**Appendix**

**Presentation 1 – Beatrice Golomb**

# Oxidative Stress, Mitochondria and Illness in GWV: A Hypothesis

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## Summary

GWV have illness characteristics thought to defy pathological explanation

It has been suggested these preclude a toxic explanation

It is proposed that oxidative stress and mitochondrial (mt) pathology provide a good fit with features, including previously perplexing features, of illness in GWV

This theory fits both the *exposures* linked to illness; and the *health profile* (symptom pattern) associated with illness in GWV.

This theory provides predictions, avenues for research, and hopes for treatment and future prevention.

## GWI Characteristics Needing Explanation

Prominence of fatigue, mood-cognition, muscle sx[1]

GWV have Sx spanning ↑ categories, relative to nondeployed controls[2]

Across GWV, ↑ rate of almost every sx[3]

[1]Fukuda *Jama*. 1998;280(11):981-8.

[2]Steele *Am J Epidemiol*. 2000;152(10):992-1002.

[3]Doebbeling *Am J Med*. 2000;108(9):695-704.

## GWI Characteristics Needing Explanation

Among GWI who served side by side, some developed problems and others did not [1]

Among persons who got illness, manifestations differ [1,2]

Variable and often long latency to development of illness [3]

[1] Steele *Am J Epidemiol*. 2000;152(10):992-1002.

[2]Fukuda *Jama*. 1998;280(11):981-8.

[3] Kroenke *J Occup Environ Med*. 1998;40(6):520-8.

## GWI Characteristics Needing Explanation

AChEi are particularly strong RFs.

But many exposures have been associated (less strongly and consistently) to risk

No single exposure is common to all ill GWV[1]

Illness is associated with the number of such exposures[1]

GWV are at increased risk of ALS[2,3]

[1] Doebbeling *Am J Med*. 2000;108(9):695-704.

[2] Haley *Neurology*. 2003;61(6):750-6. [3] Coffman *Neuroepidemiology*. 2005;24(3):141-50.

## Protean Symptoms

"The increased prevalence of nearly every symptom assessed from all bodily organ systems among Gulf War veterans is difficult to explain pathophysiologically as a single condition"

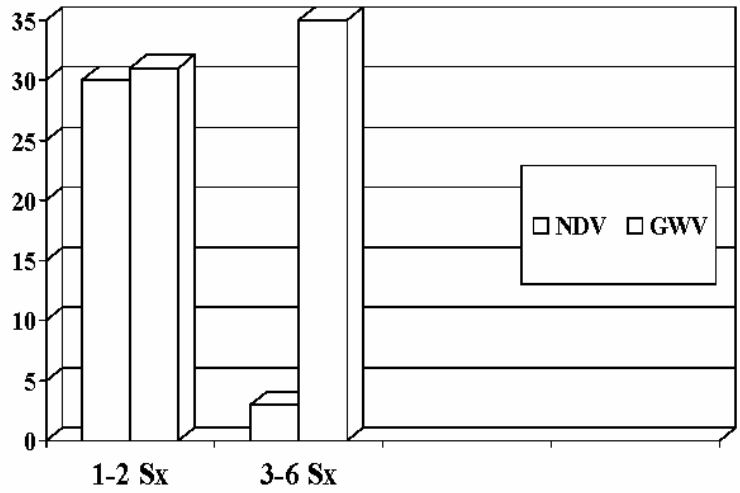
Doebbeling *Am J Med*. 2000;108(9):695-704.

## Chronic Symptoms Encompass Many Domains

| Symptom | %GW | %non | OR* | 95%CI |
|---|---|---|---|---|
| Fatigue | 36 | 12 | 4.1 | 2.9-5.7 |
| Pain muscles | 21 | 6 | 4.6 | 2.9-7.2 |
| Moderate/multiple pain | 34 | 13 | 3.6 | 2.6-5.0 |
| Recent memory | 32 | 8 | 4.9 | 3.4-7.2 |
| Irritability/outbursts | 31 | 8 | 5.2 | 5.5-7.7 |
| Concentration | 22 | 5 | 4.6 | 2.9-7.3 |
| Night sweats | 20 | 4 | 5.3 | 3.2-8.8 |
| Heat/cold intol | 18 | 6 | 3.7 | 2.3-5.9 |
| Chemical sensitivity | 17 | 4 | 4.6 | 2.7-7.8 |
| Nausea/upset stomach | 17 | 4 | 4.3 | 2.6-7.1 |
| Abd pain/ cramping | 15 | 4 | 4.2 | 2.5-7.3 |
| Mouth sores | 8 | 1 | 6.6 | 2.7-16.4 |
| Rash | 20 | 4 | 5.7 | 3.4-9.6 |
| Moderate/multiple skin sx | 19 | 6 | 4.1 | 2.5-6.6 |
| Difficult breathing/catch brth | 18 | 4 | 4.1 | 2.5-6.7 |

