOG 2016-

Mitochondrial and Metabolic dysfunction in ME/CFS

Could this approach lead to a diagnostic test for ME/CFS?

ATP levels are reduced in neutrophils from ME/CFS patients: evidence of mitochondrial impairment?



Acumen test

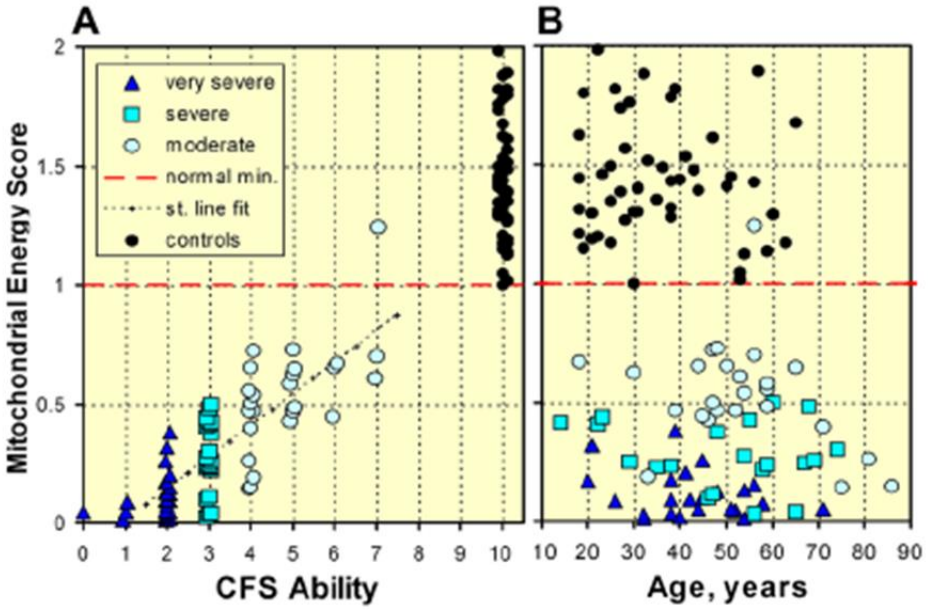


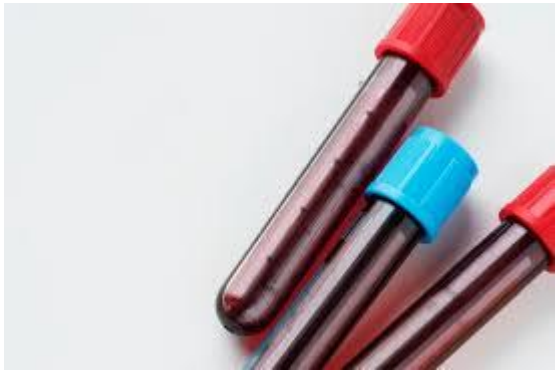
Figure 4. The Mitochondrial Energy Score. A. The Energy Score plotted against CFS Ability with a point for each patient. A point for each control is plotted at CFS Ability = 10. The horizontal dashed line at Energy Score = 1.00 is our normalisation at the minimum Energy Score for controls. Also shown is the best straight line fit to the patient data. B. The Energy Score plotted vs. Age of patients and controls.

Booth (2009) Int J Clin Exp Med 1-16

A good clinical test

- Robust used in multiple labs.
- Not affected by variation in processing.
- Show a correlation with disease

Living system



Per ml of blood
5 billion red cells
4-10 million Leukocytes
1.1-3.5 Lymphocytes

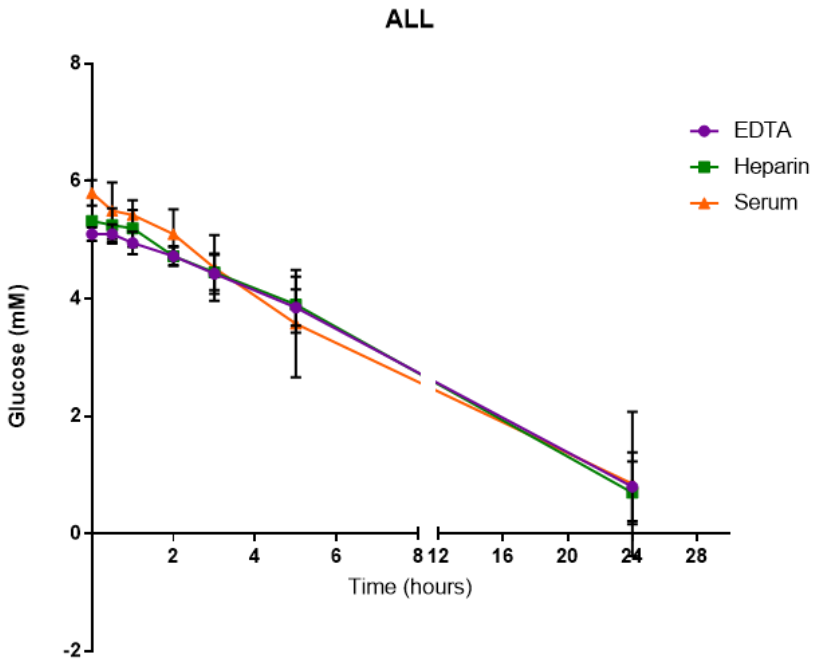
Organ failure markers come from the tissue of interest or reflect function

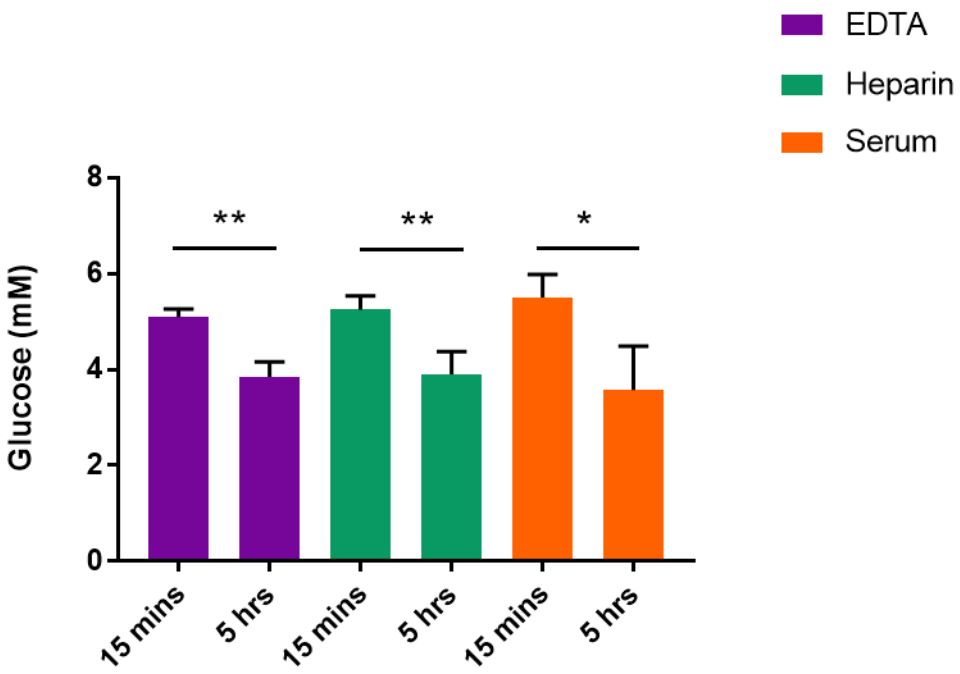
Liver: aspartate transaminase (AST) and alanine transaminase (ALT)

Heart: Troponin (released from damaged heart) or Brain Natriuetic Peptide (BNP) increased when a heart is working very hard

Problems with the acumen test: blood glucose levels drop rapidly over time!

Blood samples are left for between 24-72hrs in the blood collecting tube: What impact might this have on the data?





