Gulf War Illness:
Some BioMarker Findings

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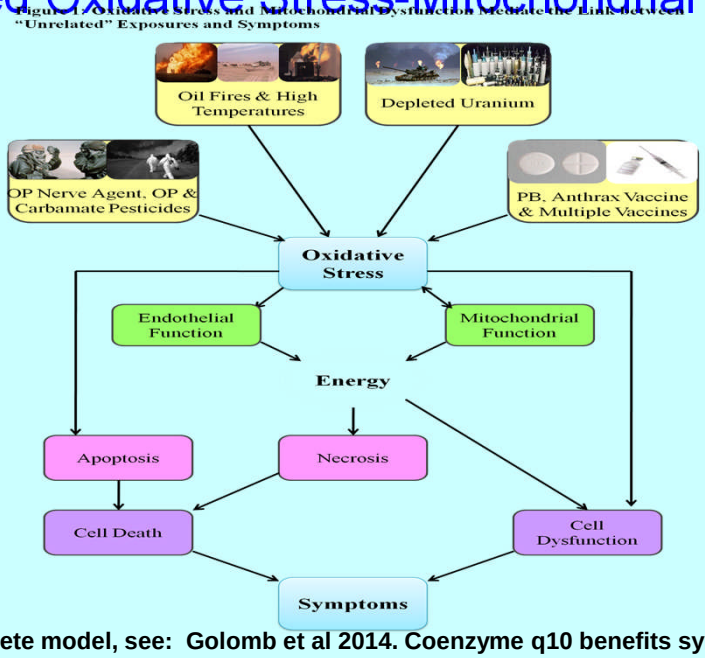
Simplified Oxidative Stress-Mitochondrial Model



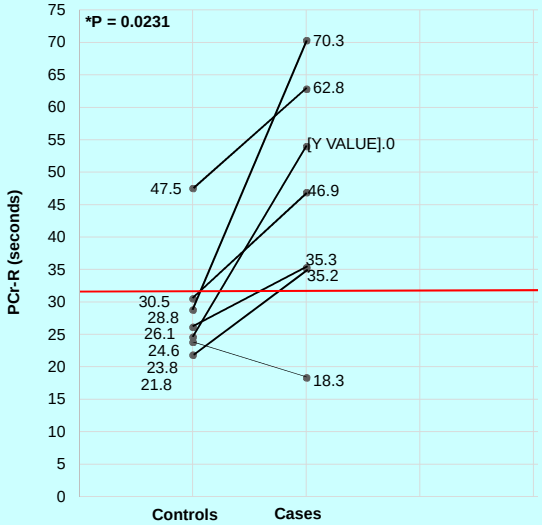
For more complete model, see: Golomb et al 2014. Coenzyme q10 benefits symptoms in gulf war veterans: results of a randomized double-blind study. *Neural Comput* 26:2594-651.

GWI Features Consistent with MD-OS

MD = mitochondrial dysfunction. OS = oxidative stress.	GWI	MD-OS
Symptoms: Fatigue, muscle, brain sx archetypal	√	MD
Symptom multiplicity+ heterogeneity: encompass GI, psych, sleep, dyspnea, vision, exert'l intol…	√	MD
Variable latency to symptom onset	√	MD
Exposures: AChEi especially strong relationship	√	OS+MD
Other chemically "unrelated" exposures contributory – common toxicity by OS	√	OS
Objective Markers: ↓ PON; ↓ HRV; ↓NK cell fxn	√	OS
↑ autoantibodies; ↑ infl+coagulation activation; ↑GGT	√	OS
Attendant Conditions: ↑ALS; ↑HTN; ↑CFS-FM-IBS	√	OS-MD

