Dr. Vance Spence (UK – Scotland)

Dr. Spence var ledende klinisk forsker med ansvar for forskningsenheden for karsygdomme (vaskulære sygdomme) ved Ninewells Hospital, University of Dundee Medical School, Dundee, Scotland, indtil hans pensionering pga. ME.
Han er aktuelt formand for og medstifter af ME Research group for Education and support (MERGE www.meresearch.org.uk) og er æres Senior Research Fellow ved Institut for Kardiovasculær forskning, University of Dundee Medical School.
Dr. Spence er en af de førende forskere indenfor ME/CFS i vaskulær sygdoms forsknings enheden, hvor han undersøger ME/CFS mht. pro-inflammatoriske cytokiner og pro-oxidant tilstand.
To nye publikationer fra hans hånd (og medforfattere) er i trykken:
"*Oxidativ stress niveau er forhøjet ved CFS og er associeret med kliniske symptomer*", samt
"*Er CFS en hyperkoagulations tilstand associeret med aktivering af blodpladerne?*"
Han har også været tæt involveret i studierne af
"*Acetylcholine medieret vasodilatation i mikrocirkulationen hos patienter med CFS*", samt
"*Øget neutrofil apoptose (programmeret celledød) ved CFS*".
Det er specielt det sidste emne aktuelle foredrag handler om. Dr. Spence er også en velkendt kommentator indenfor forskellige forskningsområder der vedrører CFS/ME patienter og folk med lignende problemer.

Hvordan definers CFS?

CFS: US CDC - 1994 (Fukuda definition)
Mest udbredte definition af CFS

Chronic Fatigue
- 6 måneders varighed
- Vedvarende eller tilbagefald
- Akut eller gradvis start
- Ikke ordentlig udhvilet efter hvile
- Ikke resultatet af vedvarende anstrengelse
- Resulterer i betydelig reduction i aktiviteter
- Fører til betydelig funktionel svækkelse

Ny startede symptomer (4/8 kræves)

Ondt i halsen	Kognitive symptomer
Ømme lymfeknuder	Muskel smerter
Multi-led smerter	Hovedpine
Ikke udhvilet efter søvn	Sygdomsfølelse efter anstrengelse

BENIGN MYALGIC ENCEPHALOMYELITIS?
Goodwin CS. Lancet Jan 3rd 1981, p 37

Symptomer og sygdomstegn i relation til muskler:
- Tilbagevendende episoder af udtalt svaghed og udmattelse
- Let trætbarhed
- Betydelig muskel ømhed

Variable involvement of the CNS:
- Pyramidebane eller kranie nerve læsioner
- Tab af perifer følesans
- Involvering af det autonome nerve system
- orthostatisk takykardi
- abnorm kolde ekstremiteter
- episoder med svedeture eller bleghed
- forstoppelse og blære forstyrrelser

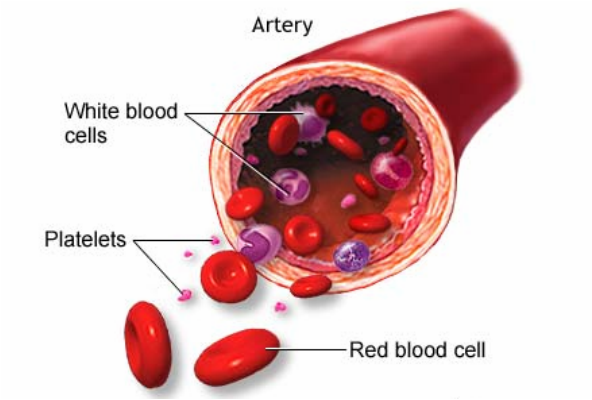
Biokemiske abnormiteter:
- øget urin creatinin
- lavt serum pyruvat
- øget serum myoglobin

Betydningen af CFS/ME

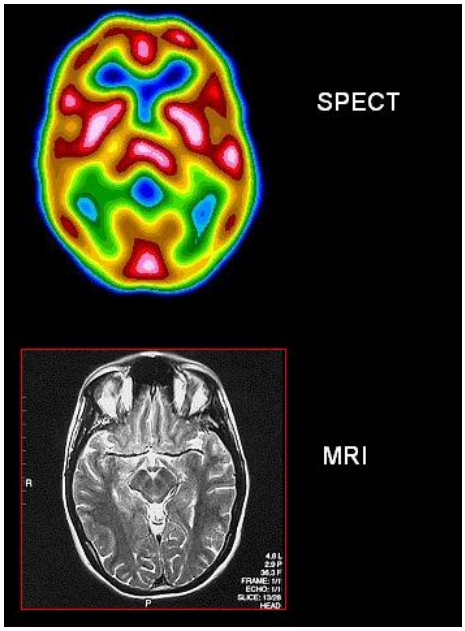
"Fuld helbredelse er SJÆLDEN"
R. Cairns & M. Hotopf. A systematic review describing the prognosis of CFS. Occupational Med 2005; 55: 20–31

