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<b>13. ABSTRACT (Maximum 200 Words)</b> We investigated the basis of symptoms of exertional fatigue in 30 symptomatic and 21 asymptomatic Gulf War (GW) Veterans matched for age, height and weight. Serum CK, incidence of elevated CK, and muscle histology and fatigue rate and level of oxygen uptake relative to O <sub>2</sub> delivery during forearm exercise were similar in both groups. In contrast, peak cycle work (126±34 versus 153±43 watts), oxygen utilization (23.5±5.5 vs 27.4±7.4 ml.kg <sup>-1</sup> min <sup>-1</sup> ), and cardiac output (173±53 vs 206±49.kg <sup>-1</sup> min <sup>-1</sup> ), and peak blood lactate (6.6±2.3 vs 8.0±2.3 mM) and potassium (5.5±0.6 vs 6.0±0.7 mM) were lower (p<.05) in symptomatic veterans. Mean resting heart rate was higher and peak exercise heart rate and activities of oxidative enzymes were lower in symptomatic veterans but these differences were not statistically significant. In response to 10 weeks of regular cycle exercise, a cohort of symptomatic veterans (n=7) showed a normal increase in work capacity (139±24 watts pre, 166±25 watts post training, p<.001) and in oxidative capacity (peak O <sub>2</sub> uptake pre 25.4±3.5 ml kg <sup>-1</sup> min <sup>-1</sup> , post 29.2±4). Our results indicate that lower oxidative capacity attributable to lower levels of cardiovascular fitness correlate with symptoms of fatigability in Gulf War veterans and can be reversed by exercise training.			
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## Introduction

A variety of illnesses have been linked to military service in the Persian Gulf Conflict, but no consistent medical syndrome has been recognized and no specific etiology is known. Muscle symptoms, in particular abnormal fatigability and myalgias, are common in affected individuals, but the etiology of these muscle symptoms is unknown. We investigated the general hypothesis that a fundamental physiologic mechanism of muscle fatigue in Gulf War veterans is an impairment of muscle oxidative metabolism. Under this general hypothesis, we addressed four specific questions:

- 1) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production?
- 2) Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals?
- 3) Is the normal increase in muscle oxygen utilization and the capacity for oxygen transport in response to regular, aerobic physical conditioning impaired in these patients?
- 4) Is there a specific pattern of impaired activities of mitochondrial enzymes to account for impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training?

Our study was designed to investigate the physiological basis of muscle symptoms, in affected individuals utilizing resources of a center devoted to the study of human muscle metabolic disorders and to investigation of the physiologic basis of exercise intolerance.<sup>1-3</sup> The study employed protocols designed to probe muscle oxidative metabolism including measurement of systemic oxygen transport (cardiac output) at rest and in exercise by means of acetylene rebreathing, evaluation of peak muscle oxidative metabolism by monitoring oxygen uptake and the production of lactate and pyruvate during cycle and forearm exercise, and by assessing activity levels of muscle oxidative enzymes.

# Body

## METHODS:

*Subject identification, recruitment:* We proposed to identify approximately 25 Gulf War veterans with muscle symptoms - including fatigability, myalgia and/or weakness - the onset of which dated to their Gulf War service. We proposed to compare exercise responses in these patients with those of a age, weight, sex matched control group of Gulf War veterans without muscle complaints.

*Experimental procedures:* The fundamental approach to evaluating muscle oxidative metabolism in Gulf War veterans involved cycle and forearm exercise testing during which physiologic and metabolic changes related to muscle capacity for oxidative phosphorylation were monitored:

a) *Cycle exercise.* Subjects underwent resting and exercise evaluation of oxidative metabolism using cycle ergometry. Studies were designed to assess peak capacity for oxygen utilization and oxygen transport (cardiac output) as well as monitoring changes in blood levels of metabolites that reflect levels of anaerobic glycogenolysis (blood lactate and lactate/pyruvate ratios) as well as heart rate and blood pressure responses to graded exercise. The exercise paradigm consisted of a ramp cycle test in which the workload was increased by 10-20 watts every 1-2 minutes. Heart rate was monitored continuously with a 12-lead electrocardiogram. Gas exchange and cardiac output were determined at rest and during submaximal and maximal workloads. Ventilation ( $V_E$ ) was measured utilizing Douglas bags and a Tissot spirometer; fractions of  $O_2$ ,  $CO_2$  and  $N_2$  in expired air were determined with a mass spectrometer (Marquette 1100); and oxygen uptake ( $VO_2$ ), carbon dioxide production ( $VCO_2$ ), and respiratory exchange ratio, RER ( $VCO_2/VO_2$ ), were calculated. Cardiac output was measured utilizing acetylene rebreathing in which the rate of disappearance of  $C_2H_2$  from a rebreathing bag is proportional to pulmonary blood flow and cardiac output (Q).<sup>4, 5</sup> Systemic arteriovenous  $O_2$  difference was calculated from the Fick equation:  $VO_2 = \text{cardiac output} \times \text{systemic a-v } O_2 \text{ difference}$ . The increase in cardiac output ( $\Delta Q$ ) relative to the increase in  $VO_2$  ( $\Delta VO_2$ ) from rest to exercise was determined as the ratio,  $\Delta Q/\Delta VO_2$ . Venous blood was sampled at rest, submaximal and maximal workloads, and at 5 and 10 minutes post exercise for lactate, pyruvate, and potassium. In addition resting serum creatine kinase (CK) and CK at 10 minutes post maximal cycle exercise were determined.