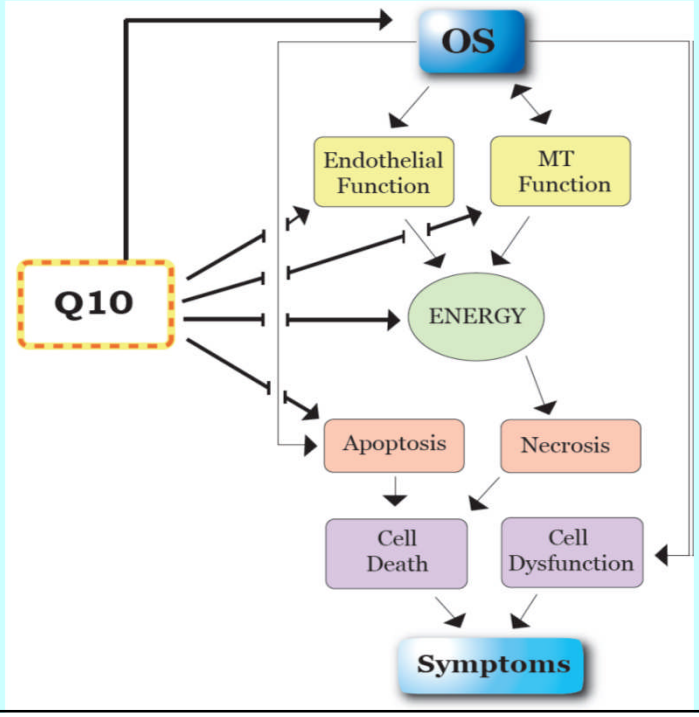
Golomb BA. Oxidative Stress and Mitochondrial Injury in Chronic Multisymptom Conditions: From Gulf War Illness to Autism Spectrum Disorder. Available from Nature Precedings <http://hdlhandlenet/10101/npre201268471> (2012).

PCr-R of GWV with GWI vs Matched Controls



GWV = Gulf War veteran; **GWI** = Gulf War illness; **PCr-R** = phosphocreatine-recovery post-exercise time constant.
Line segments connect controls to their case matches.
Note clustering of most control (but not case) PCr-R values under 31 sec, with widely distributed case values primarily > 35 seconds.

Koslik HJ, Hamilton G, Golomb BA. Mitochondrial Dysfunction in Gulf War Illness Revealed by 31Phosphorus Magnetic Resonance Spectroscopy: A case-control study. *PLoS ONE* 2014;9:e92887.



Q100 vs Placebo Benefited Symptoms and Function in GWI

Participants: 46 GWV met Kansas & CDC criteria for GWI

Intervention: PharmaNord CoQ10 vs placebo x 3.5 mos

Results (Q100 vs placebo):

- **GSRH**: Disparities at baseline. Significant in men (85%): p=0.04
- **Symptoms**: 20 sx each present in ≥50% of participants
  - 7 of 20 signif (p<0.05): all favored Q100 (sign test p=0.004).
  - 19 of 20: Direction favored Q100 (sign test p=0.00004).
    - (All but sleep problems, NS).
- **Function** (SPS): >80% vs 40% improved: p=0.025

Change coQ10 related to:

- Function (SPS) change: p=0.033 GSRH change: p=0.064

Golomb et al 2014. Coenzyme q10 benefits symptoms in gulf war veterans: results of a randomized double-blind study. *Neural Comput* 26:2594-651.

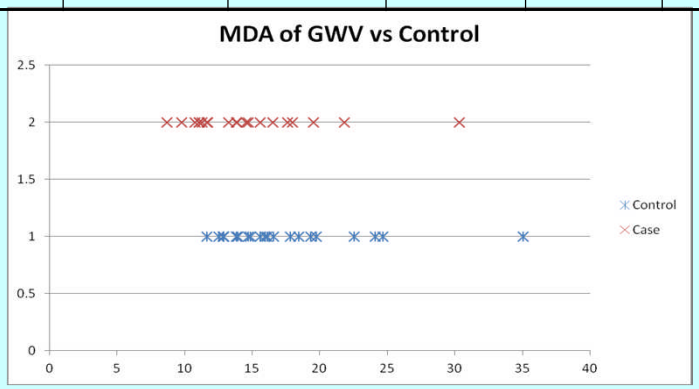
Lipid Derived Markers: Eicosanoids, products of arachidonic acid - are depressed in GWI