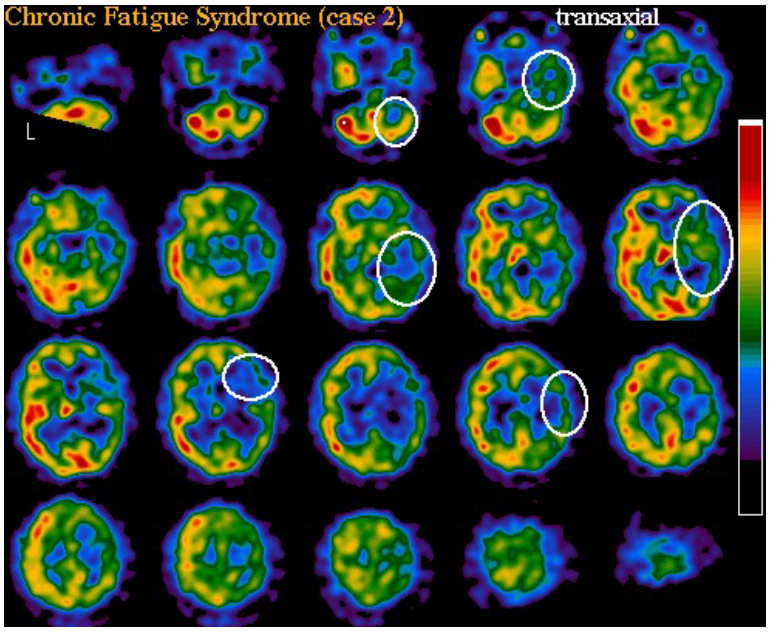
"... patienterne har svær, langtids functions-nedsættelse. Væsentlig bedring er usædvanlig (under 6%)."
Andersen MM et al. Illness and disability in Danish CFS patients at diagnosis and 5-year follow-up. J Psychosom Res 2004; 56: 217–229

Hvorfor VASKULÆR?

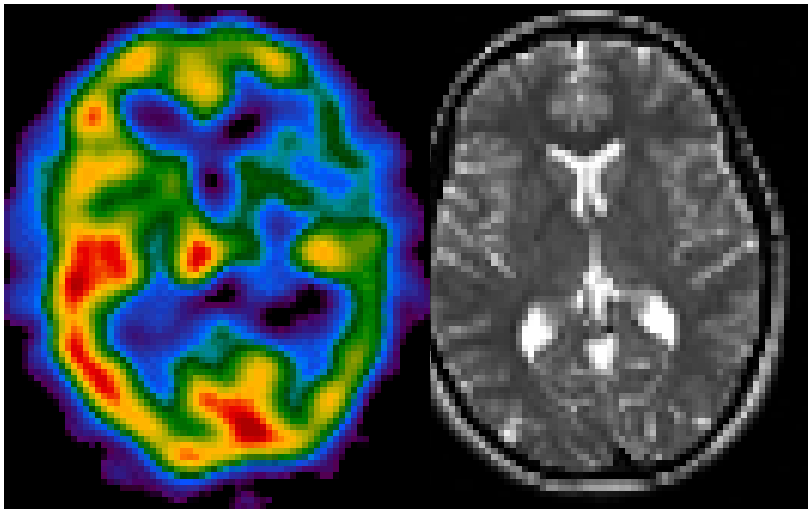


HMPAO SPECT imaging af normal hjerne – symmetrisk hjernegennemblødning [99mTc-HexaMethylPropyleneAmine Oxime] -> CFS/ME: cerebrale perfusions defekter, som involverer multiple vaskulære områder!





HMPAO SPECT and T2 weighted MRI in a patient with CFS/ME



"….. the findings are consistent with the hypothesis that CFS results from viral infection of neurons, gIia, or vasculature **….**"

Schwartz et al AJR 1994;162: 943-951

SPECT/PET imaging in Lyme Disease

- Fallon BA et al. Functional brain imaging and neuropsychological testing in Lyme disease.
  Clin Inf Dis 1997; 25: S57-S63.
- Sumiya H et al. Brain perfusion SPECT in Lyme neuroborreliosis.
  J Nucl Med 1997; 38: 1120-1122.
- Logigian EL et al. Reversible cerebral hypoperfusion in Lyme encephalopathy.
  Neurology 1997; 49: 1661-1670.
- Plutchok JJ et al. Serial brain SPECT imaging in chronic Lyme encephalopathy.
  J Nucl Med 1997; 38: 278P
- Newberg A et al. Cerebral metabolic changes associated with Lyme disease.
  Nuclear Medicine Communications, 2002, 23, 773-777
- Plotkin et al. FDG-PET studies of acute brainstem Lyme neuroborreliosis.
  J Neurosurg 102:927–929, 2005

ORTHOSTATISK INTOLERANS
[ude af stand til at blive stående pga. subjektive (symptomer) eller objektive fund som lavt blodtryk (hypotension) (tegn)]

Vance Spence and Julian Stewart
University of Dundee, UK and New York Medical College, US

Data, data everywhere and no one stops to think!
(With apologies for the adaptation to Samuel Taylor Coleridge and the line in The Ancient Mariner – 'Water, water, everywhere, Nor any drop to drink'.)