b) *Aerobic forearm exercise.* Subjects also underwent aerobic forearm exercise monitoring the pattern of contractile fatigue and changes in venous effluent metabolites that reflect the rate of glycogenolysis and adenine nucleotide breakdown via adenylate deaminase. Subjects performed maximal handgrip exercise utilizing a custom designed handgrip ergometer. Maximal voluntary contraction was considered to be the highest grip force attained in three trials. Subjects performed 30 maximal handgrip contractions in 1 minute. The force of each contraction was displayed and recorded. Venous blood from a cubital vein of the exercising forearm was sampled at rest, immediately at the end of exercise, and at 1, 2, 5, and 10 minutes post exercise for lactate and ammonia content.

c) *Near infrared spectroscopy (NIRS):* We evaluated changes in muscle oxygenation utilizing NIRS employing a custom designed NIR spectrometer<sup>6</sup> or a commercial NIR device (ISS) to identify abnormalities of oxygen utilization relative to oxygen delivery during

exercise.<sup>6-8</sup> Spectra were collected during rest and with repetitive hand gripping exercise at 50 and 100% of maximal voluntary contraction. The probe was placed to sample oxygenation of the flexor digitorum profundus. Light in the NIR range (700-1000 nm) passes readily through biological tissues including skin and bone. NIR light is diffusely scattered by tissues and photons are absorbed primarily by the iron-porphyrin complexes of oxy- and deoxyhemoglobin and myoglobin and by oxidized copper atoms of cytochrome aa<sub>3</sub>. NIR is able to detect *qualitative* changes in the reduction-oxidation state of the copper complex of cytochrome aa<sub>3</sub> (in cytochrome c oxidase) and oxygenation of tissue hemoglobin (Hgb) and myoglobin (Mgb). We utilized as our major index of oxygen extraction, changes in deoxyhemoglobin+myoglobin. Results are expressed as percentage of deoxyhemoglobin+myoglobin signal during exercise compared to the level of deoxy signal after 7 minutes of forearm ischemia.

d)<sup>31</sup>P *Phosphorus magnetic resonance spectroscopy* (<sup>31</sup>P MRS) studies of the vastus lateralis were performed on a Philips Gyroscan NT whole-body MRI/MRS system operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). All individuals lay in the supine position with feet first in the magnet on a custom-built exercise apparatus mounted on the patient table. A 6-cm diameter-surface coil was positioned over the vastus lateralis at approximately 1/3 the distance between the lateral epicondyle and greater trochanter: Short-arc knee extension exercise was performed inside the magnet by rhythmically pulling against bungee cords of various tensions that were attached to a strap around the ankle. A strain gauge connected to the bungee cords allowed for force production measurements. The exercise protocol consisted of contractions that were held for 3-5 seconds with 1-2 seconds of relaxation. The intensity corresponded to 30% of maximum voluntary contraction for the first minute followed by maximal intensity contractions until exhaustion. This non-ischemic exercise paradigm usually led to pronounced metabolic changes within 1 to 3 minutes of exercise, allowing a minimum of 15 minutes of acquisition during the recovery period for each individual. Calculation of metabolite concentrations, recovery kinetics, and intracellular pH was performed as previously reported.<sup>9</sup> We used the PCr recovery half-time after exercise (PCr  $t_{1/2}$ , the time required for PCr concentrations to reach the halfway recovery point) as the <sup>31</sup>P MRS-assessed index of mitochondrial oxidative function.<sup>10</sup> Due to complexities in recovery behaviour, PCr recovery was fitted with a second order step-response function.