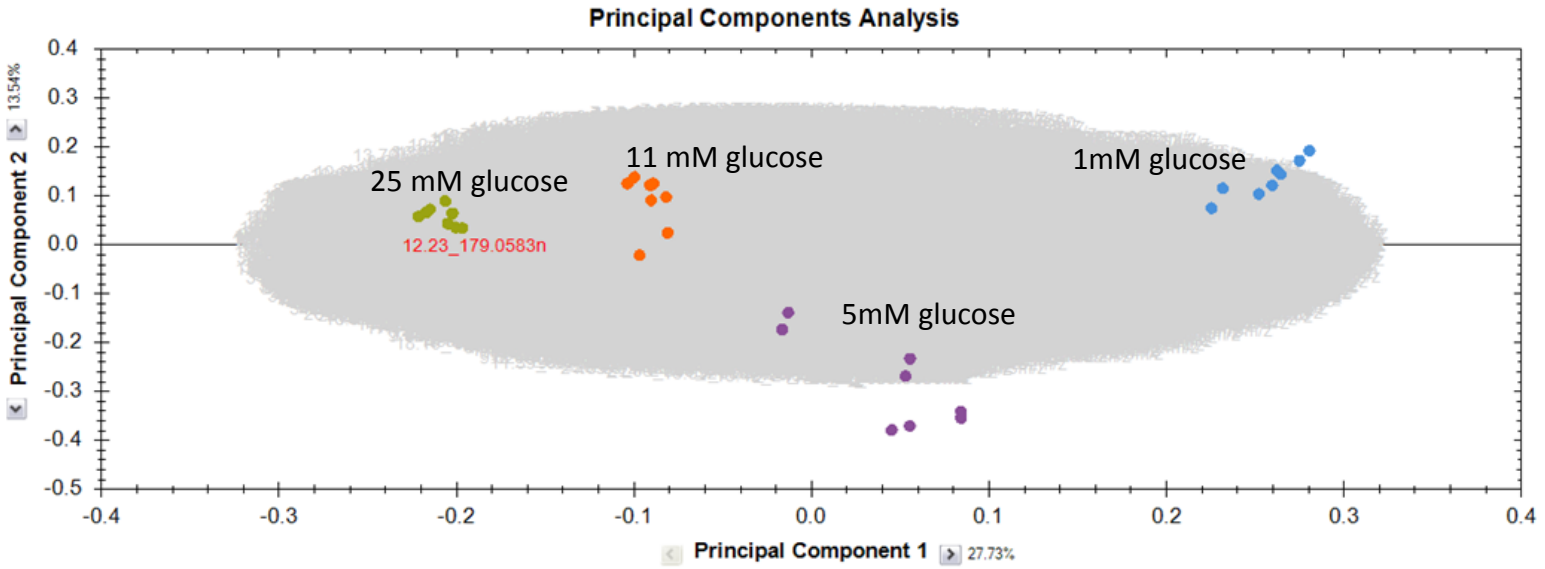
Tiffany Lodge

At different glucose concentrations cells function very differently

"Cloud of cell metabolities"

"Chemicals which make a cell tick"

"17,000 metabolites detected in each cell"



No difference was found using the acumen test on freshly processed blood samples from ME/CFS patients and controls (Newcastle/Oxford paper in preparation)

New Scientist

DAILY NEWS 3 November 2017

Blood cells in chronic fatigue syndrome are drained of energy

Blood cells in people with CFS seem as listless as it can make people feel
Mario Mitsis/Alamy

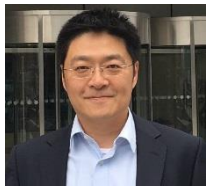
By **Andy Coghlan**

Using lymphocytes and monocytes from blood

Tomas (2017) Cellular bioenergetics is impaired in patients with chronic fatigue syndrome. PLOS ONE 12(10): e0186802.

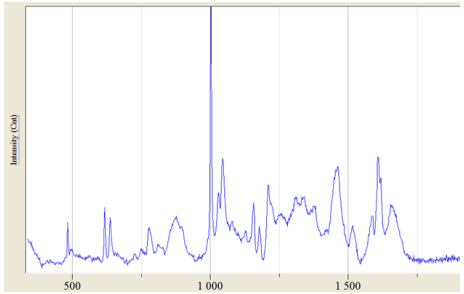
Further evidence of impaired energy metabolism in ME/CFS (Oxford)

Single cell Raman Spectroscopy



Prof Wei Huang

Jiabao Xu



- A distinct cell signature is found in ME/CFS patients (5 Patients 5 controls)
- Machine learning can detect patients with 98% accuracy
- Linked to increased levels of the amino acid phenylanine

Response to a ketogenic diet

Helen Dawes

Director Centre for Movement, Occupational and Rehabilitation Sciences

- Fuel utilisation in a post ketotic state is very different in the ME/CFS patients

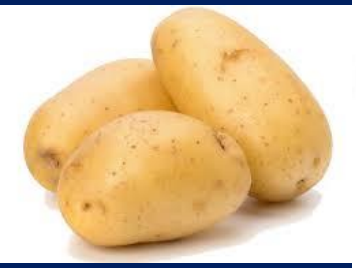
Xu (2018) Analyst DOI: 10.1039/c8an01437j

Key messages

1) There is clearly something altered regarding energy metabolism in some ME/CFS patients
   - How does this fit into the disease process?
   - Is this linked to the fatigue or is it part of a compensatory mechanism to deal with fatigue?

2) We need to work out the most appropriate test and way of carrying it out when using cells derived from blood samples!

ME/CFS patients may have a problem with carbohydrate metabolism



Glucose

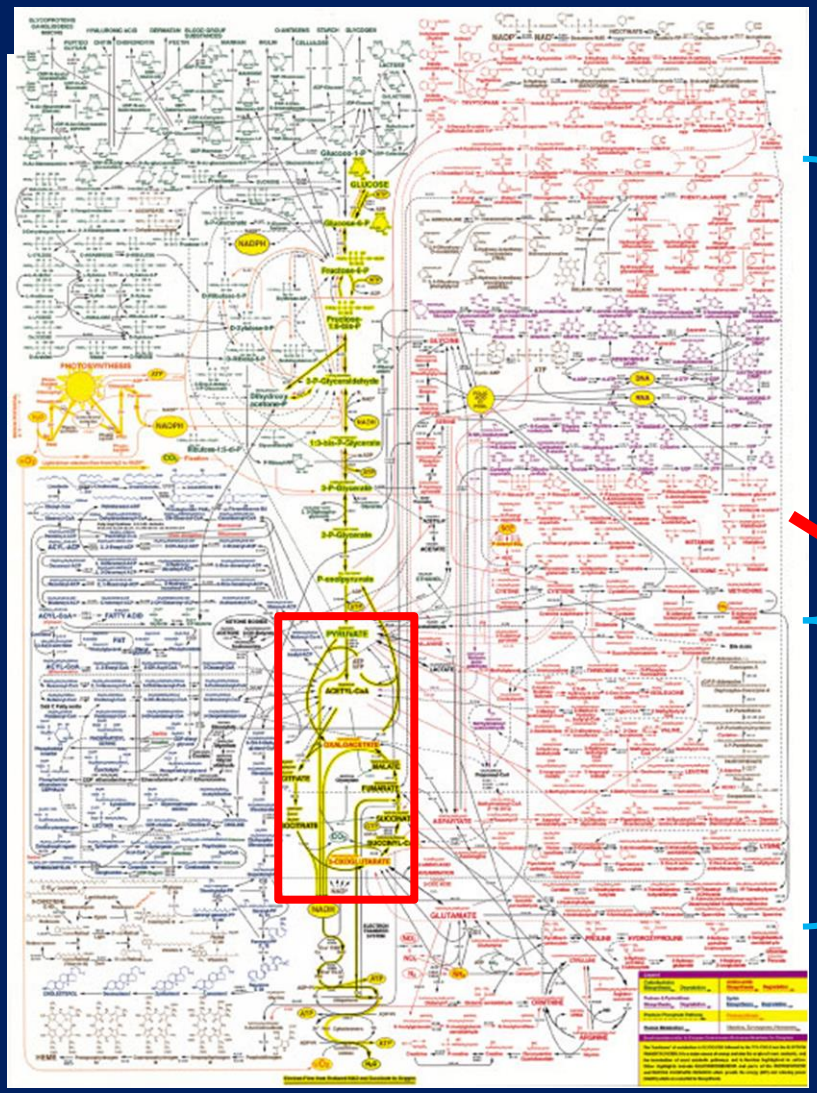
Fatty acids & monoacylglycerols

Metabolic switch may bring on chronic fatigue syndrome

Andy Coghlan, New Scientist Feb 13th 2017

Metabolic disturbances in ME/CFS

Cellular metabolic pathways



A

Enzyme X

B

Enzyme Y

C

Enzyme Z

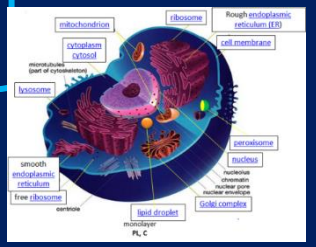
D

Glucose

Cytoplasm

Pyruvate metabolism

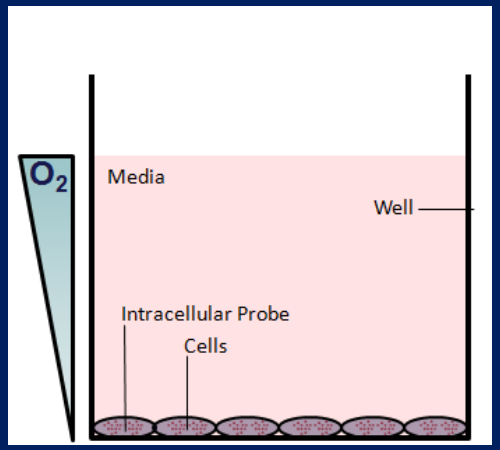
Mitochondria



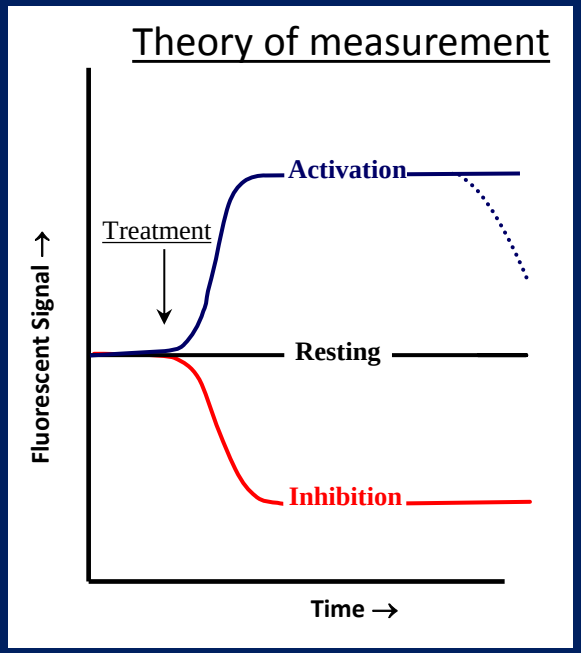
Fluge (2016) JCI Insight., Naviaux (2016) PNAS , Yamano (2016) Sci Rep

Are there circulating factors in the blood of ME/CFS patients which are preventing full recovery?