Myalgic encephalomyelitis (ME) does not receive a very good press. A recent example in a 'for' and 'against' argument in the *Daily Mail* (*Is the ME illness all in the mind?* 26 May 2003) had one correspondent stating: '*I don't believe ME exists; those who are genuinely ill and disabled possibly have something else wrong with them – the rest should get a life*'. A cruel comment – yet it has a ring of truth to it. While many claim to have the illness, they cannot prove it and the term ME is probably over-diagnosed, over-used and, for many people, over-bearing.

ME is not your conventional illness: it doesn't have diagnostic tests, it doesn't have treatment, and, most importantly, it has very few members of the 'caring professions' looking out for it. There are even arguments about the name. The term, ME, has largely been subsumed under the generic term chronic fatigue syndrome (CFS), which begged Simon Loblay, in the Medical Journal of Australia, to ask: '*Is CFS a recognisable disease entity with a unique pathophysiology, or is it a ragbag of common non-specific symptoms with many causes, mistakenly labelled as a syndrome?*' There is no straightforward answer; it depends who you ask. However, the 1998 statement by the then Chief Medical Officer, Sir Kenneth Calman, serves notice on the scale of the problem, '*I recognise that chronic fatigue syndrome is a real entity. It is distressing, debilitating, and affects a very large number of people. It poses a significant challenge to the medical profession*'. This led to a report (Department of Health, 2002), which concluded '*A programme of research on all aspects of CFS/ME is required. Government investment in research on CFS/ME should encompass health-services research, epidemiology; behavioural and social science, clinical research and trials, and basic science*'. In the context of that conclusion and in light of the recent MRC report on the illness (2003), it is prudent to ask what direction basic science research should take.

While the cause of CFS/ME remains to be elucidated, extensive literature exists on non-specific findings, including: the role of a variety of infectious agents; up-regulation of anti-viral pathways; immune abnormalities; disruption to the hypothalamic pituitary adrenal axis (HPA axis); neuropsychological impairments; dysfunction of the autonomic nervous system; oxidative stress; and lipid peroxidation. These have been recently reviewed (Afari and Buchwald, 2003), as has the role of cytokines and other immunological markers in the illness (Patarca-Montero *et al.*, 2002). None of these biological findings, however, can possibly be afforded the status of a marker since they are not necessarily part of the major disease pathway. They are, then, no more than factors somehow involved in the

Title image. Tilt table testing can be used to assess orthostatic intolerance associated with CFS/ME.

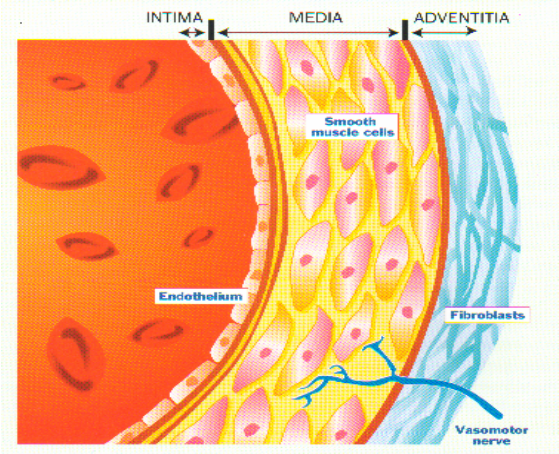
Udelader her grafer og fotos der viser blodtryksfald / pulsstigning og synlige ændringer i hud-gennemblødning under ortotatisk blodtrykstest.