Because of the lack of established norms for quadriceps exercise and <sup>31</sup>P MRS evaluation, we studied 14 healthy control individuals before recruiting symptomatic and asymptomatic Gulf War veterans for the study. Thus for this protocol, Gulf War veterans with muscle symptoms were compared to both healthy non-veteran subjects and to asymptomatic Gulf War veterans.

e) *Muscle biopsy - histochemical and biochemical evaluation.* We originally proposed to evaluate muscle histology and biochemistry only in a cohort symptomatic and control subjects undergoing exercise training. However this portion of the protocol was expanded to include histology and muscle biochemistry from vastus lateralis muscle samples in all subjects enrolled in the study. To accomplish this, we adopted the needle biopsy technique to acquire samples ranging from 70-200 mg. We adapted our histology techniques to permit histochemical analyses of such samples and miniaturized spectroscopic techniques of enzyme analysis to enhance the amount of biochemical information available from needle biopsies. We performed initial needle biopsies on 51 veterans; in an additional 10 veterans we performed repeat needle biopsy after completion of a 10 week aerobic training protocol. For muscle histology, we utilized stains designed to interrogate morphology (H&E, modified trichrome), fiber type distribution (NADH tetrazolium reductase, alpha glycerol phosphate dehydrogenase, alkaline and acid myosin ATPase), and fiber oxidative/metabolic capacity (SDH, cytochrome c oxidase, oil-red-o). We also used a combined stain in which muscle is processed first for COX followed by counterstaining with SDH. The rationale for this procedure is that it permits detection of fibers containing mitochondrial DNA mutations. Common mitochondrial DNA mutations when present in sufficient proportions result in deficiency of cytochrome c oxidase (due to deficiency of mtDNA encoded COX subunits) with

retained expression of SDH (which is nuclear encoded). Fibers containing significant levels of mutant DNA (typically > 60% mutant mtDNA) stain blue (SDH positive, COX negative) whereas normal fibers stain brown (COX positive). For subjects undergoing training, repeat muscle biopsies with histological and biochemical analyses was performed. Biochemical, enzyme assays performed included citrate synthase, hydroxyacyl CoQ dehydrogenase (HAD), succinate dehydrogenase (SDH), and cytochrome c oxidase (COX).

f) *Aerobic training* - We proposed to investigate the the hypothesis that the subjects with symptoms of fatigability show altered capacity to adapt to conditioning exercise. Our original plan was to study 10 symptomatic individuals and 10 control subjects after 10 weeks of aerobic conditioning performed on a stationary bicycle. In order to assure compliance with exercise training, subjects were issued recording, Polar heart monitors that permitted downloading of heart rate data for each exercise training session.

g) Statistics: Results are expressed as mean  $\pm$ SD. We compared mean results for symptomatic and control subjects using a two tailed Students t test for unpaired data. Differences were considered significant if  $p < .05$ . Pre and post training responses were compared using paired t tests.

## RESULTS

*Subject identification, recruitment:* . We recruited 77 veterans who agreed to participate in the study and were scheduled for initial evaluation. 26 veterans never reported for evaluation despite repeated attempts to encourage participation and often multiple appointments. Thus 51 veterans ultimately were evaluated, including 30 symptomatic and 21 control subjects, Of the symptomatic individuals, 27 were men and 3 were women. Of the control subjects, 18 were men, 3 were women. Overall, these subjects were well matched with respect to age, weight and height (Table 1).

Table 1: characteristics of symptomatic (patients) and asymptomatic (controls) GW veterans