	Case	Control	Difference		
Test	**Mean**	**Mean**	**Mean**	**SE**	**P**
Ln(**PGF2a**)	3.3	4.3	-.94	.26	0.001
Ln(**LB4**)	2.1	3.1	-1.0	.35	0.009
PGF2a	43.4	104	-60.8	20.8	0.006
Ln(**PGD2**)	4.4	5.6	-1.2	.45	0.01
LB4	19.1	51.8	-32.6	14.6	0.03
PG D2	310	1001	-692	312	0.03
13,14-dihydro-15-**keto-PGD2**	5.2	16.5	-11.3	5.5	0.05

Dr. John Repine, PI, DoD CDMRP GW093021

MDA-- *also a product of arachidonic acid* - is depressed in GWI

	Case	Control	Difference		
Test	**Mean**	**Mean**	**Mean**	**SE**	**P**
Ln(MDA)	2.6	2.8	0.18	0.057	0.004
MDA	15	18	2.9	0.097	0.004



What is MDA

A product of arachidonic acid metabolism – like eicosanoids:

"Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids.[3]

It is a prominent product in Thromboxane A2 synthesis, wherein cyclooxygenase 1 or cycloxygenase 2

metabolizes arachidonic acid **{emphasis added}**

to prostaglandin H2, by platelets and a wide array of other cell types and tissues."

Wikipedia, "Malondialdehyde" 2015-09-28

Candidate "General" OS Markers designated by NIH group[1] predict GWI negatively, positively, and neutrally. (We will see there is widespread lipid dysregulation.)

	Coeff (SE)	P	95% CI
Ln (MDA)	-3.5 (1.5)	**0.024**	-6.5, -0.47
8-OHDG	2.7 (1.3)	**0.039**	0.14, 5.2
F2iso	*0.003 (0.003)*	***0.34***	*-0.003, 0.008*

R^2 = 0.20 Sample: Age-, sex-, and ethnicity matched GWI cases and controls (N=40)

Logistic regression: Outcome = GWI Case Status.

Adjusts for age and sex (these are still a source of variance, though not a confounder)

DoD CDMRP GW093063.

[1]Kadiiska et al. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005;38:698-710.

OS and CAC (+lipid-based) Markers Predict GWI Case Status

	Coeff (SE)	P	95% CI
Ln (MDA)	-5.5 (1.7)	**0.001**	-8.9, -2.1
8-OHDG	2.7 (1.2)	**0.022**	0.39, 5.0
Citrate	-0.33 (0.17)	**0.045**	-0.66, -0.0076
Ln (Fumarate)	4.7 (2.0)	**0.016**	0.88, 8.6

R^2 = 0.35 Sample: Age-, sex-, and ethnicity matched GWI cases and controls (N=40)
Logistic regression: Outcome = GWI Case Status.
Adjusts for age and sex (these are still a source of variance, though not a confounder.)
DoD CDMRP GW093063. Thanks to Dr. Richard I. Kelley for CAC marker assessment.

OS and CAC Markers Predict GWI Case Status

	Coeff (SE)	P	95% CI
Ln (MDA)	-5.6 (1.7)	**0.001**	-8.9, -2.3
8-OHDG	6.1 (2.6)	**0.019**	0.99, 11.1
Ln (PGF2a)	-2.4 (1.4)	**0.081**	-5.0, 0.29
Ln (Fumarate)	4.2 (1.9)	**0.028**	0.46, 7.9
Citrate	-0.29 (0.16)	**0.069**	-0.075, 0.27

R^2 = 0.47.
Sample = GWI cases and age-, sex-, and ethnicity matched controls (N=40)
Logistic regression: Outcome = GWI Case Status.
Adjusts for age and sex (these are still a source of variance, though not a confounder.)
DoD CDMRP GW093063; Also, DoD CDMRP GW093021 (Repine) for PGF2a.
Thanks to Dr. Richard I. Kelley for CAC marker assessment.