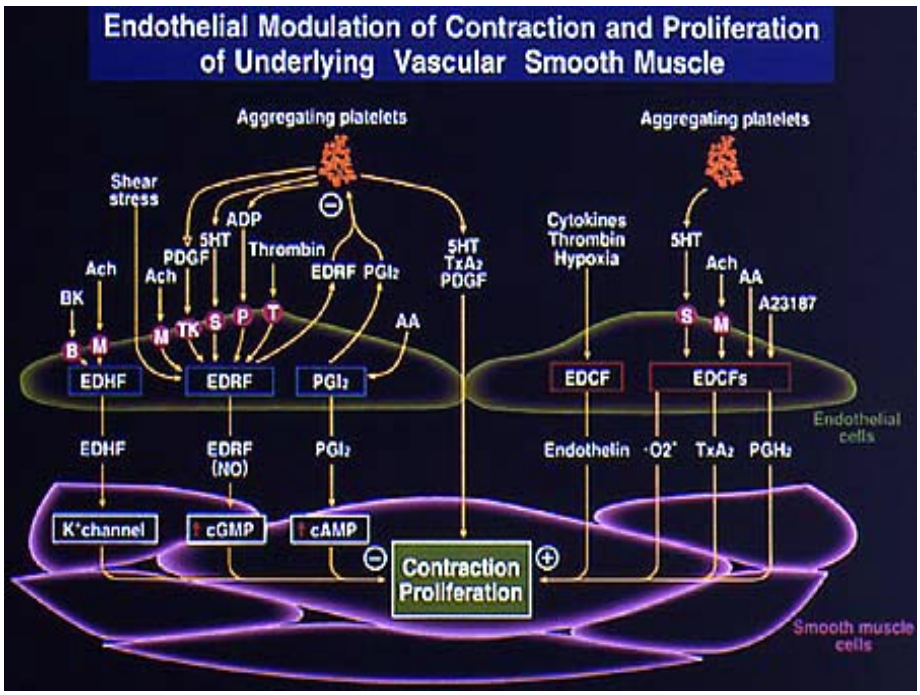
Er blodgennemstrømnings problemet ved CFS/ME CENTRAL eller LOKAL (peripheral)?

"Der er reduceret hjertefunktion (cardiac output) ved svær CFS/ME **hvor blodtrykket opretholdes** på bekostning af nedsat gennemblødning resulterende i nedsat cirkulation / langsomt blodflow"

Peckerman et al. Abnormal Impedance Cardiography Predicts Symptom Severity in CFS. Am J Med Sci 2003;326:55–60

Karvæggen





Endothel (indre karvæg) modulerer sammentrækningen og væksten af den underliggende vaskulære muskulatur

Biokemiske og vaskulære markører ved CFS/ME, OPIDN & GWS

- **<u>Del i</u> Klinisk undersøgelse –** SF36, MOS, historie, symptomer
- **<u>Del II</u> Blod gennemstrømning –** Respons på NO, ACh & MCh
- **<u>Del III</u> Blod prøver**
  - i) Lipid analyse
  - ii) Enzymer (AChE, BChE, PON)
  - iii) Inflammatoriske markører
  - iv) Oxidativ stress: a) muskel b) endothel c) fagocytotiske celler
  - v) Neutrophil apoptosis (programmeret celle død)
  - vi) Arteriel eftergivenhed
  - vii) Koagulations markører

Patienter

- **3 <u>grupper</u> som alle opfyldte 1994 CDC kriterierne for CFS**
- **103 patient indvilligede i deltagelse**
- **6 frafaldne/udelukkede – opfyldte ikke CDC 1994 CFS kriterier**

Patient grupper

1) CFS/ME [uden kendt udsættelse for OP] (n=48)
2) Organophosphate (OPIDN) [Land arbejdere] (n=25)
3) Gulf War Illness (GWS) [Golf krigs Veteraner] (n=24)

Demographic data for CFS, OP & GWS groups

	No.	Age Mean (Range)	Sex F:M	Smoking Y:Ex:N	BMI [kg/m^2] Mean (Range)
CFS/ME patients	48	47.5 (19-63)	30:18	6:2:40	26.2* (16.3-39.6)
CFS/ME controls	34	45.9 (19-63)	21:13	5:2:27	23.9 (18.9-32.9)
OP patients	25	47.7 (33-64)	3:22	2:4:19	28.0** (22.9-35.2)
OP controls	18	45.9 (30-59)	3:15	3:2:13	25.2 (20.6-29.9)
GW patients	24	39.7 (29-52)	1:23	12:4:8	30.6 # (18.1-38.6)
GW controls	18	40.6 (29-52)	1:17	8:0:10	25.3 (20.8-33.2)

Kennedy G et al. Annals of Epidemiology 2004; 14: 95–100

KLINIK

1994 FUKUDA CDC classification symptoms (%)

	CFS/ME n=48	OP n=25	GW n=24
Sore Throat	52%	48%	46%
Concentration/Memory Problems	100%	96%	100%
Swollen/tender Glands	56%	24%	38%
Muscle Pain	94%	24%	38%
Joint Pain	77%	76%	96%
Headaches	58%	68%	88%
Sleep	90%	72%	96%
Post Exercise Fatigue	100%	96%	96%

Kennedy et al Ann Epidemiol 2004; **14**: 95–100.

… SF-36 – alle 3 grupper markant lavere end raske kontroller

Biokemiske og vaskulære markører:

INFLAMMATIONS MARKØRER

Høj Sensitiv C-reaktiv protein (hsCRP) (p > 0,005 for ME/CFS Mann-Whitney U-test) klart forhøjet ved ME/CFS ikke ved OP eller GWS!

