in vivo *19*: 387-390 (2005)

Exercise Capacity and Immune Function in Male and Female Patients with Chronic Fatigue Syndrome (CFS)

CHRISTOPHER R. SNELL[1], J. MARK VANNESS[1], DAVID R. STRAYER[2] and STACI R. STEVENS[3]

[1]*University of the Pacific, Department of Sport Sciences, Stockton, CA 95211-0197;*
[2]*Hemispherx Biopharma, Philadelphia, PA;*
[3]*Workwell Physiology Services, Inc., Ripon, CA 95366, U.S.A.*

Abstract. *Hyperactivition of an unwanted cellular cascade by the immune-related protein RNase L has been linked to reduced exercise capacity in persons with chronic fatigue syndrome (CFS). This investigation compares exercise capacities of CFS patients with deregulation of the RNase L pathway and CFS patients with normal regulation, while controlling for potentially confounding gender effects. Thirty-five male and seventy-one female CFS patients performed graded exercise tests to voluntary exhaustion. Measures of peak VO_2, peak heart rate, body mass index, perceived exertion, and respiratory quotient were entered into a two-way factorial analysis with gender and immune status as independent variables. A significant multivariate main effect was found for immune status ($p<0.01$), with no gender effect or interaction. Follow-up analyses identified $VO_{2\ peak}$ as contributing most to the difference. These results implicate abnormal immune activity in the pathology of exercise intolerance in CFS and are consistent with a channelopathy involving oxidative stress and nitric oxide-related toxicity.*

Despite increasing recognition as a serious, often disabling illness, chronic fatigue syndrome (CFS) remains a diagnostic enigma. Problems of patient heterogeneity and a dearth of quantifiable pathological findings frustrate both clinicians and researchers alike. However, the discovery of abnormalities in the immune cells of some CFS patients (24) suggests a cellular etiology and offers the promise of a definitive biological test for this controversial condition.

The presence of a novel 37-kDa RNase L in the peripheral blood mononuclear cells (PBMC) of certain CFS subpopulations is indicative of deregulation in the 2-5A/RNase L antiviral defense pathway and implies the presence of chronic viral infection (4). Presence of the 37-kDa RNase L correlates positively with proinflammatory cytokine levels in persons with CFS, providing support for a viral hypothesis (24). One or more of the various cytokines produced to fight the viral infection may be responsible for the profound fatigue and other symptoms that typify CFS (14).

Deregulation of the 2-5A synthetase/RNase L pathway is associated with lower general health status among CFS patients (24). Elevated levels of the 37-kDa RNase L have been shown to predict fatigue, muscle pain, and depressed mood in persons with CFS (15). Enhanced immune function and clinical improvement has been seen in patients expressing the 37-kDa RNase L contingent with immunomodulatory drug therapy aimed at normalizing RNase L activity (23). Such indications of immune function abnormalities have prompted some researchers to suggest a channelopathy in CFS (17).

In a previous study, we found that the presence of deregulated RNase L activity could be linked to low exercise tolerance in persons with CFS. Both peak oxygen consumption ($VO_{2\ peak}$) and exercise duration were significantly reduced in CFS patients with 37-kDa RNase L expression compared to those not expressing the protein (22). However, it is possible that these results were confounded by a failure to adequately control for gender differences in $VO_{2\ peak}$ and a greater proportion of women among the patients who expressed the 37-kDa RNase L protein. Other researchers have noted that exercise studies in CFS commonly pool gender data. This error is compounded when gender distribution between study groups is unequal, which may explain findings of reduced exercise capacity in CFS patients reported by some studies (21). The purpose of this present study was to further examine the relationship between immune function and exercise capacity with an expanded subject population while controlling for possible gender differences in exercise performance.

Correspondence to: Christopher R. Snell, Ph.D. Department of Sport Sciences, University of the Pacific, Stockton, CA 95211, U.S.A. Tel: 209-946-2703, Fax: 209-946-3225, snells@juno.com

Key Words: RNase L ratio, functional impairment, immune dysfunction, gender.