Intracellular O_2 Measurement Principles
[MitoXpress® Intra]



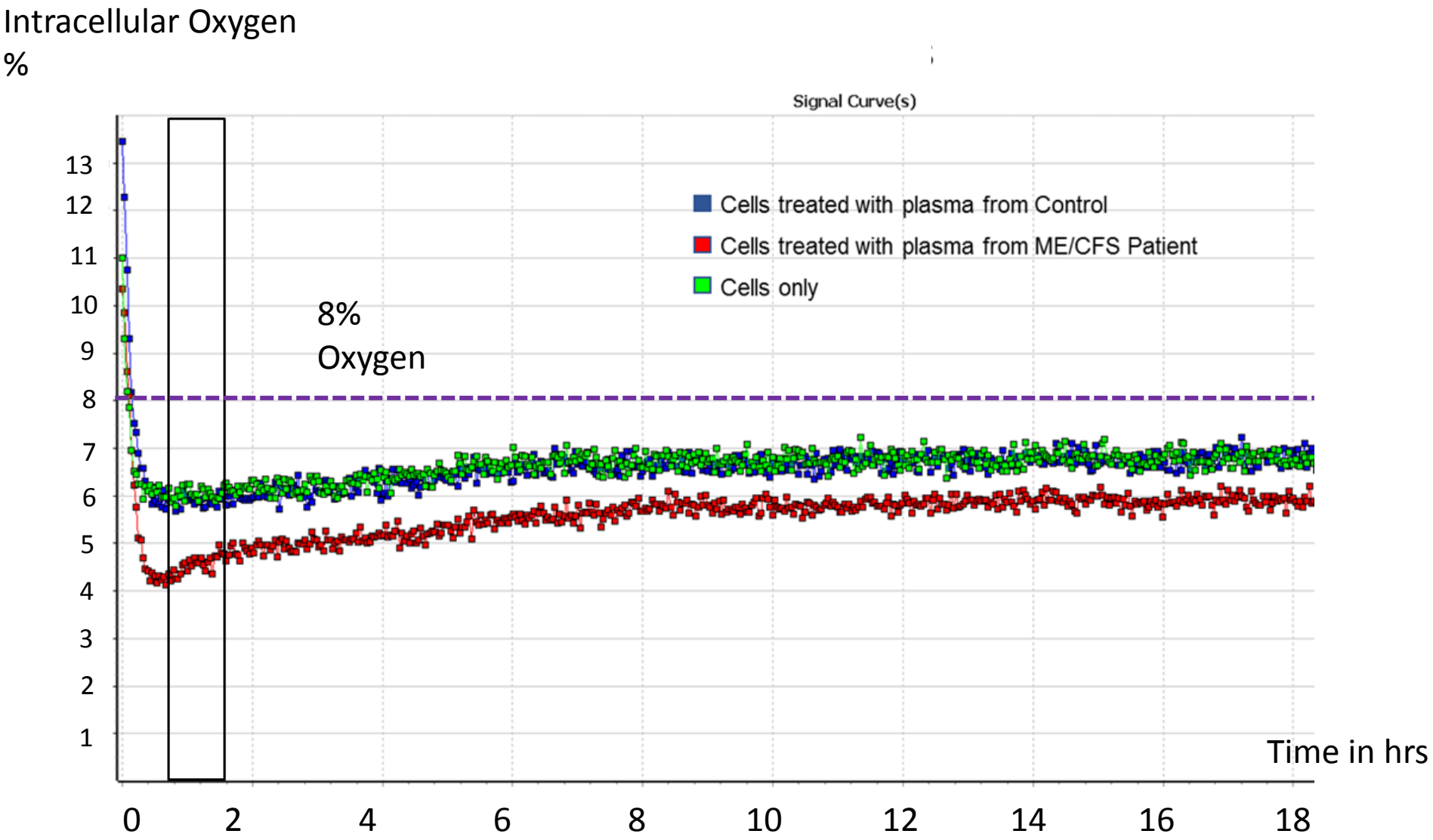
FLIM image of a 96 Well Plate well seeded with HepG2 loaded with Intracellular Probe



O_2 consumption

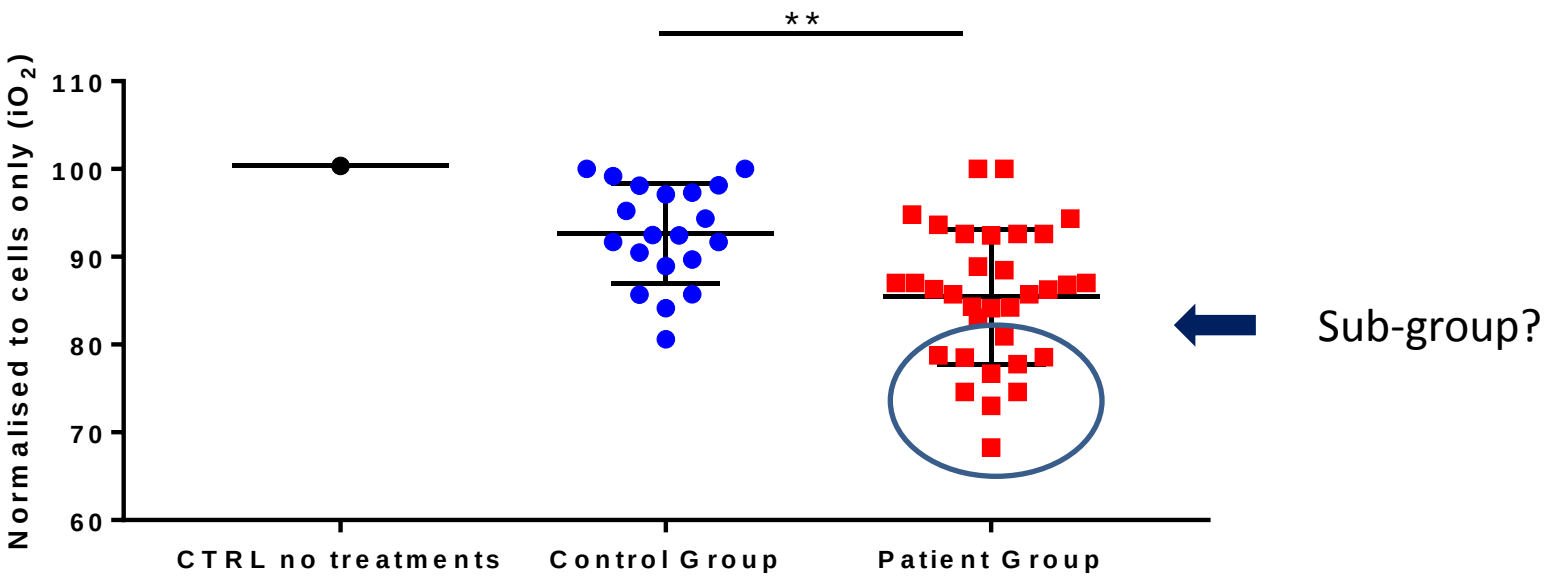
- Easy to use (simply add probe to cells – no chemistry)
- Cell penetrating, real-time, live cell measurement of oxygen concentration
  - *measurement of local oxygen concentration*
  - *study of drug effects and metabolism under controlled (known) oxygen environment*
  - *multiple drug additions during study*
- Ratiometric measurement on a dual/sequential-delay TR-F plate reader

The effect on intracellular oxygen concentrations when ME/CFS plasma is added to human control muscle cells



ME/CFS plasma in a sub-group of patients increases muscle oxygen consumption

Tiffany Lodge



NB:

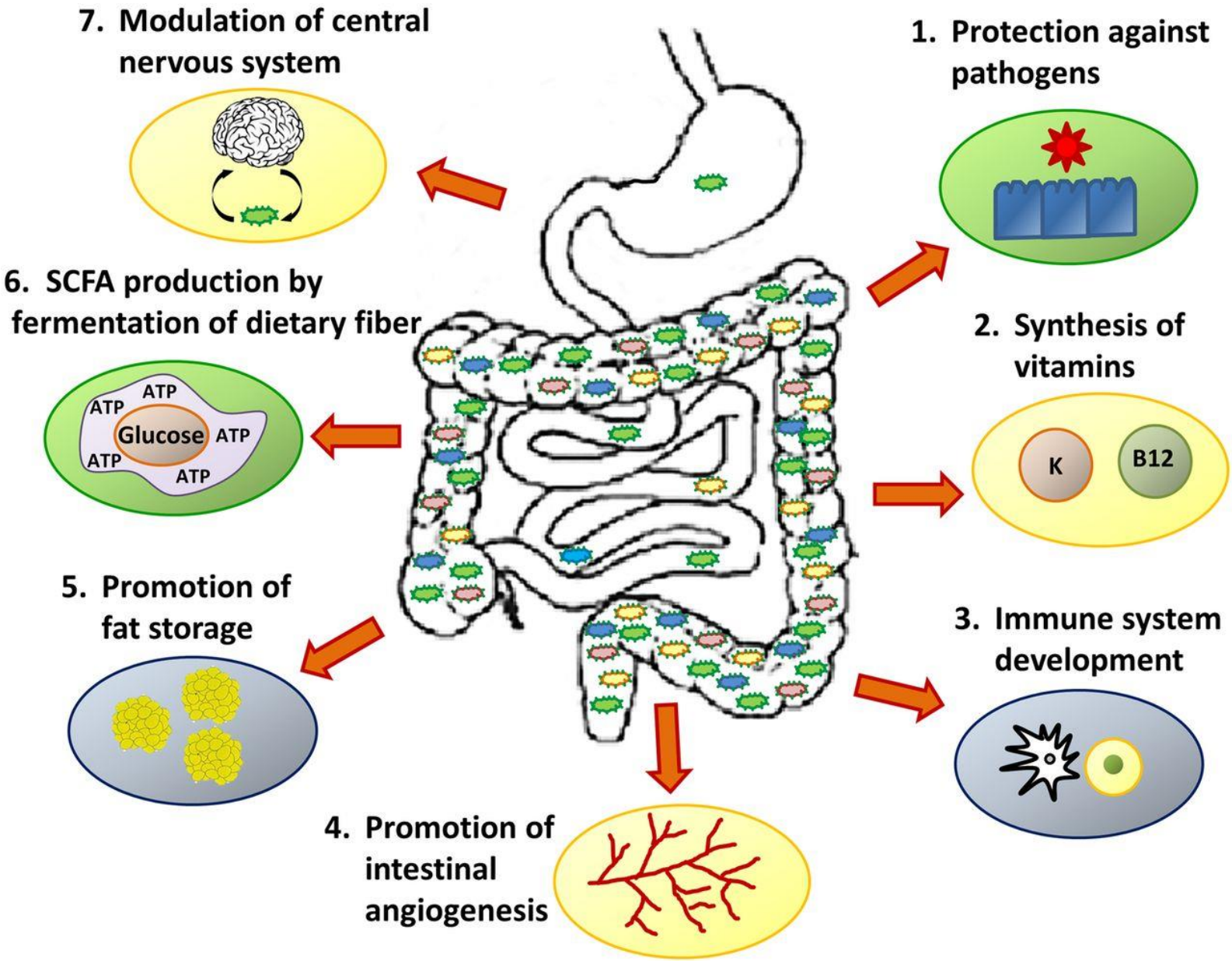
-This would be the predicted response of a cell if suddenly it was unable to use glucose or carbohydrates.

What is the ME/CFS factor which can change the energetic function of control muscle cells?

- Small molecule
  (i.e. chemical)
- Antibody
- Vesicle delivering cargo
- miRNA
- Signalling molecule

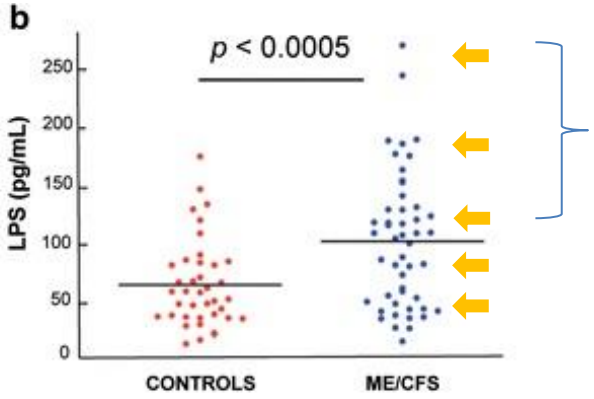
The microbiome

The role of microbes in the gut



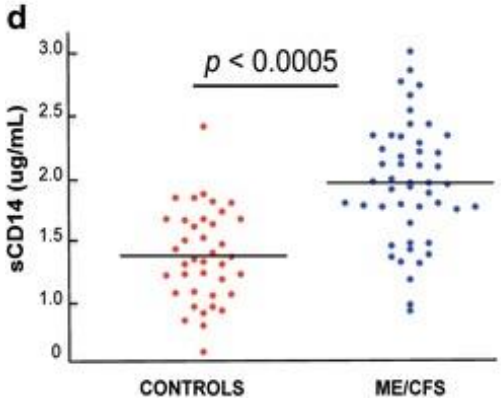
Evidence for a leaky gut in ME/CFS

LPS = Bacterial cell component in the blood



Sub-group?

Markers in the Blood



sCD14=Inflammatory marker produced by the liver in response to LPS

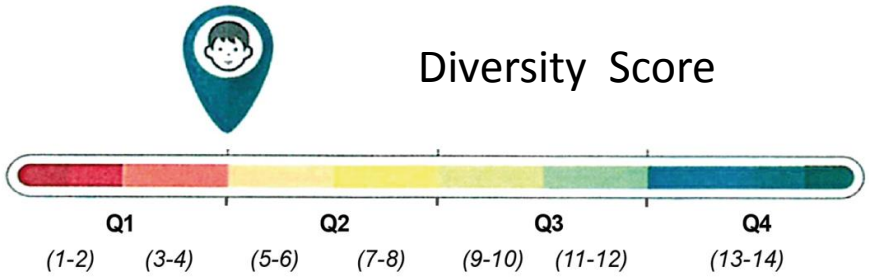
The diversity of bacteria in the ME/CFS microbiome is reduced