TGFβ-1 signifikant højere for ME/CFS sml. med kontroller

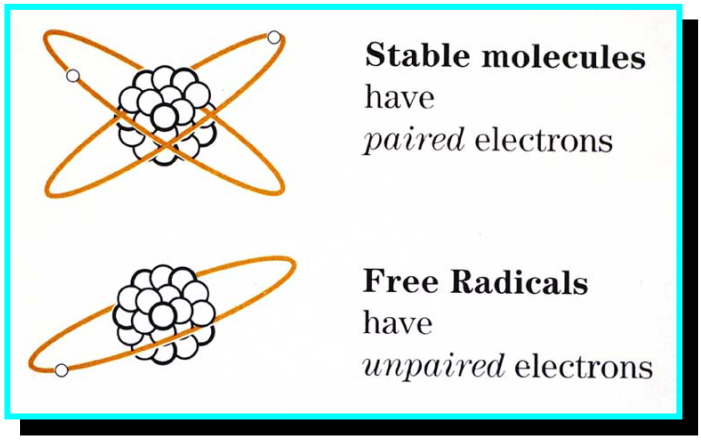
TGFβ-1 er forhøjet ved både tidlig og sen neuroborreliose i forh. til kontroller
Widhe M et al. Cytokines in Lyme borreliosis: lack of early TNF-alpha and TGF-beta1 responses are associated with chronic neuroborreliosis. Immunology 2002 107 46–55

TGFβ-1 er ikke forhøjet ved GWS!

Konklusion: ME/CFS er pro-inflammatorisk – hvilket har betydning for endothel og glat muskelcelle biologi

OXIDATIONS MARKØRER

FRIE RADIKALER:



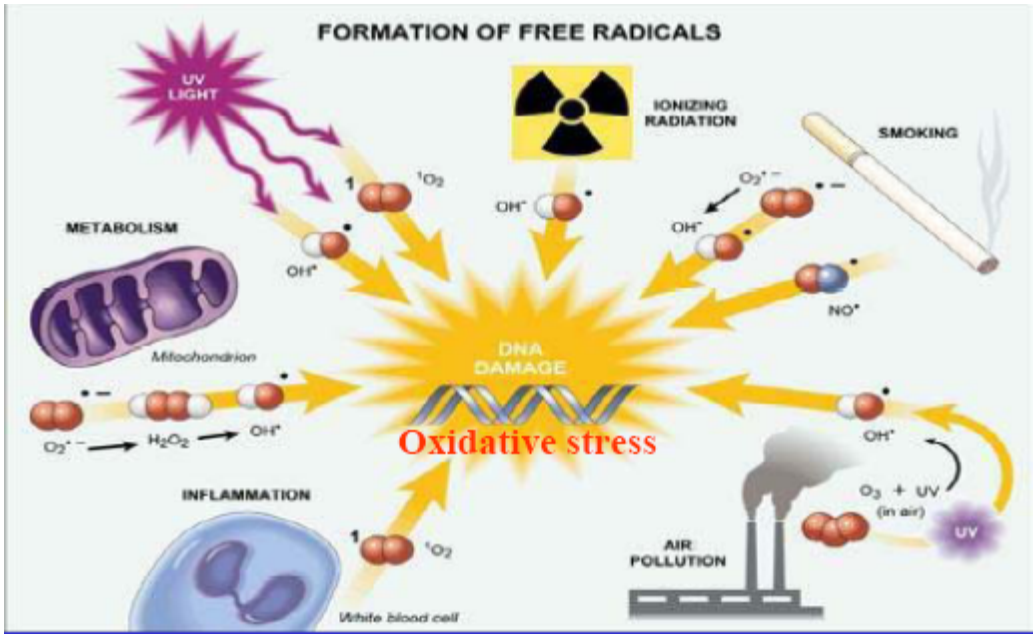
100 millioner frie radikaler dannes hver dag i vores krop

- **10,000 DNA skader per dag per celle**
- **Defekter der slipper forbid de front-linie forsvaret skal repares og skadede organeller skal fjernes**

Oxidativt stress: ubalance mellem pro- og antoioxidant systemet der normalt holdes i balance

ANTI-OXIDANTER: Superoxide-dismutase, Glutathione-peroxidase, Katalase, Vitaminer E og C
PRO-OXIDANTER: Superoxide anion peroxynitrit, oxideret glutathion, hydrogen peroxid, hydroxyl radikal, lipid peroxider.

Frie Radikal skabende faktorer: Ioniserende stråling, luft forurening, rygning, metabolisme, inflammation



Nyere arbejder om oxidativt stress ved CFS/ME:

- Øget fri radikal dannelse ved CFS/ME patogenese er foreslået *(Richards et al 2000*)
- Øget følsomhed for lipid peroxidering *(Manuel et al 2001, Keenoy et al 2001, Fulle et al 2000)*
- Øget NO dannelse ved CFS/ME – reagerer med superoxid radikal og danner den potente oxidant peroxynitrit *(Pall 2000)*
- Sammenhæng med CFS symptom præsentation *(Richards et al 2000,* Vecchiet *et al* 2003) *Oxidative damage to muscle (Fulle et al 2000)*

Blod test & Metoder:

Test

- Lipid profil (HDL & total cholesterol)
- Ox-LDL, GSH & plasma isoprostanes, MDA

Metoder

- Lipider – målt på en Cobas Bio analyser
- Ox-LDL målt ved ELISA
- Rød blod celle GSH (glutathion) målt med et spektrofotometer
- Plasma isoprostaner malt på en GCMS(?)