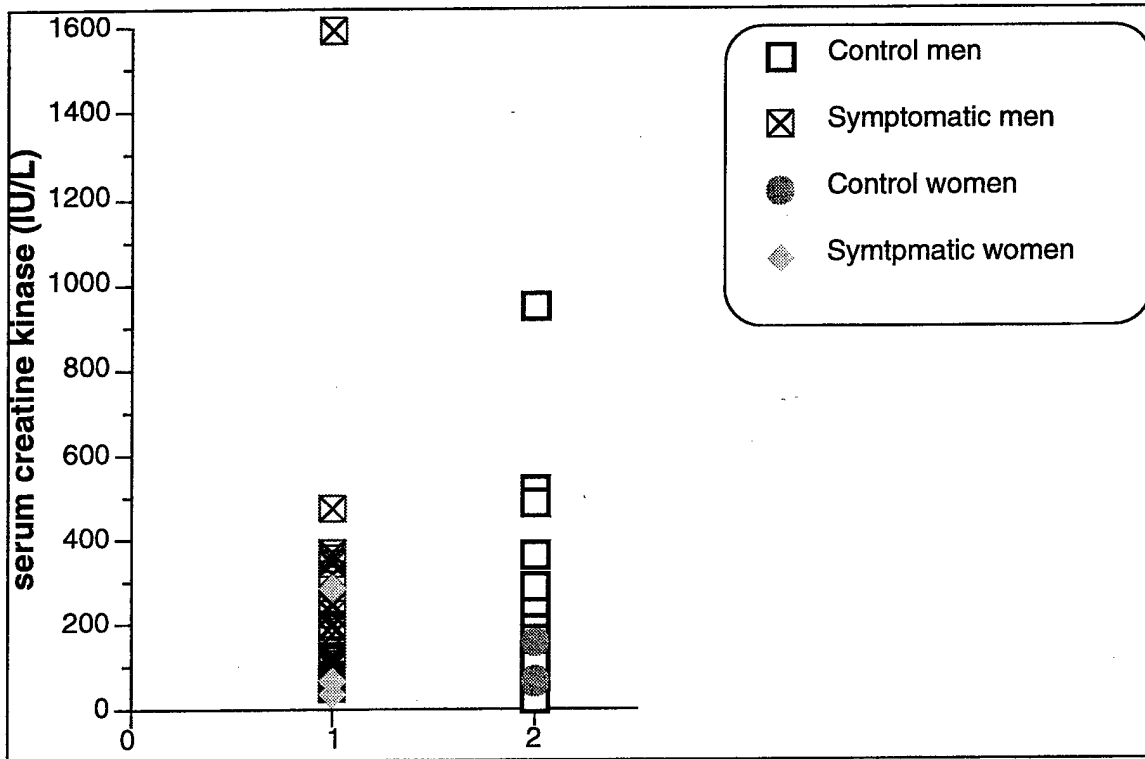
variables	patients	controls	p value
male	27	18	
female	3	3	
age	40 $\pm$ 9	40 $\pm$ 9	0.9132
height (cm)	174 $\pm$ 7	177 $\pm$ 8	0.242
weight (kg)	89 $\pm$ 17	89 $\pm$ 10	0.979

Table 2: serum CK levels in subjects.

creatine kinase	*patients (n = 29)	controls (n = 21)	p value
resting mean $\pm$ SD	177 $\pm$ 111	220 $\pm$ 211	0.357
resting range	34-469	22-943	
number above normal ( $\leq$ 269)	6	5	
post exercise mean $\pm$ SD	221 $\pm$ 135	253 $\pm$ 218	0.425
post exercise range	40-564	22-985	
number above normal ( $\leq$ 269)	8	6	

We measured resting and post exercise CK levels in 50 of our subjects (table 1, figure 1). There was no significant difference in CK levels in the two groups. Elevated CK levels (normal for our laboratory  $\leq 269$  IU/L) were detected in 12 subjects (5 controls, 7 patients) prior to maximal exercise. The subject with the highest CK (1586) was unable to perform cycle exercise due to limb pain. Post exercise, 14 subjects, 8 patients and 6 control, had elevated CK. The highest control CK level in a control subject (943 at rest, 985 post exercise) was in a 37 y.o. black man who had lifted weights within days of his evaluation. Both patients and control showed a trend toward increased CK levels after maximal exercise testing. Mean post exercise CK levels increased about 20% in patients, from 177 to 221, and a comparable amount in control subjects, from 220 to 253.

Figure 1: Range of resting CK levels in Gulf War veterans



*Data addressing specific hypotheses:*

1. Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production?