C-reactive protein levels are normal evidence of low level inflammation

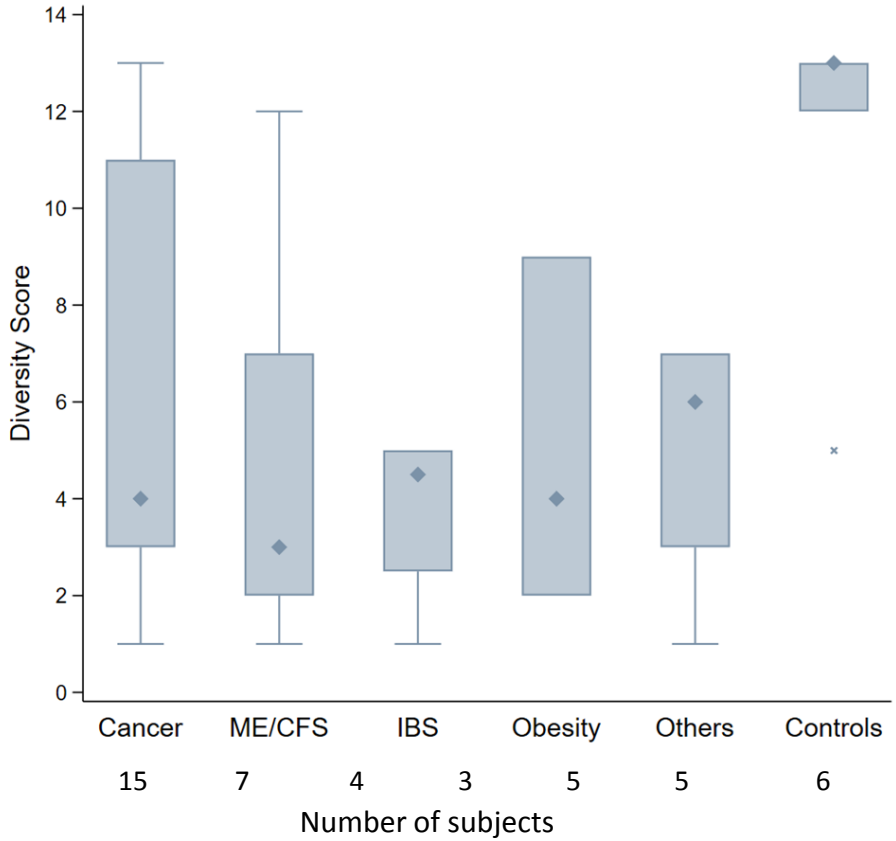
Giloteaux (2016) Microbiome 4:30

Many chronic conditions present with a low gut bacterial diversity score

Morten 2018 Human Microbiome Journal, Vol 10



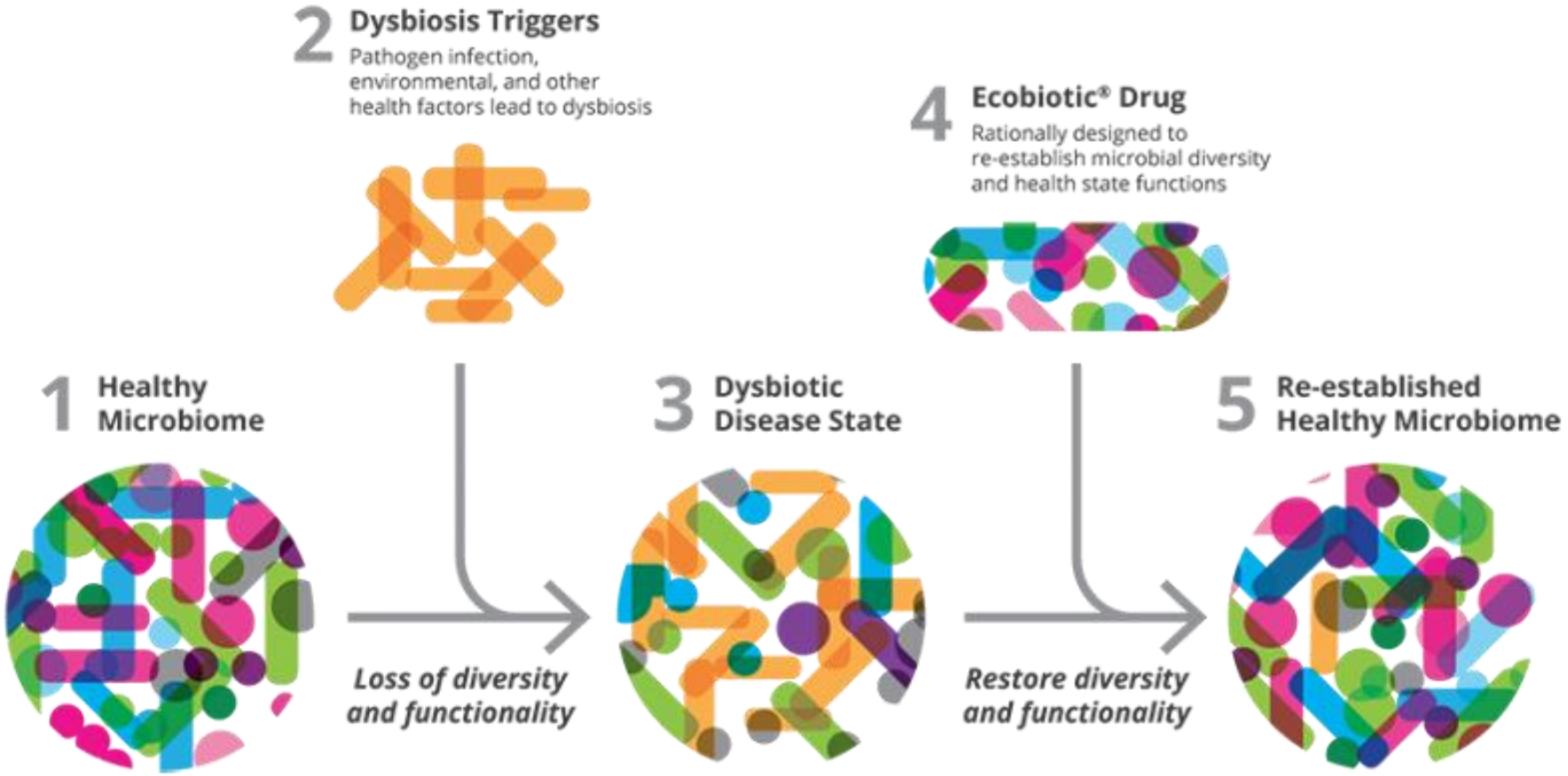
Map My Gut : Professor Tim Spector



Dr Julian Kenyon

The Dove Clinic for Integrated Medicine

Restoring a healthy microbiome: can this help ME/CFS patients?



Diet /Faecal Microbiota transplant (FMT)

FMT case report (10 treatments)

Female Aged 49 (JE4918)	• History of Chronic Fatigue Syndrome of at least 20 years. • Importantly in this patient's previous history, at the age of 14 she had surgery for a ruptured appendix, which was clearly followed by Peritonitis. • Also, at the time of the operation she developed a wound infection due to pseudomonas and was given intravenous antibiotics. • We carried out FMT in July 2018 and within a month of carrying out this treatment her energy levels returned to normal and her symptoms of Chronic Fatigue also disappeared. • That improvement has been maintained till now.

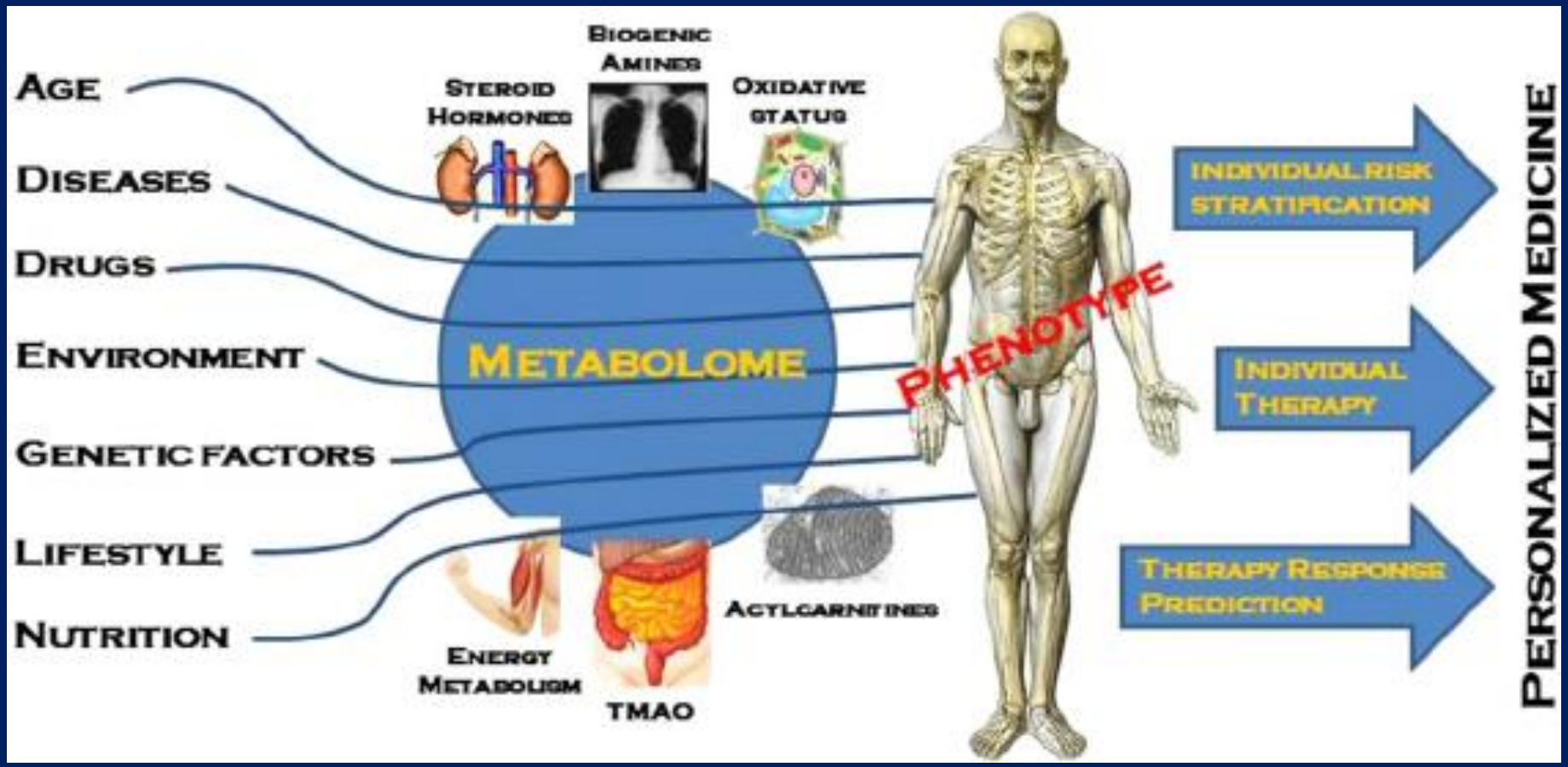
Are the improvements sustainable?

A Norwegian clinical FMT trial in ME/CFS started in Aug 2018

Current/Future research

Biomarker discovery: identifying sub-groups

Metabolomics is closer to the phenotype



Can all the A, B, C. D's found floating in blood give us clues as to what is going on in the body?