ISOPROSTANER er stabile og pålidelige markører for oxidant stress in vivo
Celle membran phospholipider -> Arachidon syre (ω-6 EFA's) -> **COX-1** -> prostaglandiner -> eg. Prostacyclin => VASODILATOR
Celle membran phospholipider -> Arachidon syre (ω-6 EFA's) -> **FRIE RADIKALER** -> Isoprostaner -> e.g. 8-iso PGF2 alpha -> VASOKONSTRIKTOR

Frie Radikaler og Arachidon syre kaskaden (detaljer udeladt) fører til : PGI2, TxA2, PGE/F

RESULTATER: Markører på oxidation ved CFS/ME, OP & GWS patienter:

Measurement	*CFS/ME Patients*	*OP Patients*	*GWS patients*
Cholesterol	NS	NS	NS
HDL	↓↓↓	↓	NS
oxLDL	↑↑	NS	NS
GSH	↓ & =	NS	NS
F2-isoprostanes	↑↑↑	NS	NS

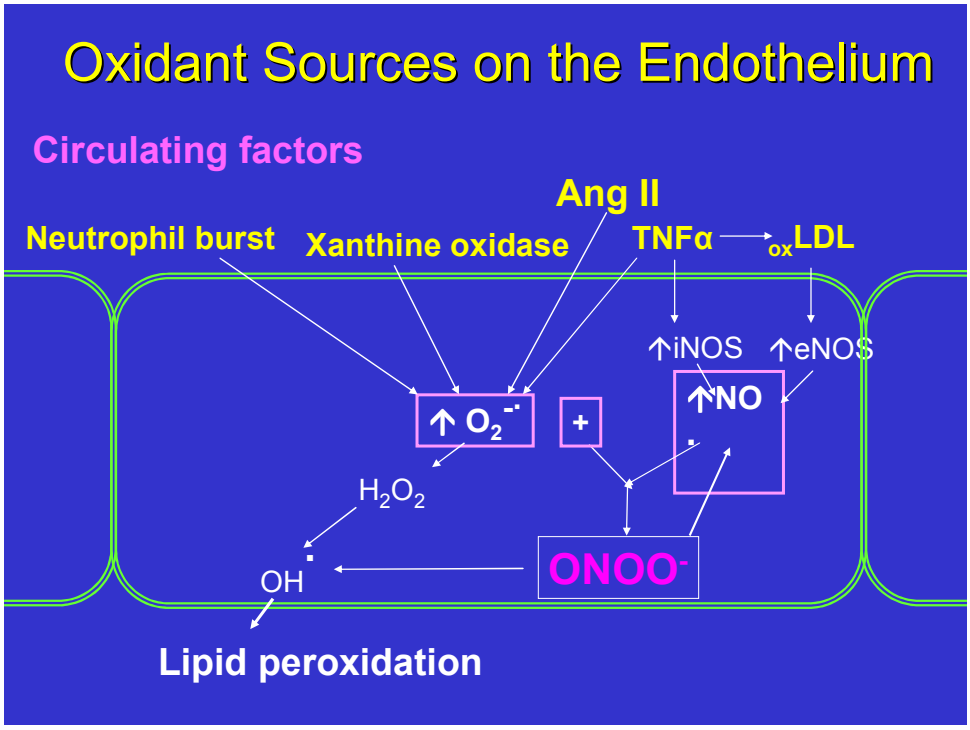
Øget isoprostan niveau er associeret med post-anstrengelses muskelsmerter hos CFS/ME patienter

"Isoprostan (8-epi-PGF2α) er ofte forhøjet hos patienter med muskel smerter som er på "sikker" statin (cholesterol-sænkende) terapi"
<u>Atorvastatin (A) & Fuvastatin (F) øger 8-epi-PGF2a</u>
Sinzinger H et al. J Clin Pharm & Ther 2001; <u>26</u>: 303-310