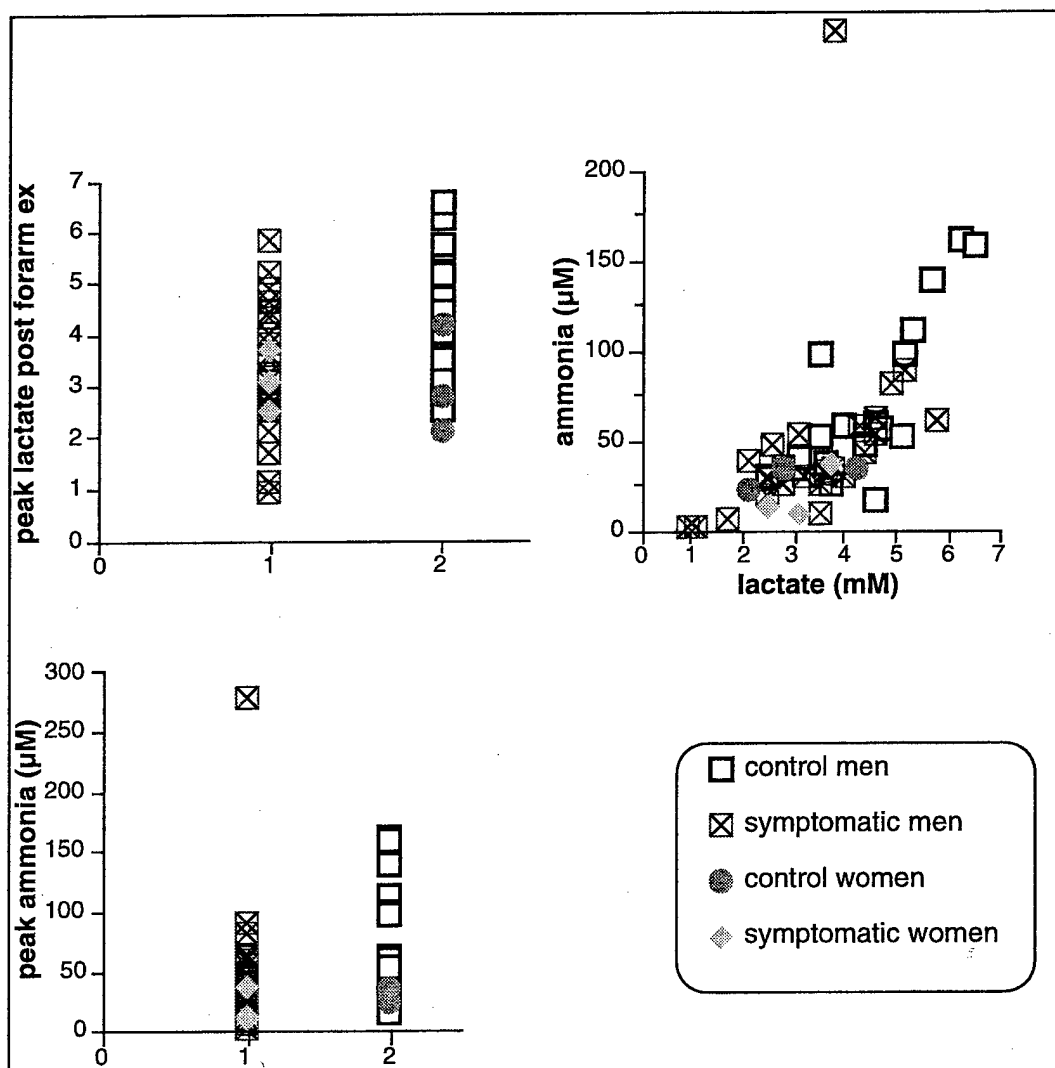
We addressed this question by evaluating the decline in grip force during maximal aerobic hand grip exercise, by evaluating peak work capacity during cycle exercise, and by monitoring lactate and ammonia responses to such exercise. Values for peak grip force and for the decline in grip force as a percentage of initial force were similar in symptomatic and control subjects (table 3). Resting lactate and ammonia levels were the same in both subject groups. However peak lactate levels after aerobic handgrip exercise were significantly lower in patients compared to controls. Peak ammonia levels also were lower in patients, but this difference did not reach statistical significance. 3 patients had peak lactate levels of less than 2mM. In each of these individuals there

was no increase in ammonia levels (figure 2, upper left). This pattern implies that poor effort was responsible and contributes to the low post-exercise lactate response for the group as a whole.<sup>3</sup>

Table 3: Aerobic forearm exercise

variables	patients	controls )	p value
initial force (kg)	37.±10.6	42.3±10.2	0.090
% initial force at 1 sec	93±11	94±9	0.680
% initial force at 30 sec	80±15	82±6	0.637
% initial force at 60 sec	71±16	72±7	0.684
resting lactate (mM)	0.89±0.2	0.88±0.30	0.928
peak lactate p exercise (mM)	3.3±1.1	4.2±1.2	0.009
resting ammonia (µM)	12±13	12±9	0.979
peak ammonia p exercise (µM)	42±49	64±45	0.115

figure 2: lactate, ammonia responses to forearm exercise



2. Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals?

We addressed this question by employing cycle ergometry and measuring oxygen utilization and cardiac output (systemic O<sub>2</sub> transport) during peak exercise and by calculating peak systemic arteriovenous O<sub>2</sub> difference. One symptomatic individual was not able to perform this test because of complaints of leg and hip pain. The mean resting heart rate was higher in symptomatic than in asymptomatic veterans but the difference did not reach statistical significance. Peak work, oxygen utilization, and cardiac output (expressed in ml/kg/min) were all significantly lower in symptomatic veterans compared to asymptomatic subjects (table 4) Peak systemic a-v O<sub>2</sub> difference patients and controls was similar (Table 4). The relationship between the increase in cardiac output ( $\Delta Q$ ) and the increase in oxygen uptake from rest to peak exercise were similar between symptomatic and asymptomatic veterans and to previously published norms for healthy subjects.<sup>5</sup> Mean peak lactate, pyruvate, and potassium were all significantly higher in controls than in symptomatic veterans. Peak exercise heart rates were lower in symptomatic individuals but the difference was not statistically significant.