A

Enzyme X

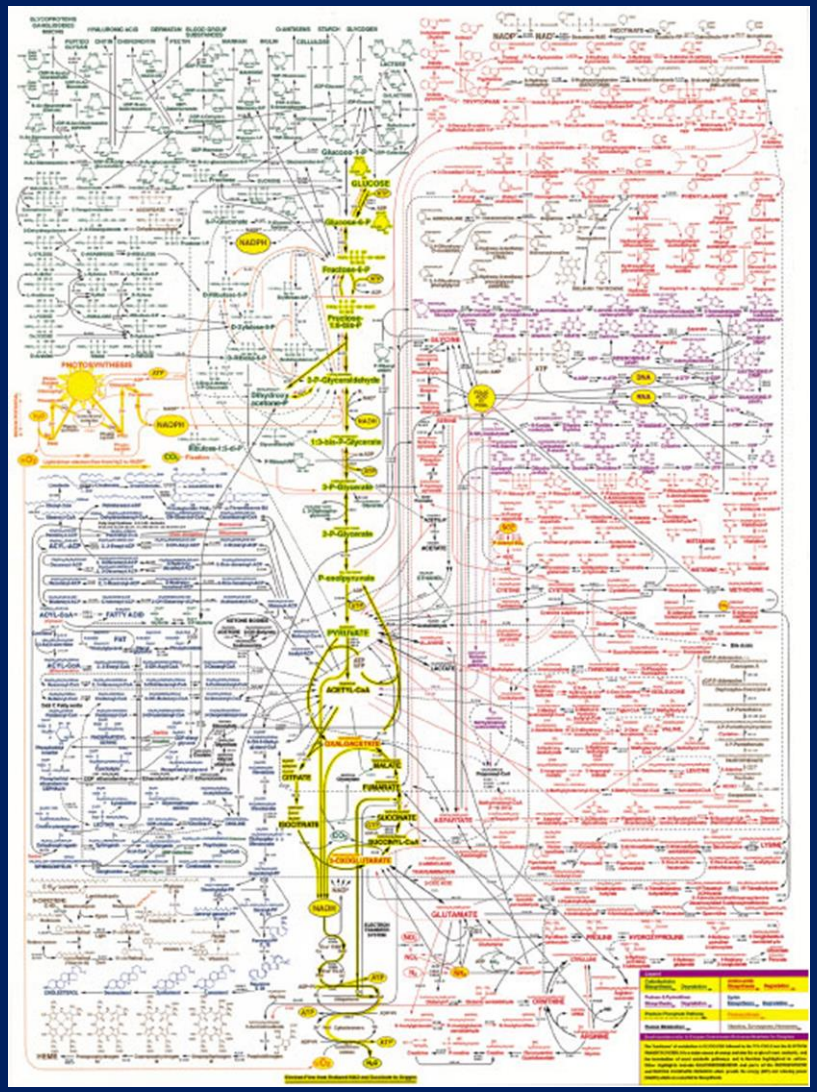
B

Enzyme Y

C

Enzyme Z

D



A

B

C

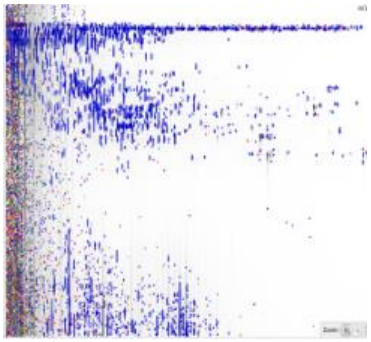
D

Cell

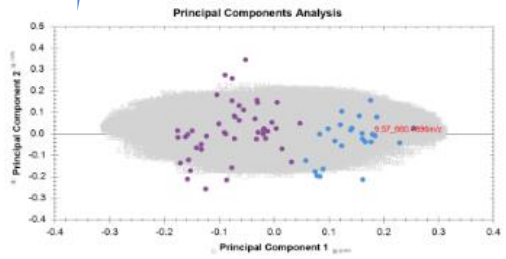
Metabolomics - Experimental Workflow

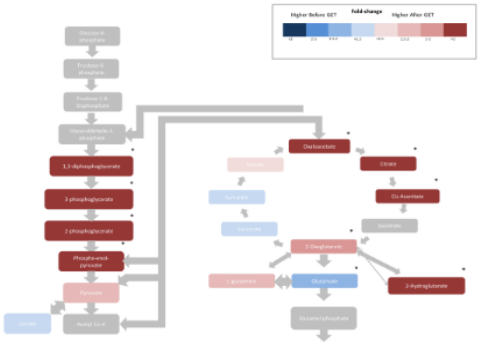
Sample preparation

Sample analysis



Metabolite identification





Polish samples

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ

DR PAWEL ZALEWSKI

- Healthy controls (n=24)
- Base Line ME/CFS patients (n=59)
- ME/CFS patients who have completed GET (6 month sample) (n=28)

- Clinical data Pre and Post GET
- Standard Biochemistry Pre and Post GET

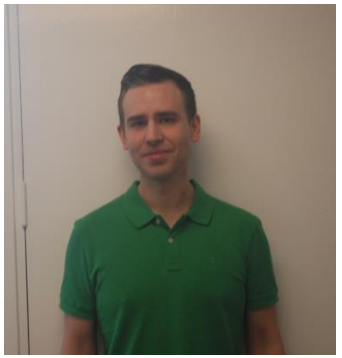
UNIVERSITY OF OXFORD

Mass Spectroscopy/Metabolomics on blood plasma

Prof James Mccullagh

Joe Harvey

Tom Ranger



Jamie Strong

Examples of clinical variables assessed

Basal metabolic rate (TANITA MA-180)

Autonomic nervous system function (Task Force Monitor)

Aortic stiffness

Aerobic capacity assessment (Spiroergometry)

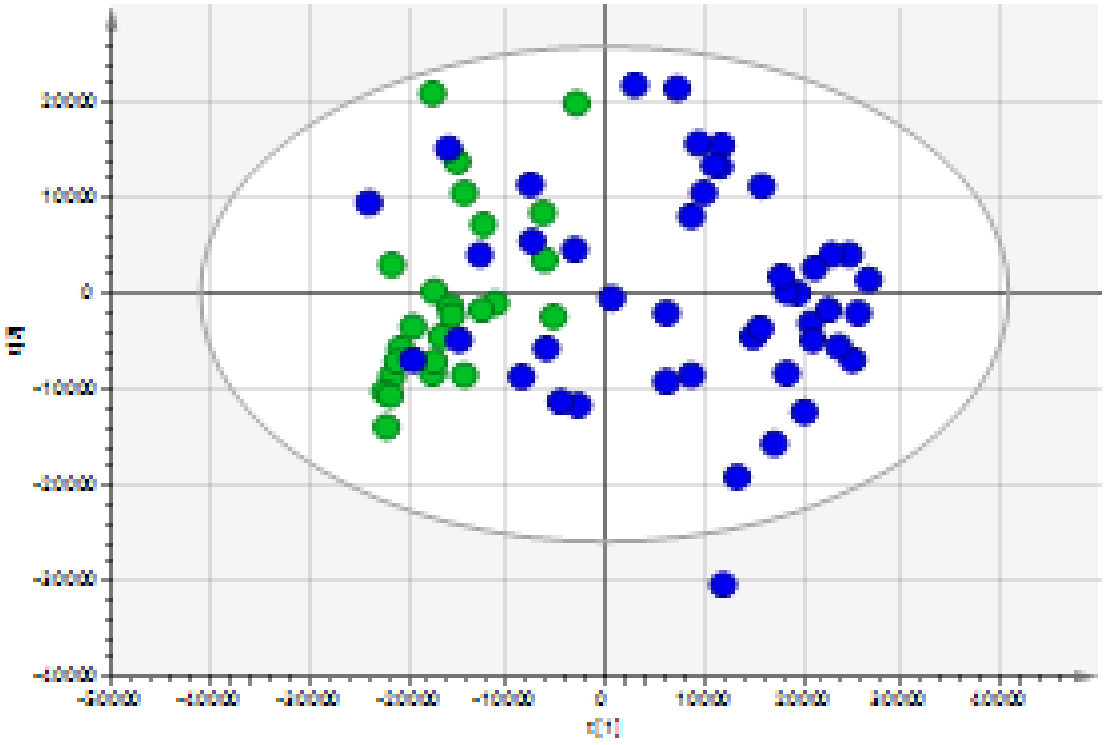
VO_2 Max

RER

HRmax

A principle component analysis can separate ME/CFS and the control groups based on differences in plasma metabolites