*Steele 2000 Am J Epi 152 992-1002 (check Steele vs Unwin)*

## Multiple Symptoms Together




Y axis: % reporting this number symptoms. *Steele 2000. Am J Epi 152:992-1002*

## Delayed Onset

**"Symptom onset was often delayed, with two-thirds of symptoms not developing until after individuals returned from the Gulf War and 40% of symptoms having a latency period exceeding one year" (many exceeding three years)**

**Kroenke** *J Occup Environ Med*. 1998;40(6):520-8.

## Elevated Risk of ALS in PGW

**ALS Death, Military Service: RR = 1.5 (1.1-2.1) p = 0.007** [1]

**ALS incidence, diagnosis before age 45 (young ALS), GW vs expected** [2]

| | | |
|---|---|---|
| **1991-4** | **0.9 (0.3-2.4)** | **p = 0.6** |
| **1995-8** | **2.3 (1.3-3.9)** | **p = 0.006** |
| **1998** | **3.2 (1.03-7.4)** | **p = 0.02** |

**∴increasing (test of trend)**

**ALS national case ascertainment, Gulf War** [3]

| | |
|---|---|
| **Deployed** | **1.9 (1.3-2.8)** |
| **Deployed active duty** | **2.2 (1.4-3.4)** |
| **Deployed Air Force** | **2.7 (1.2-5.8)** |
| **Deployed Army** | **2.0 (1.1-3.8)** |

**Confirmed elevated in GWV in capture-recapture study** [4]

[1] Weisskopf 2005 Neurology 64:32. Method; CanPrevenxStudyII cohort of AmCancerSoc, F/U1989-98 ALS mortal. 200 ALS cas
[2] Haley 2003 Neurology 61:750. Method: ALS Registries plus publicity campaign. 20 GW ALS, 17 age < 45.
[3] Horner 2003 Neurology 61:74: Method: national case ascertainment: press releases, internet, veterans service organizations, brochures to neurologists, extant VA and Department of Defense (DoD) inpatient, outpatient, and pharmacy medical records with ICD-9, use of riluzole, drug used for ALS.
[4] Coffman 2005 Neuroepidemiology 24:141

## Defy Pathological Explanation

Sx Prominence: of fatigue, mood-cognition, muscle sx

Sx Multiplicity: Persons with problems have Sx spanning ↑ categories, relative to nondeployed controls with problems

Sx Diversity: Across GWV, ↑ rate of almost every sx

Variable Development: Among veterans who served side by side, some developed problems and others did not

Variable Manifestations: Among persons who got illness, manifestations differ

Long/ Variable latency: Sx develop often years after deployment

## Defy Pathological Explanation???

Viewed through the context of environmental exposures inducing damage via oxidative stress and mt dysfunction, each of these features transforms from a conundrum to an expected illness feature.

## Exposures were Common

"New" exposures included

- PB (~250K), a carbamate AChEi
- Nerve agent (~100K??), an OP AChEi
- BT vaccine, a mercury containing vaccine
- Anthrax vaccine, a highly reactogenic vaccine (150K)
- DU, with heavy metal and low level radioactivity features
- Smoke from oil well fires

## "Highlighted" exposures included

- Pesticides & insect repellants, aggressively used, including carbamate and OP pesticides (AChEi)
- Fumes from missiles
- Tent heaters
- Solvents
- CARC paint
- Heat: desert + exertion + NBC suits

## Exposures are linked to illness

**AChEi exposures show particularly strong and consistent link to GWI (as previously reviewed) (later slide)**

**Dose response data show a significant relation between dose and illness for PB, the discretely dosed AChEi (later slide)**

**Other exposures show somewhat weaker and less consistent relationship**

**Still, the number of such exposures reported is linked to illness[1]**

[1]**Kroenke** *J Occup Environ Med*. 1998;40(6):520-8.