These data suggest that symptomatic individuals as a group have lower oxidative capacity. These results are likely explained in part by a lower level of physical conditioning. Our results also suggest that, as a group, these subjects stop exercise at a lower relative work intensity as indicated by lower peak heart rates and lower blood levels of lactate, pyruvate, and potassium.

Table 4: Oxygen uptake, extraction, and transport during peak cycle exercise.

variables	patients (n = 28)	controls (n = 22)	p value
peak work (watts)	126±34	153±43	0.0165
peak watts/kg	1.43±0.35	1.75±0.54	0.0140
peak VO <sub>2</sub> (L/min)	2.06±0.56	2.40±0.56	0.0410
peak VO <sub>2</sub> (ml/kg/min)	23.5±5.5	27.4±6.8	0.0313
peak cardiac output (L/min)	15.3±3.7	16.8±3.2	0.1432
peak cardiac output (ml/kg/min)	173±53	206±49	0.0311
resting heart rate (bpm)	70±12	65±11	0.185
peak heart rate (bpm)	161±21	169±15	0.108
peak systemic arteriovenous diff	13.7±2.3	14.2±2.9	0.4660
$\Delta Q/\Delta VO_2$	5.85±1.6	5.88±1.1	0.9454
RER	1.10±0.11	1.11±0.08	0.6838
peak lactate (mM)	6.55±2.3	8.0±2.3	0.0280
peak pyruvate (mM)	0.276±.06	0.316±.04	0.0109
peak L/P	27.0±6.0	29.4±6.2	0.1615
peak K+	5.5±0.6	6.0±0.7	0.0074

We also addressed oxygen extraction relative to oxygen delivery utilizing near infrared spectroscopy by measuring deoxyhemoglobin at 50% and 100% MVC expressed as a percentage of the maximal levels achieved during resting ischemia. No systematic differences in NIR spectroscopy have been detected (Table 5).

*Table 5: Deoxy hemoglobin plus myoglobin percent of ischemia*

variables	patients (n = 19)	controls (n = 12)	p value
50% MVC	33.07±3.77	27.72±4.97	0.3922
100% MVC	44.94±2.79	42.32±4.75	0.6140

Additionally, we directly assessed muscle energy metabolism and oxidative capacity in a cohort of 5 patients and 5 control Gulf War veterans utilizing <sup>31</sup>P magnetic resonance spectroscopy to evaluate resting, exercise and recovery responses of the quadriceps femoris. We compared the responses of the Gulf War subjects to a group of 14 healthy control subjects (non GW controls). These results are presented in table 6.

There was no statistically significant difference in any measured <sup>31</sup>P MRS variable between the two groups of Gulf War veterans. Similarly, there was no statistically significant differences in spectroscopy values for GW patients and non GW controls.

*Table 6: 31P MRS at rest and in recovery from leg extension exercise.*

variables	non GW controls	GW patients	GW controls	p value (comparison of GW patients vs GW controls)
resting PCr/Pi	7.87±1.55	6.73±2.80	7.60±2.23	0.604
resting pH	7.05±.02	7.06±.02	7.06±.01	0.373
resting PCr	32.2±2.43	32.4±3.1	32.5±2.0	0.999
resting Pi	4.29±0.93	5.43±1.83	4.48±0.88	0.329
resting PDE	3.81±1.34	7.06±1.46	5.06±1.46	0.062
resting PME	1.03±0.54	1.59±1.51	1.35±0.65	0.757
resting ADP	18.06±5.39	18.55±8.01	17.75±4.88	0.854
resting PP	119356±53158	106034±61549	112458±46070	0.856
t1/2 PCr	0.54±0.15	0.64±0.23	0.87±0.47	0.345
t1/2 Pi	0.37±0.10	0.62±0.18	0.64±0.26	0.871
t1/2 ADP	0.17±0.06	0.19±0.05	0.17±0.06	0.499
V	30.98±9.1	25.59±4.4	19.91±9.3	0.251
Qmax	39.3±10.1	35.7±8.5	25.4±10.5	0.129
end exercise pH	6.57±0.18	6.63±0.19	6.52±0.258	0.471
end exercise PCr	8.98±4.6	10.06±6.1	9.68±9.8	0.943

PCr = phosphocreatine; Pi = inorganic phosphate; PDE = phosphodiester (usually attributable to phospholipids); PME = phosphomonoester (usually attributable to sugar phosphates); ADP = adenosine diphosphate; PP = phosphorylation potential, [ATP]/[ADP][Pi]<sup>1</sup>; t 1/2 PCr - time in minutes for PCr to recover to 1/2 the resting level; t 1/2 Pi, t 1/2 ADP - half time in seconds for Pi and ADP respectively; V = initial rate of PCr resynthesis : Q = the apparent maximum rate of oxidative ATP synthesis<sup>11</sup>

3. Is the normal increase in muscle oxygen utilization and the capacity for oxygen transport in response to regular, aerobic physical conditioning impaired in these patients? We were able to complete exercise training in 10 veterans (7 patients, 3 controls). An additional 10 veterans (5 control, 5 symptomatic) agreed to participate in training but dropped out without completing the training protocol.