<u>KONKLUSION:</u> CFS/ME er såvel Pro-oxidant som Pro-inflammatorisk

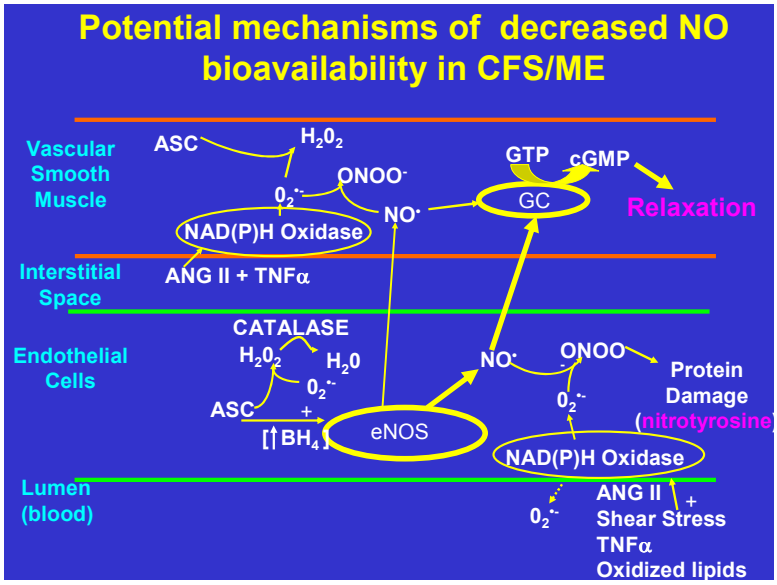
<u>Mål for ME/CFS forskningen:</u> FINDE KILDERNE TIL FRIE RADIKALER: Muskel, endotel, inflammatoriske/immun celler

McArdle F et al. Intracellular generation of reactive oxygen species by contracting skeletal muscle cells. FRBM 2005 In Press



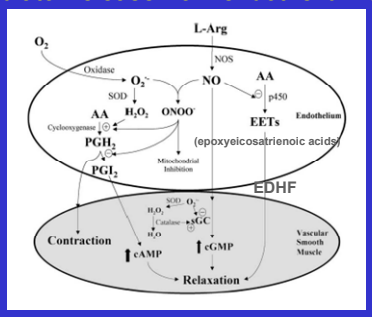
Effekten af øget ONOO-:

- **Bioatilgængelighed af nitrit oxid (NO)**
- **Udtrykke iNOS via NFkB**
- **Prostacyclin syntase udtrykkes**
- **Aktiverer matrix metalloproteinaser (MMPs)**
- **MMP-2 danner et nyt vasokonstriktor peptide, medium endothelin (ET-1)**
- **Calcitonin gen relateret peptide (CGRP)-mediated vasodilatation**

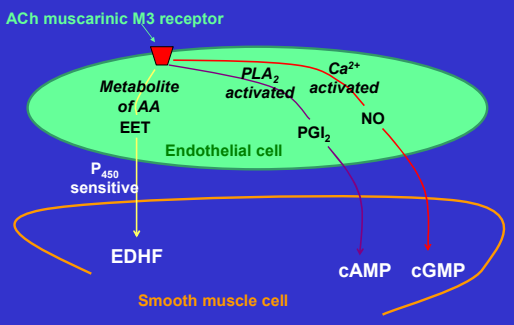


Nitrotyrosin rester - et "fod aftryk" efter ROS/RNS skade

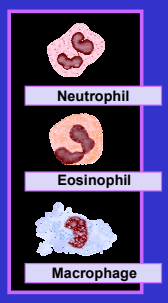
Superoxide interactions modulating vasoactive mediator release from endothelium and SMC



Vasodilator pathways via the endothelium



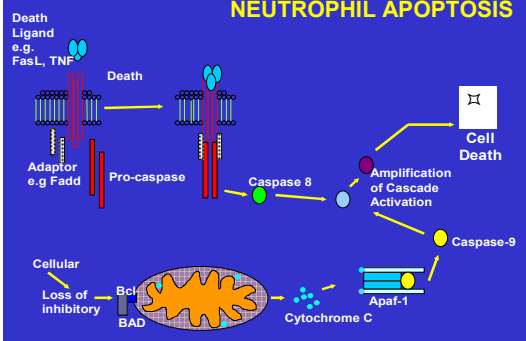
Oxidative Stress: inflammatory cells



Oxidants Released

$O_2^{\cdot -}$
H_2O_2
HOCl
$^{\cdot}NO$
$ONOO^-$
$^{\cdot}NO_2$
$HO^{\cdot}$
$^1O_2$

NEUTROPHIL APOPTOSIS



Neutrofile

- **50-70% af alle hvide blodceller**
- **"Første line forsvar" mod infektiøse substancer**
- **Kan forlade blod cirkulationen og vandre ud i vævene og finde bakteria el. fremmedlegemer**
- **Ødelægger bakterier, virus og fremmedlegemer via en proces kaldet fagocytosis**