## Acetylcholinesterase Inhibitors

| Author | PB | Pesticide | Nerve Agent |
|---|---|---|---|
| Australian | + | + | + |
| Gray | *+ (all)* | *+ (all)* | *+ (2 of 3 models)* |
| Haley | + | + | + |
| Kang | + | + | + |
| Nisenbaum | + | + | + |
| Unwin | + | + | + |
| Cherry | + | + | |
| White | | + | + |
| Wolfe | + | + | |
| Proctor | ° | + | + |
| McCauley | | | + |
| Schumm | + | | |
| Sullivan | + | | |
| Steele | | | + |

+ = statistically significant positive association; ° = No significant association
- = statistically significant negative association (no instances). Blank = not assessed.
Two very small studies showed no signif effect (Bell strong RR but comparison Ns 9 and 14; Spencer small N)

## PB dose response

| Study | Significant Dose response? |
|---|---|
| Australian[1] | + |
| Schumm female[2] | + |
| Schumm male[3] | + |
| Wolfe[4] | + |

[1] Commonwealth Dept of Veterans' Affairs. Australian Gulf War Veterans' Study http://www.dvn.gov.au/media/publicat/2003/gulfwarhs/html/exexecutive_summary.htm
[2] Schumm Psychol Rep 2001: 88: 306-8
[3] Schumm Psychol Rep 2002 90: 707-21
[4] Wolfe 2002 J Occup Environ Med 44: 42-54

## Anthrax Vaccine Epi

| | N | RR/OR | Adjusted | Nation |
|---|---|---|---|---|
| DoD[a] | 5394 A | 1.6** | No | US |
| Unwin[b] | 4248 | 1.5* | Yes | UK |
| Hotopf[b] | 900 | 1.4* | Yes | UK records |
| Schumm[c] | 900 | 1.2** | Yes | US |
| *Gray[d]* | *3831* | *3.7*** | *No* | *US* |
| Kang[e] | 11441/15891 | 1.3** | No | US |
| *Mahan[f]* | *4601/2979* | *2.13*** | *Yes* | *US* |
| Mahan[g] | 353/2979 | 1.56** | Yes | US records |

*p < .01; **p < .001. a. Navy/marines: registry participation in ill recipients vs overall. b. UK servicemen. c. Ohio et al reservists. d. Seabees; saturated & backwd elim model loses signif, 1.01. e. Registry vs surveyed veterans, rate = rate vaccines in ill vs well, chi-2. f. Stratified sample of 15K GWV. Functional impairment. Lower for less severe outcomes. g. Exposed are subset with known anthrax vaccination history; relative to reported-no

## Anthrax Vaccine Epi

| | N | RR/OR | Adjusted | Nation |
|---|---|---|---|---|
| *Goss-Gil*[h] | *3113* | *1.9*** | *Yes* | *Canada* |
| *Steele*[i] | *1548/482* | *3.8*** | *Yes* | *US* |

*p < .01; **p < .001.

h. Chronic fatigue by "nonroutine" vaccines, RR 1.3* cognitive dysfcn.

i. Ns are GW and nonGW. Any vaccine. Kansas def GWI; 2.4** CDC def.

## These exposures induce oxidative stress

**AChEi are particularly potent oxidative stressors, and confer their toxicity and lethality by this means.**

**"Anticholinesterase compounds, organophosphates and carbamates… exert their toxicity in mammalian system primarily by virtue of acetylcholinesterase inhibition at the synapses and neuromuscular junctions... However the mechanism(s) involved in the brain/muscle damage appear to be linked with alteration in antioxidant and the scavenging system linked to free radical-mediated injury"[1]**

**→ Also: antioxidants protect against this toxicity and lethality[2]**

[1]**Milatovic** *ScientificWorldJournal.* 2006;6:295-310.[2]**Pena-Llopis** *Aquat Toxicol.* 2003;65(4):337-60.

## Other exposures induce oxidative stress

Other exposures exert their toxicity via oxidative stress, toxicity often shown defended against by antioxidants

- Reactogenic vaccines [1]
- Heavy metal (DU, mercury in vaccines) [2]
- Petroleum products (paints, solvents, exhausts, fumes) [3]
- Sleep deprivation [4]
- Heat [5]
- Radiation, many types [6]
- Mental stress [7]

[1] Clapp *Cardiovasc Res.* 2004;64(1):172-8. [2] Leonard *Free Radic Biol Med.* 2004;37(12):1921-42. [3] Piotrowska *Acta Pol Pharm.* 2002;59(6):427-32. [4] Ramanathan *Neuroreport.* 2002;13(11):1387-90. [5] Lin *Comp Biochem Physiol A Mol Integr Physiol.* 2006. [6] Park *Prep Biochem Biotechnol.* 2006;36(1):19-35. [7] Sivonova *Stress.* 2004;7(3):183-8.