Table I. *Pooled within-groups correlations with function, univariate F's, and P values.*

Variable	Structure matrix	*F*	*P*
$VO_{2\ peak}$(ml/min/kg)	1.00	12.84	0.00
HR_{peak} predict (%)*	0.49	5.81	0.02
RQ_{peak}*	0.40	0.68	0.41
RPE_{peak}*	0.12	4.77	0.03
BMI*	-0.11	1.25	0.27

* These variables did not enter the discriminant analysis

Table II. *Means and standard deviations for gender by RNase L groups.*

	M (SD)			
	Deregulated		Normal	
Variable	M (n=20)	F (n=50)	M (n=15)	F (n=21)
$VO_{2\ peak}$ (ml/min/kg)	21.2 (4.4)	18.0 (4.4)	23.2 (4.1)	22.5 (3.5)
HR_{peak} predict (%)	77.3 (12.2)	77.9 (12.8)	84.0 (10.8)	84.2 (13.3)
RQ peak	1.05 (0.15)	1.05 (0.12)	1.07 (0.10)	1.07 (0.11)
RPE peak	18.0 (1.42)	18.4 (1.35)	18.9 (1.25)	18.6 (1.49)
BMI	29.0 (0.67)	29.2 (0.86)	29.2 (0.80)	29.0 (0.67)

Materials and Methods

Study group. The study group comprised 106 CFS patients (35 males, 71 females, mean age 43 ± 10 years) from nine sites across the United States, all of whom underwent rigorous diagnosis using the criteria developed by Holmes *et al.* (13) and Fukuda *et al.* (10). All subjects signed an informed consent document prior to beginning testing. Subjects and testers were blind to the expression of RNase L as blood test results were not available until all testing was completed. Upon receipt of the blood test results, 37 kDa RNase L expression was present in 70 patients (20 males, 50 females), but not in the remaining 36 (15 males, 21 females).

Measures. Venous blood samples for all patients were collected and analyzed at the Molecular Genetics Institute in Montpellier using the techniques described by De Meirleir *et al.* (4). A ratio of 37 kDa protein to 80 kDa protein >0.5 was taken as an indication of RNase L pathway deregulation (4).

Cardiopulmonary exercise testing was employed to evaluate $VO_{2\ peak}$. The test comprised a graded treadmill protocol modified for subjects with compromised exercise capacities (9).

Procedures. The entire procedure for cardiopulmonary exercise testing was explained in detail to each patient prior to testing. Exercise continued either to volitional fatigue, until the subject could no longer maintain position on the treadmill, or the test technician stopped the test to comply with ACSM guidelines. All patients performed at least two exercise tests, two weeks apart. A third test was required if the duration for the first two tests differed by more than 10%. Criteria for maximal exertion were based on a respiratory quotient (RQ) greater than 1.00 and a perceived exertion rating (RPE) greater than 18. The highest value for $VO_{2\ peak}$ obtained from the tests was used in the analysis. A detailed description of these procedures is included in an earlier article (28).

Statistical analysis. Pearson product moment correlation coefficients ranged from 0.01 to 0.58 among the dependent variables: $VO_{2\ peak}$, body mass index (BMI), percentage of predicted peak heart rate (HR_{peak} predict), RQ and RPE. With no evidence of multicolinearity, *i.e.* any correlation above 0.9 (11), all variables were entered into a 2 (Gender) x 2 (Immune Status) MANOVA. As the follow-up to a significant multivariate effect, discriminant functions and univariate *F*'s were employed to examine the relative contribution of each dependent variable to differences between groups. Significance levels were set at $p<0.05$ for the initial multivariate analysis. To protect against the inflated type-I error rates produced when conducting multiple ANOVAs following a significant MANOVA, significance levels for the follow- up univariate analyses were adjusted using the Bonferroni procedure: dividing alpha by the number of follow-up comparisons to be made (0.05/5=0.01) (26).

Results

A significant multivariate main effect was found for immune status, Wilks' Lambda=0.85, F (5, 98)=3.34, $p<0.01$. No gender effect or interaction was observed (both $p>0.05$). Follow-up discriminant function and univariate analyses identified $VO_{2\ peak}$ as the variable contributing most to the difference between groups. $VO_{2\ peak}$ was the only variable to enter the significant discriminant function, eigenvalue=0.18, R_c=0.39. Similarly, with alpha levels adjusted to protect against Type-I errors, $VO_{2\ peak}$ was the only variable for which ANOVA was significant, F (1, 102)=12.84, $p<0.01$. Mean $VO_{2\ peak}$ for the deregulated RNase L group was lower than for the normal group (19.6 *vs.* 22.8 ml/kg/min). Pooled within-groups correlations with function, univariate *F*'s and *p* values are contained in Table I. Means, standard deviations and group n's are shown in Table II.