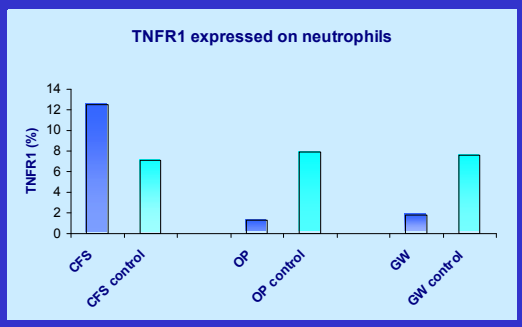
Neutrofil apoptose

- **Fagocytose af intakte bacterier resulterer i↑ apoptose af den neutrofile (i.e. den neutrofiles død)**
- **↑ Neutropfil apoptose – observes i patienter med infektion**
- **Simpelt forklaret – den specielle sekvens af hændelser der leder til neutrofil apoptose udløses af faktorer der er tilstede i blod, som binder til receptorer udtrykt på den neutrofiles overflade**

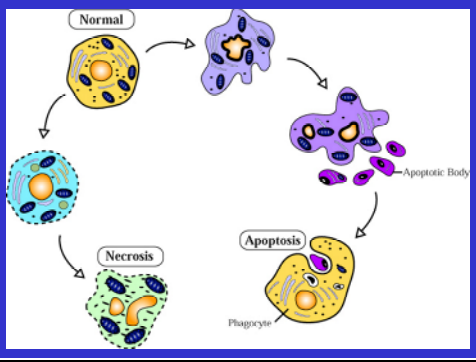
Neutrophil Apoptosis Results

	CFS/ME patients	*OP patients*	*GWS patients*
Apoptosis receptor TNF-R1	↑↑↑	NS	**NS**
Apoptosis initiator Annexin V	↑↑↑	**NS**	**NS**
% of neutrophils undergoing apoptosis	↑↑↑	**NS**	**NS**

Neutrophil apoptosis in CFS/ME, OP, GW



Neutrophil cell death/ apoptosis vs necrosis



Neutrophil apoptosis in CFS/ME

NEUTROPHILS	CFS/ME patients	Controls	p <
Normal Viable cells (Range)	63 % (56 - 70)	77 % (71 - 81)	0.002
Early apoptotic cells % (Range)	32 % (26 – 38)	20 % (16 – 25)	0.002

Kennedy et al J Clinical Pathology 2004;57:891–893

<u>Øget neutrofil apoptose fandtes KUN i CFS/ME gruppen IKKE i OPIDN eller GWS -kunne skyldes:</u>

- Toxisk stadie
- Reprogrammering af den apoptotiske vej
- Hurtigere neutrofil omsætning
- Persisterende viral eller bakteriel infektion

↑ apoptotiske neutrofile i CFS/ME => ↑ respiratorisk (oxidative) burst => ↑ FRIE RADIKALER
=> **Celle & systemiske skader**
Frie radikaler => H_2O_2 => ↑ apoptotiske neutrofile

Er CFS en bakteriel infektion?
– ja sandsynligvis for mange CFS pt.
– Problemet er hvilken infektion i hvilken pt.

Hel-genom DNA Mikroarray Analyse af Gen udtryk i CFS (Gow & al. Glasgow) <u>konklusioner</u>:

- Skift af immunrespons med fortrinsvis antigen præsentation til MHC class II receptorer IL-13 & IL-6 biosyntese; Antigen processering via MHC class II (MHC II DP a1 & DR a)
- Nedregulering af MHC class I system medførende suppression af NK celler og T-celle receptor CD158K (LL), HCP5 (HLA complex 5) T-cell receptors a,b,d,g
- Øget celle-membran prostaglandin-endoperoxidase syntase aktivitet medførende ændringer i oxygen transport
- Prostaglandin syntase 1 & 2; haemoglobin a,b,d,g
- Makrofage activering
- Toll factors 4 & 8, IL1, CD16a
- Neutrofil apoptosis proteins
- Phosphatidylserine, thrombospondin, defensin α

INDICES of STIFFNESS

	CFS/ME N=47	Controls N=34	P value
Augmentation (mmHg)	9.5 (6.4)	6.6 (6.6)	0.05
Sub-endocardial viability ratio (%)	166 (32)	196 (30)	0.005
Aortic MAP (mmHg)	119 (18)	105 (13)	0.003

CFS/ME patients have significantly increased arterial stiffness.

Arterial eftergivenhed / stivhed i ME/CFS – puls bølge analyse:

CFS/ME patienter har signifikant øget stivhed af arterier

Van de Putte EM et al. Is chronic fatigue syndrome a connective tissue disorder? A cross-sectional study in adolescents. Pediatrics. 2005; (Apr)115:415-22

- Bindevævet normalt
- Ingen led hypermobilitet
- Normal collagen omsætning

MEN … dette til trods findes arterierne I unge med CFS langt stivere end forventet, hvilket kræver yderligere undersøgelser!

<u>Kennedy, Spence et al :</u>

Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms.
Free Radic Biol Med. 2005 Sep 1;39(5):584-9.

Increased neutrophil apoptosis in chronic fatigue syndrome.
J Clin Pathol. 2004 Aug;57(8):891-3.
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