## Thus, may explain link of number of exposures to illness

True across classes of exposure[1]

True within classes of exposure (next slide)

[1]Doebbeling *Am J Med.* 2000;108(9):695-704.

## MULTIPLE VACCINES

| | HI | LOW | RR |
|---|---|---|---|
| UNWIN | ≥7 | 0 | 1.8*** |
| UNWIN w/records≥ 7 | 0 | 1.9*** | |
| HOTOPF postdepl | ≥ 5 | 0-1 | 5.0*** |
| CHERRY | ≥10 | 0 | 2.25* |
| CHALDER | ≥7 | 0-2 | 2.8**/ 1.8* |
| AUSTRALIAN-a ≥10 | 0 | 1.5** | |
| AUSTRALIAN-b ≥10 | 0 | 1.3** | |

Unwin:CDC; Hotopf: CDC; Cherry: sx severity (also peripheral score);Chalder: belief have GWI (unadjusted/adjusted); Australian-a: functional impairment; Australian-b: # health symptoms. Also PCS score; MCS score. Australian signif by dose response for those with 1+ vaccines

## ROS cause damage

ROS damage proteins, lipids, DNA and RNA

Genova *Ann N Y Acad Sci.* 2004;1011:86-100.
Mandavilli *Mutat Res.* 2002;509(1-2):127-51.

## ROS can Damage Mt

Oxidative stress, via ROS, can impair mt fxn and induce MtDNA damage [1]

MtDNA are especially vulnerable – mutate 1000x more than nuclear DNA [2]

[1] Staniek *Free Radic Res.* 2002;36(4):381-7.

[2] Khaidakov *Mutat Res.* 2003;526(1-2):1-7.

## Mt Damage Affects Cells/Organs

1. Reduce cell energy [1] (necrosis)
2. Increase oxidation (ROS) [2] (apoptosis)

Once mtDNA are damaged, there can be self-perpetuation due to increased production of ROS (when resp chain proteins are damaged) [2]

[1] Yamamura *Antioxid Redox Signal.* 2001;3(1):103-12

[2] Mandavilli *Mutat Res.* 2002;509(1-2):127-51.

## Clinical Effects Can Commence to Appear

As mt damage accrues, clinical effects arise.

There is variable vulnerability due to:

- Levels of inherited mtDNA mutation; and mutations of nDNA related to mt fxn
- Levels of acquired mtDNA mutation
- Differences in current antioxidant levels
- Differences in prior and current other oxidative stressors
- Differences in clearance of prospective toxins

## Classic Mt Disease "Matches" GWI

Mt encephalomyopathy prominently features [1]

- Fatigue
- Cognitive
- Muscle

Brain&muscle are esp aerobically dependent [2]

But all cells need energy; any organ can be affected; mt ds is often multisymptomatic [3]

[1] Fukuda K *Jama.* 1998;280(11):981-8.

[2] Erecinska *Respir Physiol.* 2001;128(3):263-76.

[3] Wei *Proc Natl Sci Counc Repub China B.* 1998;22(2):55-67.

## Classic Mt Disease is Multisymptomatic[1]

Affected Domains Can include:

- GI
- Sleep
- Neuropathy sx
- Psychiatric
- Heart
- Breathing
- About everything else

[1] Wei *Proc Natl Sci Counc Repub China B.* 1998;22(2):55-67.

| Symptom/ Condition | Evidence for Occurrence in GWV | Evidence for Occurrence w Mitochondrial Ds | *Reports of Benefit to Sx w/ Antioxidant* |
|---|---|---|---|
| Fatigue | (3-6, 20) | (189-192) | *(193-195). Q10 →greatest benefit among suite of things tried (193)* |
| Muscle sx | (3-6, 20) | (192, 196-201) | *(195, 202, 203)* |
| Cognitive | (3-6, 20) | (198, 200, 204, 205) | *(194, 195, 206)* |
| GI | (3, 4, 6, 20, 207) | (200, 208) | *(203, 209)* |
| Psychiatric | (4, 5, 20) | (210) | *(211)* |
| Sleep | (3-6, 20) | (212-216) | *(217)* |
| Migraine/ Headache | (3-6, 20) | (172, 218-221) | *(222)* |
| Shortness of breath | (3, 4, 6, 20) | (223) | *(195)* |