All of the subjects who did train demonstrated an increased work and oxidative capacity attributable to increased cardiac output, increased peak systemic a-v O<sub>2</sub> difference, or both (Tables 6 and 7).

The average increase in oxidative capacity was approximately 15%, a level comparable to results in previous studies of healthy individuals.<sup>12</sup>

*Table 6: Training effects for patients (n=7) in oxygen uptake, extraction, and transport during peak cycle exercise.*

<b>variables</b>	<b>Pre</b>	<b>Post</b>	<b>p value (paired t test)</b>
peak work (watts)	139±24	166±25	<.0001
peak watts/kg	1.56±0.212	1.91±0.261	<.0001
peak VO <sub>2</sub> (L/min)	2.26±.26	2.53±.32	.0027
peak VO <sub>2</sub> (ml/kg/min)	25.4±3.5	29.2±4.0	.0007
peak cardiac output (L/min)	17.36±0.2.29	18.62±4.3	0.2036
peak heart rate (bpm)	162±6	169±7	0.4674
peak systemic arteriovenous diff	13.18±1.6	13.96±2.4	0.2794
ΔQ/ΔVO <sub>2</sub>	5.80±1.1	5.73±1.7	0.8496
RER	1.140±.119	1.130±.060	0.7329
peak lactate (mM)	7.7±2.5	8.1±1.7	0.5091
peak pyruvate (mM)	0.292±.038	0.322±.069	0.2801
peak L/P	27.0±4.6	32.7±7.1	0.0939

These results indicate that training resulted in a normal increase in work and oxidative capacity in response to exercise training and indicate no evidence of impaired capacity to adapt normally to regular exercise. These data also support the view that a lower level of physical conditioning explains to a large extent the differences between symptomatic and asymptomatic individuals. In this patient cohort, pre training physiological and metabolic responses were similar to the symptomatic group as a whole. After training, peak work, oxygen uptake, as well as peak lactate and pyruvate were equal or greater to the mean values for asymptomatic subjects prior to training.

*Table 7: Training effects for controls (n=3) in oxygen uptake, extraction, and transport during peak cycle exercise.*

<b>variables</b>	<b>Pre</b>	<b>Post</b>	<b>p value</b>
peak work (watts)	160±36	180±45	0.5804
peak watts/kg	1.9±0.483	2.11±0.499	0.6229
peak VO <sub>2</sub> (L/min)	2.66±0.603	2.93±0.753	0.6432
peak cardiac output (L/min)	17.63±2.40	17.69±7.03	0.9889
peak heart rate (bpm)	183±3	186±3	0.5811
peak systemic arteriovenous diff	14.98±1.30	17.17±3.11	0.3216
ΔQ/ΔVO <sub>2</sub>	5.42±0.50	4.44±0.712	0.1179
RER	1.14±0.107	1.12±0.033	0.7361
peak lactate (mM)	9.73±2.12	10.73±2.05	0.5865
peak pyruvate (mM)	0.395±0.144	0.341±0.027	0.5932
peak L/P	30.58±6.44	37.17±5.95	0.3533

4. Is there a specific pattern of impaired activities of mitochondrial enzymes to account for impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training? Are there morphological abnormalities in skeletal muscle that correlate with elevations in serum CK, with clinical symptoms of fatigue, or with physiological abnormalities?

Analysis of these muscle histology has revealed only occasional minor and non specific abnormalities such as occasional atrophic muscle fibers and increased variation in fiber size. Muscle histochemistry revealed only rare abnormalities with no definite relationship to symptoms or to the laboratory finding of elevated serum CK. Three asymptomatic (ages 47,53 and 55) and two symptomatic (ages 52 and 60) veterans revealed isolated or occasional SDH positive, COX negative fibers. The most abundant of these changes was in the 52 year old symptomatic veteran who displayed 10-12 such fibers per low power field. SDH positive, COX negative fibers are a characteristic features of heteroplasmic mitochondrial DNA mutations. They may be seen in otherwise healthy control subjects, particularly in the elderly. They usually are attributed to de novo mutations (usually large scale deletions) that expand focally. When they reach a critical threshold, they impair the synthesis and assembly of respiratory chain components coded on the mitochondrial genome. The abundance of these fibers in the single symptomatic veteran is intriguing but this isolated finding does not permit a firm conclusion as to its significance.