Markers Predict GWI Case Status. …

	Coeff (SE)	P	95% CI
Ln (MDA)	-5.6 (2.7)	**0.012**	-10.0, -1.2
PGF2a	-0.038 (0.015)	**0.014**	-0.068, -0.0076
Ln (mal)	-7.7 (3.1)	**0.014**	-13.9, -1.6
Ln (akg)	7.5 (3.2)	**0.019**	1.2, 13.8

R^2 = 0.47.
Sample = GWI cases and age-, sex-, and ethnicity matched controls (N=40)
Logistic regression: Outcome = GWI Case Status.
Adjusts for age and sex (these are still a source of variance, though not a confounder.)
DoD CDMRP GW093063; Also, DoD CDMRP GW093021 (Repine) for PGF2a.
Thanks to Dr. Richard I. Kelley for CAC marker assessment.

CAC and OS Markers Shift in Parallel

	Controls		GWI Cases	
	r	P	r	P
AKG & Uric Acid	-0.11	0.56	**+0.55**	**0.001**
Isocitrate & GGT	-0.0095	0.96	**+0.48**	**0.006**
Fumarate & F2iso	0.066	0.78	**+0.66**	**0.0015**
AKG & MDA	**+0.69**	**0.0007**	0.21	0.38

∴ New Correlations between OS and CAC Markers are forged. Normal ones are lost.

Metabolomics of Gulf War Illness

Preliminary Draft for Beatrice Golomb
Jane C. Naviaux, Kefeng Li, A. Taylor Bright,
William A. Alaynick, Robert K. Naviaux
University of California, San Diego School of Medicine

Study

- **Sample N = 40**
  - **20 male GWV with GWI (CDC+Kansas)**
  - **20 male age-sex-ethnicity matched controls**
- **Plasma assessed by NextGen Metabolomics using liquid chromatography with tandem mass spectrometry (LC-MS/MS) on an ABSCIEX 5500***
- **617 analytes targeted**
  - **450 analytes were detectable**
  - **167 analytes below detectable limits**

"While two compounds may have similar UV spectra (liquid chromatography) or similar mass spectra, it is uncommon for them to have both. The two orthogonal sets of data can be used to confidently identify, confirm, and quantify compounds." *

Findings May Have Treatment Implications

E.g.: Inactive 25-OH Vitamin D was strongly increased

Suggests a block in renal mitochondrial 1-alpha hydroxylation and consistent with a chronic oxidative state

-- Supports observations of benefit with vit D or CLO (and attempted treatment trial submissions!)

E.g. Prominent role for phospholipids, including phosphatidylcholine

-- Supports observation of veteran member of RAC who cited benefits from lecithin

-- Supports my observations of added benefit with WGO

Summary: Pre-Metabolomic

- **Evidence of dysregulation affecting mt & OS-related parameters**
- **Evidence of coordinated shifts in OS and mt-related markers, leading new relationships to be forged**
- **Evidence of widespread dysregulation affecting lipid based markers, arachidonic acid products -- eicosanoids and MDA**
- **Lipid based markers of OS change relation to other markers of OS in GWI**
- **With just a couple markers of mt fxn, and a couple more of OS and/or lipid alteration, able to separate GWI from control with $R^2 = 0.47$**
- **Can't exclude factors/ exposures related to our sample**

Summary: Metabolomic

Widespread dysregulation in phospholipids, sphingolipids, sterois.

Strong link to membrane status. Powerful resemblance to mt metabolomics.

Also markers bear on OS protection, mt fxn, apoptosis, myelin production

Provide a new lens for existing objective alterations (e.g. corticosteroid alterations)

Provide a new lens for observed treatment benefits (lecithin, CLO, WGO)