| Symptom/ Condition | Evidence for Occurrence in GWV | Evidence for Occurrence w Mitochondrial Ds | *Reports of Benefit to Symptom with Antioxidants* |
|---|---|---|---|
| **Temperature dysregulation (sweats, chills, fevers)** | **(3, 5, 20)** | **(224)** | ***No studies identified*** |
| **Hypertension** | **(3, 4, 20)** | **(90, 198, 225, 226)** | ***(227, 228)*** |
| **Hearing Loss** | **(3, 5)** | **(200, 212, 229-231)** | ***(232, 233)*** |
| **Weight Gain** | **(20)** | **(205, 234-238)** | ***(194)*** |
| **Gum Problems** | **(4, 6, 20)** | | ***(239-242)*** |

| Symptom/ Condition | Evidence for Occurrence in GWV | Evidence for Occurrence with Mt Disease | *Reports of Benefit to Symptom with Antioxidants* |
|---|---|---|---|
| Seizures | (4) | (198, 204, 205, 243-246) | *(247) (Note: Q10 deficiency assoc'd with szs (248-251); so Q10 may benefit sz)* |
| Neuropathy or painful paresthesias | (3-5) | (208, 243, 252) | *(195, 217, 253)* |
| Vision | (4) | (201, 254, 255) and macular dystrophy (210, 231, 256, 257) | *(258)* |
| Sexual | (3, 20) | (205, 259) | *No studies identified* |
| Tachycardia or palpitations | (4) | (192) | *(217)* |
| Asthma or Bronchitis | (3, 4, 20) | (260, 261) | *(262)* |
| CFS | (3, 20, 207, 263) | (189-191, 264) | *(193)* |
| Fibromyalgia | (207) | (196); (197, 265, 266) | *(267)* |
| Heart Rate Variability abnl | (268, 269) | (270-273) | *(274)* |

## May explain: Link acute AE to chronic sx

Indeed, persons with greater acute effects are more likely to develop chronic effects – potentially compatible with greater oxidative impact at time of exposure

- Reported for vaccinations
- Reported for PB

(May be a signal that prooxidant effects exceeded antioxidant defenses)

## Acute AE of Anthrax Vaccine: Linked to Chronic Health

| | | | | AE/HEALTH LINK |
|---|---|---|---|---|
| AUTHOR | | N | OUTCOME | |
| SHUMM | US | 900 | Subj Health | YES |
| UNWIN | UK | 4248 | CDC GWI | YES |
| HOTOPF | UK | 923* | CDC GWI | YES |

*WITH RECORDS
‡Schumm reg coefficient negative, P < 0.001; Unwin OR 2.2 (1.6-3.1);
Hotopf: Adjust for vaccine AE attenuates effect of multiple vaccines

## Moreover, AE to Exposure is Predicted by Factors Linked to Oxidative State

## Health State & Meds Predict AEs

Medical problems or meds linked to AE to anthrax vaccine*.
Overall AE rate

| | N | %AE | P |
|---|---|---|---|
| Healthy | 1480 | 45 | |
| Med problem | 121 | 65 | <.01 |
| No meds | 1245 | 41 | |
| Meds | 357 | 66 | <.01 |

**Hoffman 2003 Vaccine 21: 4399*

## Health State & Meds Predict AEs

∴ Existing oxidative state may determine vulnerability to acute AE with an oxidative stressor, in turn signaling degree of oxidative stress insult (oxidative load in excess of antioxidant protection), which in turn may condition likelihood of triggering a self-perpetuating condition.

## Variable Symptoms

Variability in symptom domains may arise from:

Preexisting heteroplasmy (leading to variable loci of vulnerability within an individual, affecting vulnerability of specific organs)

- Different organ targeting by diff environmental agents

"Although everyone in a maternal lineage will harbor the same mutation, the nature and severity of the symptoms vary markedly among individuals." [1]

[1] Wallace *Epilepsia* 1994;35 Suppl 1:S43-50.