= a single individual

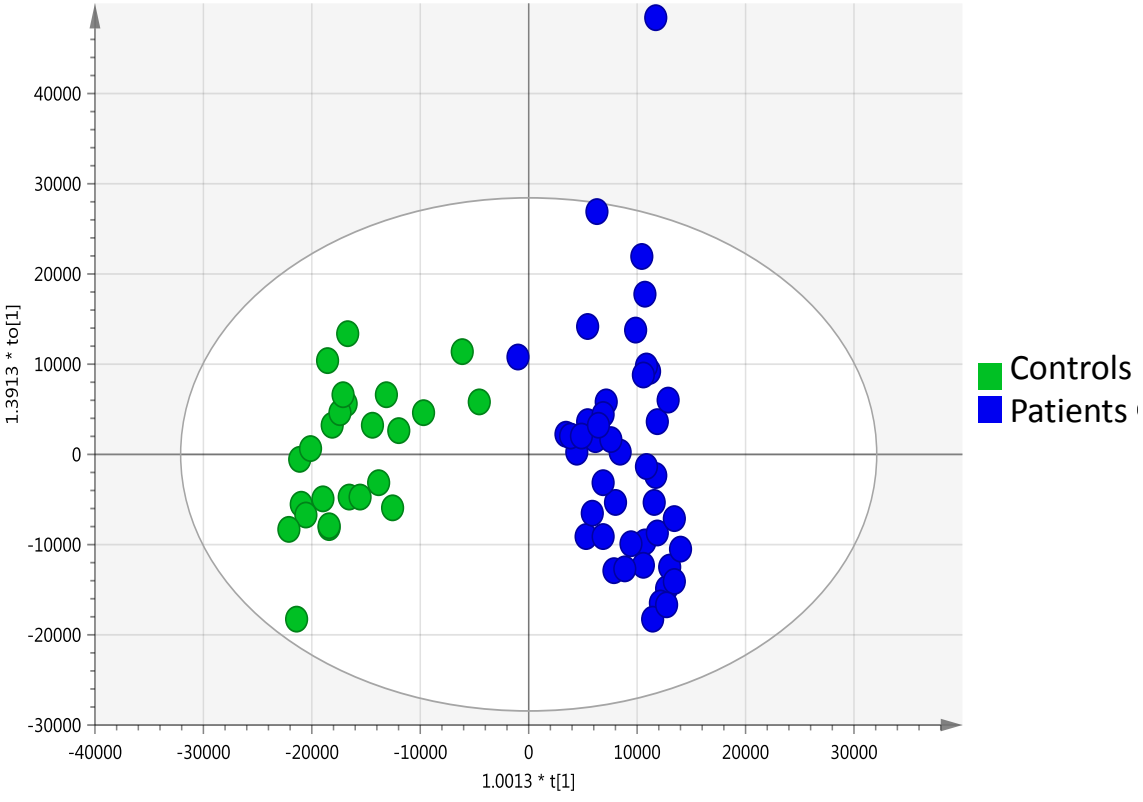


= One patient/control on which we have a profile of 11,000 plasma metabolites

37,000 variables

When we compare the top differences metabolomics data clearly discriminates between healthy controls and ME/CFS patients

OPLS-DA plot based on ~10,000 metabolites



Controls ME/CFS

VIPs with Accepted Description - Fold Difference +/- 0.2 (highlighted)	
	ME/CFS Baseline vs Controls Fold Change
Glutamine	0.61
PC(20:4/0:0)	0.59
Glutamic acid	5.97
PE-NMe(18:1(9Z)/18:1(9Z))	0.76
PC(16:0/20:4)	0.79
PC(16:0/22:6)	0.67
Isoleucine	1.22
PC(22:6/0:0)	0.53
LysoPC(P-18:0)	1.54
Histidine	1.46
SM(d18:1/24:1(15Z))	1.46
PC(O-18:0/0:0)	1.36
Sarcosine	1.39
SM(d18:1/22:1)	1.27
PC(P-16:0/0:0)	1.30
Methionine	0.75
Methyl linoleate	1.21

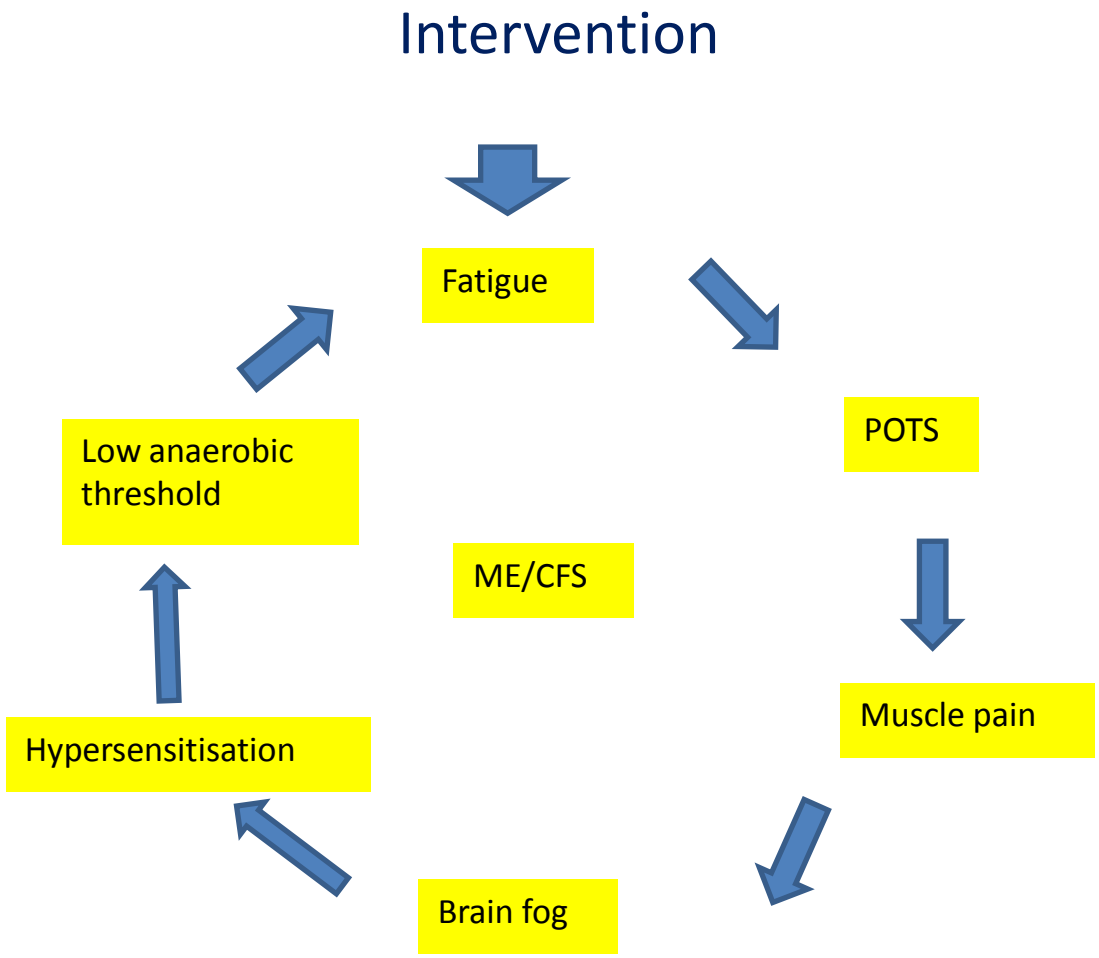
Summary of data so far

- Currently identifying the key metabolic differences between ME/CFS and controls.
- Our metabolomics data supports previous work.
- GET does not significantly impact on the key differences between ME/CFS and controls.
- Most of the key compounds that are different are unknown!

What do we plan to do next and the value of intervention studies?

- Intervention studies look to improve or worsen symptoms
- In a small study we are looking for sub-groups of patients who show a clinical and/or bio-marker response?
- Aim to identify what is important in driving the illness!
- Follow up larger studies on subgroups

Can we manipulate the key symptoms and link to our highly relevant metabolites and blood bourne factors?



Ice baths for tired damaged muscles reducing inflammation

Twickenham November 2018
“ The try that was!”

Whole Body Cryo-stimulation (WBC)

"Impacts on the immune system, reduces Inflammation, modulates cardiovascular and autonomic function "

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ

Newcastle University

POLISH SOCIETY OF CFS/ME RESEARCH

UNIVERSITY OF OXFORD

Chronic Fatigue Syndrome – A Whole-Body CryoStimulation (WBC) treatment

PROF. JULIA L. NEWTON, Newcastle University

DR KARL MORTEN, Oxford University

DR PAWEL ZALEWSKI, Nicolaus Copernicus University in Torun

"ME/CFS does not exist in Poland" Pawel Zalewski

WHOLE-BODY CRYOSTIMULATION (WBC) IN PATIENTS WITH CFS PILOT STUDY

- **Goal:** To assess the effects of 10 days of whole-body cryostimulation (WBC) combined with stretching training on cardiac, autonomic and immune responses