Suggest new treatment approaches

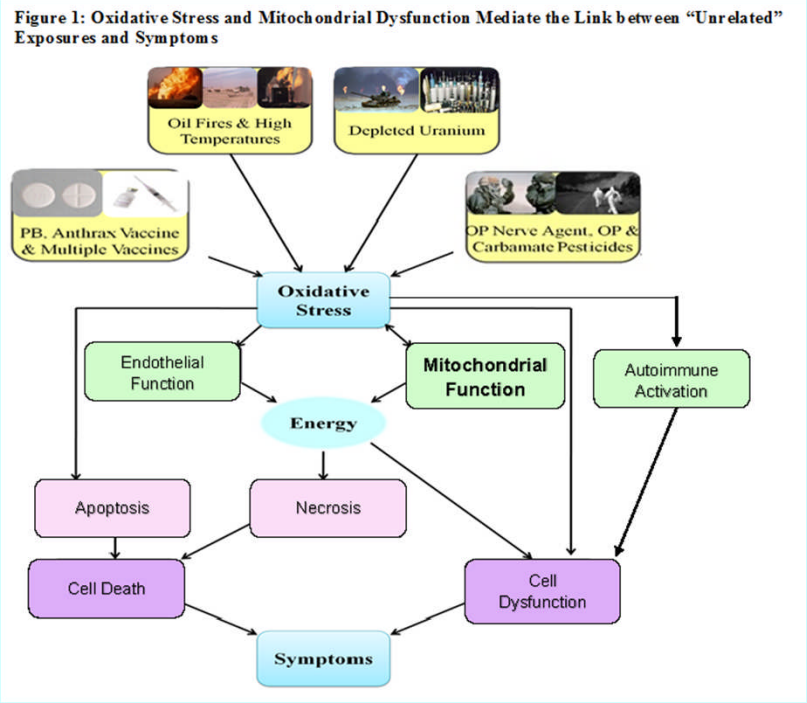
Profiles differentiate GWI from other groups with controversial tie to GWI:

– CMI (CFS); "war related illness" (PTSD)

Metabolomics able to completely separate GWI from control

Corroborate/ extend premetabolic focus on lipids, OS, mt.

Thank You



Sphingomyelin (SPH)

- a type of sphingolipid found in animal cell membranes, especially in the membranous myelin sheath that surrounds some nerve cell axons. It usually consists of phosphocholine and ceramide or a phosphoethanolamine head group.Can also be classified as sphingophospholipids.[1] In humans, SPH represents ~85% of all sphingolipids, and **typically make up 10-20 mol % of plasma membrane lipids.**
- Sphingomyelins are present in the plasma membranes of animal cells and are **especially prominent in myelin,** a membranous sheath that surrounds and insulates the axons of some neurons—thus the name "sphingomyelins.

Niacin Niacinamide NAD

1. Nahid A Khan, Mari Auranen, Ilse Paetau, Eija Pirinen, Liliya Euro, Saara Forsström, Lotta Pasila, Vidya Velagapudi, Christopher J Carroll, Johan Auwerx and Anu Suomalainen. *EMBO Molecular Medicine*, April 2014 **Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3**.
2. Depeint F[1], Bruce WR, Shangari N, Mehta R, O'Brien PJ. Chem Biol Interact. 2006 Oct 27;163(1-2):94-112. Epub 2006 May 1.**Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism.**

PGF2alpha Exposure Relations

Exposure	r	P-value
Degreasing solutions	-0.35	0.003
Pesticides on clothes/ bedding	-0.35	0.003
Radioactive chemicals	-0.35	0.003
Petroleum products (e.g., oil)	-0.34	0.004
Diesel or petrochemical fuel on skin	-0.33	0.006
DEET (e.g., insect repellant)	-0.33	0.006
Asbestos	-0.33	0.006
Burning fuels	-0.33	0.006
Solvents	-0.32	0.007
Diesel or petrochemical fumes	-0.31	0.01

MDA Exposure Relations (incl cases + ctrls – in cases separately, PB was the strongest, -0.33, 0.037)

Exposure	r	P-value
DEET (e.g., insect repellant)	-0.29	**0.0087**
Head lice treatment (e.g., Lindane)	-0.25	**0.028**
Petroleum products (e.g., oil)	-0.24	**0.029**
Stored fuels	-0.23	**0.037**
Solvents-Thinners	-0.23	**0.043**

Multivariable Model – Cases. R2 = 0.36

Exposure	**β (SE)**	**P**
Solvents-thinners	-6.1 (2.6)	**0.026**
Ciprofloxacin	-6.4 (2.6)	**0.019**
PB pills	-6.7 (2.6)	**0.015**
Atorvastatin	5.3 (2.6)	**0.051**