## Variable Latency

Phenotypic Threshold effects: Clin manifestations depend on a threshold of cells being affected (or dead) [1]

Achievement of that threshold depends on prior state, past injury, heteroplasmy; and ongoing oxidative stressors and antioxidant defenses

Thus: In one kindred with a heritable mtDNA defect, "the age of onset of major neurological disturbance varied from 3-70 years" [2]

[1] Shoffner, Lott, Wallace *Rev Neurol (Paris)* 1991;147(6-7):431-5.
[2] Crimmins *J Neurol Neurosurg Psychiatry*. 1993;56(8):900-5.

## ALS arises at Elevated Rates

Genetic variants of enzymes that clear OP AChEi – thus determining magnitude of oxidative injury in the face of exposure – have been linked to ALS risk[1]

Oxidative stress[2] and mt pathology[3,4] have increasingly been implicated in ALS

Protection against oxidative stress, via administration of coQ10, has protected against animal models of ALS [5]

[1] Saeed *Neurology*. 2006.
[2] Bowling *J Neurochem*. 1993;61(6):2322-5.
[3] Mancuso *Neurosci Lett*. 2004;371(2-3):158-62.
[4] Menzies *Brain*. 2002;125(Pt 7):1522-33.
[5] Beal *Free Radic Res*. 2002;36(4):455-60.

## ∴ The Theory is Explanatory

1. Primary sx are fatigue, cognitive, muscle
2. Many symptoms occur across all systems
3. Manifestations are commonly multisymptom
4. Illness is strongly linked to AChEi; and is linked to number of exposures experienced
5. Illness arose differentially in personnel with same exposure
6. Affected persons differ in symptom patterns
7. Latency to sx development is variable
8. ALS arises at elevated rates.

## The Theory is Predictive

1. Other conditions linked to oxidative stress may be found at increased age-adjusted rates: e.g. Parkinson's disease (already linked to pesticides; to oxidation; to paraoxonase variants); *cancer; birth defects*
2. As veterans age, increased mortality may arise in settings of illness, injury, surgery due to reduced energetic reserve; and altered oxidant/antioxidant balance
3. Increased vulnerability to oxidative stressors may lead further exposures to have disproportionate impact
4. This may include increased vulnerability not only to pesticides etc; but to medications and procedures

## Other predictions provide hope

1. Approaches to objective diagnosis may ensue
2. Vulnerability testing may become possible
3. Onset may be prevented: Antioxidants given a) routinely low dose; b) prior to or immediately after exposure, high dose may relatively protect (and have done so for AChEi lethality in animals; and for toxicity of others of the exposures in ppl)

**Potential Preventive Measure Suggested: Q10 Confers Benefit Against Exposures and Potential Mechanisms**

| Type of Exposure | Q10 protects |
|---|---|
| Radiation | (70, 180, 328, 378, 379) |
| Herbicide | (380) |
| Electric field | (381) |
| Magnetic field | (382) |
| Pesticide/herbicide neurotoxicty protection | (393) |
| Protect vs animal models of PD & ALS | (396) (395) (397) (142) (140) (139) (149) (398) (399) |
| Slow progression of neurodegeneration (PD) in people | (187) |
| Stress induced injury protection | (401, 402) |
| Thermal stress protection | (403) |
| Prescription drug toxicity | (383-390) |

## Other predictions provide hope

4. Severity of existing sx may be attenuated: by measures to improve cell energy and reduce cell oxidation (e.g. Q10, perhaps carnitine, selenium). Q10 has benefited energy, muscle and cognition in mt disease and in other settings; and may do so here.
5. Development of new symptoms may be reduced: by supporting energetics and protecting against oxidation.
6. Neurodegenerative disease risk may be mitigated: Q10 protects against inception of neurodegeneration in animals; and retards progression of early PD in humans (testing in ALS underway). Protection expected to be stronger in *preclinical* stages.

## Summary

GWV have illness characteristics thought to defy pathological explanation; and to preclude a toxic etiology

It is proposed that oxidative stress and mitochondrial (mt) pathology provide a good fit with features, including previously unexplained features, of illness in GWV

This theory fits both the *exposures* linked to illness; and the *health profile* (symptom pattern) associated with illness in GWV.

## Implications

A paradigm is proposed – that environmental exposures to oxidative stressors may cumulatively damage mt and increase risk of a range of health problems.

It is explanatory and offers testable predictions.

It offers new directions for research into illness in GWV.

This paradigm has implications to current military personnel; and may advance understanding of chronic multi-symptom health problems outside the military setting.

## Thank You