*Table 8: Muscle oxidative enzymes*

<b>variables</b>	<b>patients</b>	<b>controls</b>	<b>p value</b>
Citrate synthase	9.72±2.25	10.47±2.11	0.2455
Cytochrome Oxidase	3.17±1.42	3.54±1.59	0.4077
HAD	30.33±6.7	28.08±6.5	0.2499
Succinate Dehydrogenase	1.61±.54	1.66±.48	0.7466

Oxidative enzymes have been measured in all biopsy samples. There is a trend to slightly lower oxidative enzyme activities in symptomatic individuals compared to veterans without muscle symptoms, but no differences reached statistical significance (table 8). In symptomatic individuals undergoing exercise training, mean activity levels of most oxidative enzymes were higher after training (table 9). But differences were not statistically significant. Responses in symptomatic and asymptomatic veterans were similar (tables 9 &10).

*Table 9: Muscle oxidative enzymes pre and post training, Patients (n=7)*

<b>variables</b>	<b>pre</b>	<b>post</b>	<b>p value (paired t test)</b>
Citrate synthase	11.6±5.2	12.44±3.2	0.5625
Cytochrome Oxidase	2.46±0.1.05	3.32±0.86	0.0602
HAD	29.52±7.4	30.60±2.77	0.6676
Succinate Dehydrogenase	1.42±0..488	1.23±0.11	0.3199

*Table 10: Muscle oxidative enzymes pre and post training, Controls (n=3)*

<b>variables</b>	<b>pre</b>	<b>post</b>	<b>p value (paired t test)</b>
Citrate synthase	9.06±1.52	13.36±3.58	0.3311
Cytochrome Oxidase	2.57±0.62	3.15±0.69	0.5668
HAD	24.07±5.14	32.33±8.28	0.4445
Succinate Dehydrogenase	1.52±0.22	0.98±0.06	0.0771

## Key Research Accomplishments

- Our results indicate that veterans of the Persian Gulf War who have experienced symptoms of exertional fatigue have lower aerobic work capacity with lower peak levels of oxygen consumption and lower peak cardiac output than asymptomatic veterans.
- Our data suggest that the primary mechanism of reduced aerobic capacity is deconditioning and this deconditioning is reversible with regular aerobic exercise.
- We found no significant differences between symptomatic and asymptomatic veterans with respect to blood levels of creatine kinase, muscle morphology, forearm exercise performance, or muscle oxidative enzyme profiles.

## Reportable Outcomes

No abstracts or manuscripts related to this research have been published to date.

## Conclusions

Our results reveal only minor differences between Gulf War veterans with symptoms of easy fatigability, muscle pain, and/or weakness and those without such symptoms with respect to muscle function, metabolism, or structure. Despite the fact that symptomatic veterans complained of easy fatigability, we were not able to demonstrate statistically significant differences with respect to work capacity with forearm exercise or in most measures of leg exercise.

We did find that symptomatic veterans had lower peak work and oxidative capacity and lower peak level of cardiac output during cycle exercise and that symptomatic veterans stopped cycling at a lower mean heart rate and lower mean peak blood level of lactate and pyruvate. Two factors may contribute to these findings. First, lower mean heart rate responses and lower lactate, pyruvate responses suggest that patient effort was less, possibly related to constitutional factors that magnified perceived exertion. Second, lower peak work, oxygen uptake, and cardiac output may be explained by a lower level of physical conditioning in symptomatic veterans.

Our data also does not support the hypothesis that Gulf War veterans with muscle symptoms have any abnormalities with respect to adapting to regular physical training since patients displayed comparable increases in exercise and oxidative capacity and in cardiovascular performance as healthy subjects undergoing similar levels of training.<sup>12, 13</sup>

Our failure to identify an objective physiological or metabolic basis for muscle symptoms of fatigability, pain, or weakness is similar to the result of Amato and coworkers who evaluated 20 Air Force and Army Persian Gulf veterans with symptoms of weakness, fatigue and muscle pain.<sup>14</sup> Interestingly, Amato and coworkers found a similar incidence of elevation of serum CK in their population. With a normal CK level of  $\leq 220$ , they found that 6 of 20 patients had moderate CK elevations ranging from 223-778. This 30% incidence of above normal CK values is virtually the same as our result. However, our study indicates a similar incidence of CK elevation is found in asymptomatic Gulf War veterans. Amato found most of the CK elevations in muscular black men, in whom the normal range of CK may be higher than for other populations.<sup>15, 16</sup> In our study population, only 5 of 14 individuals with elevated CK levels were African American with the remainder white or Hispanic.

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**Appendix**

**None**