Stage 1:
Assessment 01

Stage 2:
10 sessions
WBC
streching

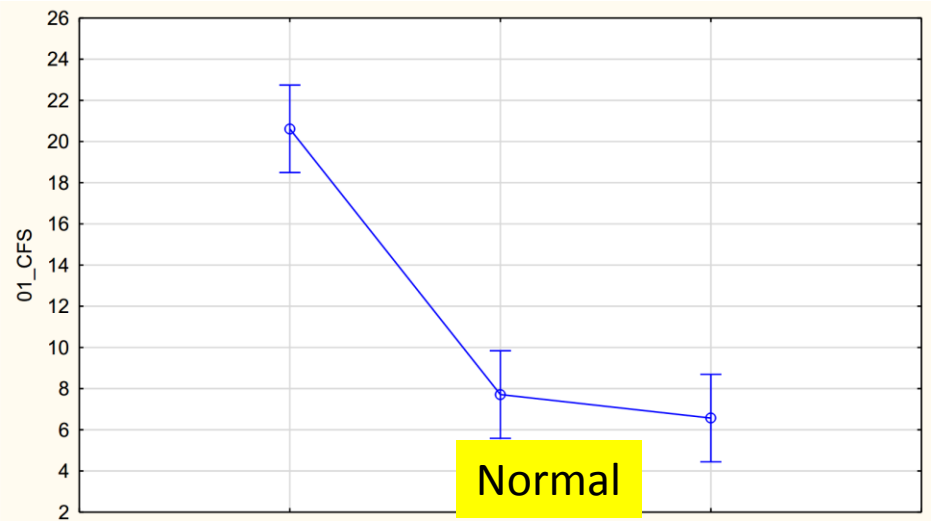
Stage 3:
Assessment 02

Stage 4:
Follow up after 3-6 months

100% of patients come back for subsequent treatments compared to 50% with GET

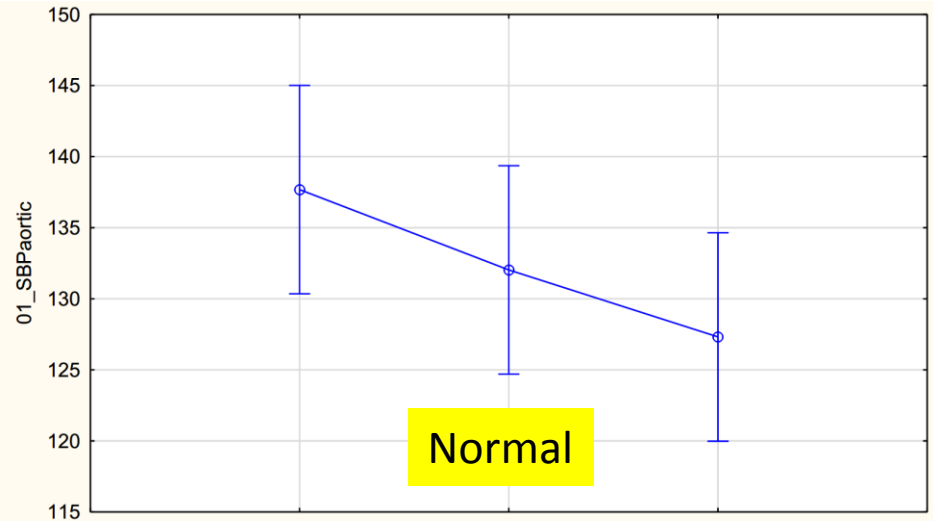
Cryotherapy study: clinical data

Chalder fatigue scale



Pre Post 1 month

Aortic Stiffness



Pre Post 1 month

12 month follow up and sequential treatment plans proposed

Grant proposal (£1.6 million project)

MS

Control

Mild

Moderate

Severe

UK
ME-CFS
Biobank

ME/CFS Viral trigger (NIH/US)

Longitudinal study 12 month
(Oxford / Royal Free)

Main objective: To identify the key disease pathways & sub-group the patients

Lymphoma
Chemo &
Non-chemo
Related fatigue
(Oxford)

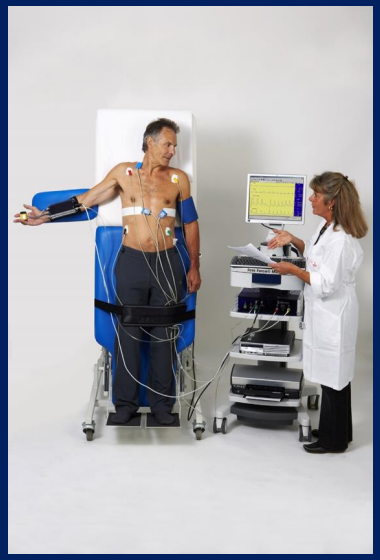
Total subjects = 1021

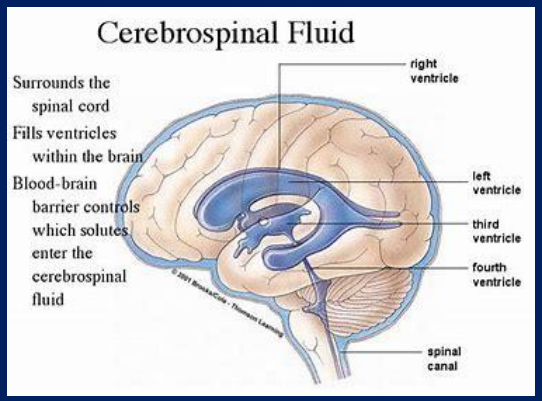
Mitochondrial
Disease
(Oxford)

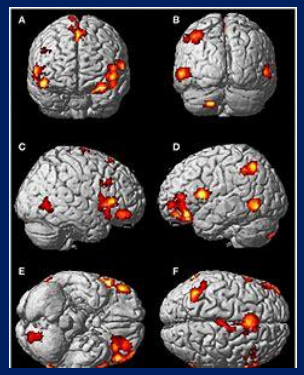
Interventions
Cryotherapy (Poland)
Exercise (Double)/
Immune stimulation (Sussex)
Antibiotics (UTAH)

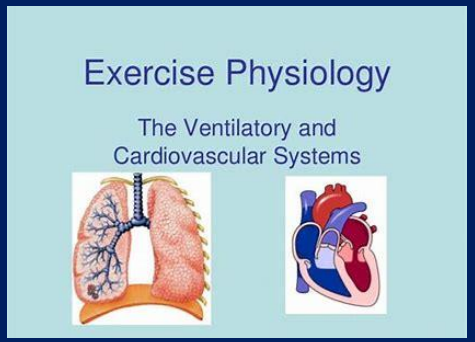
Clinical assessments

Fatigue questionaires (4)









Basic Research

Screen for the presence of the plasma energy altering factor and identify!

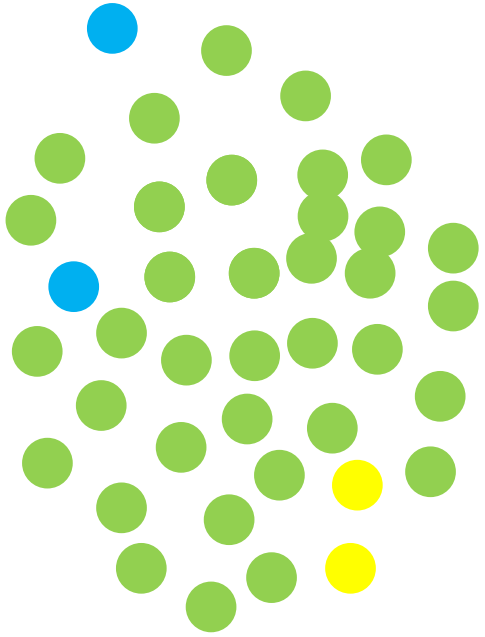
Identify Key unknowns and disease pathways

Is glucose a poor fuel for ME/CFS patients?

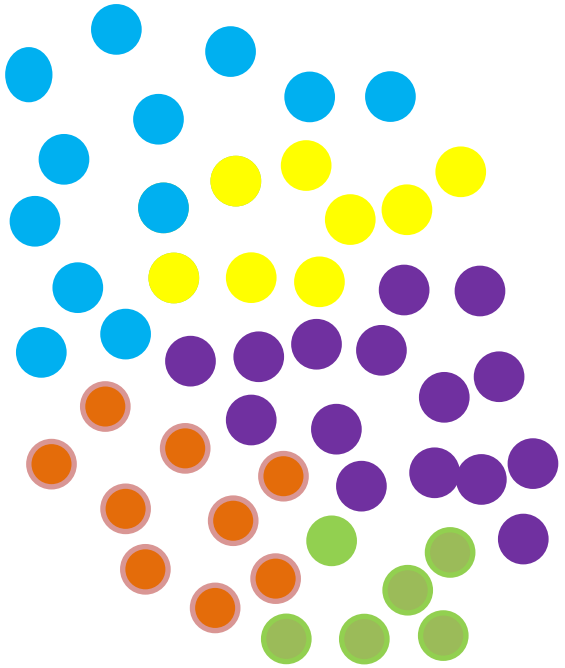
Do L-form bacteria in the blood correlate with metabolites/plasma factors?

Defining the ME/CFS cohort with biomarkers

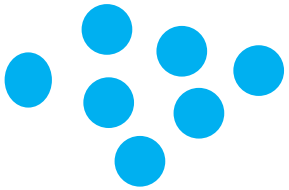
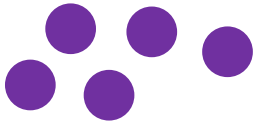
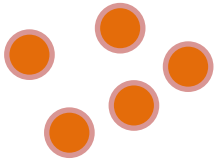
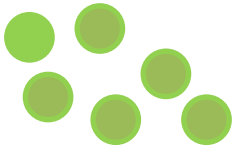
Control



ME/CFS



Using biomarkers and potential disease mechanisms to select patients for clinical trials

	Treatment
 Inflammation linked to altered microbiome	Specific antibiotic treatment/ or FMT
Systemic reduction in carbohydrate utilisation	Ketone supplement
 Low level inflammation of the brain	Acupuncture
 Auto-immune problem	Immune therapy
 No clear biomarker or problem	GET works for this group

Summary/Future

- ME/CFS appears to have a biological basis.
- Mitochondrial and metabolic dysfunction associates with ME/CFS. Cause or consequence?
- We need to identify biomarkers combined with improved clinical diagnosis to assign patients into sub-groups.
- Run knowledge driven clinical trials involving the pharmaceutical industry.
- Galvanise the patients, clinicians and scientists to work together!

Acknowledgements

Morten Group
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-Thomas Ranger

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-Ning Wang
-Hanqing Jin

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Pawel Zalewski (Poland)
Luxcel Biosciences
BMG Labtech
Julian Kenyon (Dove Clinic)
UK ME/CFS Biobank
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