Discussion

The results of this study support the value of 37-kDa RNase L expression as a biological marker for CFS. They also add to the body of knowledge linking biochemical deregulation

in the 2-5 A/RNase L pathway to clinical correlates in a subpopulation of CFS patients. The apparent relationship between 37-kDa RNase L expression and low oxygen consumption in CFS suggests a pathogenesis that may include both abnormal oxidative metabolism and attenuated activation of the autonomic nervous system.

A possible mechanism for this may involve increased nitric oxide (NO) production consistent with suggested deregulation of immune activity related to cellular stress, *e.g.*, viral or bacterial infections, toxic chemical exposure, *etc.*, and concomitant with abnormalities in the 2-5 A pathway (5). Nitric oxide regulates many physiological processes including neuronal communication, blood vessel modulation, oxidative metabolism, and immune response (16).

Increased pro-inflammatory cytokine levels consistent with viral or bacterial infection have been found in a subset of CFS patients (19). It is hypothesized that these cytokines are responsible for the RNase L anomaly also observed in some CFS patients (17). This deregulation of the RNase L antiviral pathway is further hypothesized to induce nitric oxide synthase (iNOS), leading to increased NO levels (18). Subsequent disruptions to ion channel transport may then explain many of the symptoms associated with CFS (6). An increasing number of human diseases are being associated with NO-induced ion channel defects. Among these channelopathies are Huntington's disease, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (27).

Elevated NO levels increase exposure to oxidative stress. The production of peroxynitrite, a potent oxidant, results from heightened levels of both NO and superoxide (3). Peroxynitrite and NO can compromise normal blood brain barrier permeability (BBBP) and disrupt central nervous system (CNS) neuronal transmission (1). Increased levels of NO and peroxynitrite inhibit glutamate transport and depress HPA axis activity through diminished secretion of corticotrophin releasing hormone (18). It has been proposed that many symptoms of CFS result from a complex interaction between the incidence of low circulating cortisol and abnormalities of central neurotransmitters involved in HPA axis function (2). Decreased oxygen consumption resulting from defective cellular respiration has also been linked to biochemical mechanisms involving NO. Superoxide generated by the electron transport system combines with mitochondrial NO to produce peroxynitrite. Subsequent exposure to oxidative stress can irreversibly inhibit mitochondrial respiration and ultimately lead to mitochondrial collapse and cell death (30). Englebienne (7) suggests that this cell apoptosis further accelerates RNase L deregulation to perpetuate a self-sustaining pathological cascade.

The results of this study are consistent with a hypothetical channelopathy in CFS. It is possible to explain differences in exercise capacity between the subsets of patients in terms of the documented immune abnormalities present in one of the groups. Comparable measures of effort between the groups and the absence of a significant gender effect add further credibility to such an explanation. A mechanism involving elevated NO and the oxidant peryoxinitrite has been suggested as a common etiology for the often overlapping symptoms of chronic fatigue syndrome, multiple chemical sensitivity, and posttraumatic stress disorder (18). This hypothesis is consistent with research showing a correlation between symptom severity in CFS and the extent of oxidative damage to the immune function (15). Although the role of cellular stress and increased NO was not specifically examined in this current study, the observed relationship between oxygen consumption and immune deregulation appears compatible with the biochemistry of nitric oxide synthase leading to increased NO levels. Future research in this area is certainly warranted; in particular, research that can combine assessment of the immune function and the chemistry of ion transportation with valid measures of physical functioning.

Acknowledgements

We thank the following physicians for their support during data collection: Lucinda Bateman, MD, Joseph R. Bellesorte, DU, Paul J. Cimoch, MD, Joseph F. John, MD, Robert H. Keller, MD, FACP, Alex Mercandetti, MD, Daniel L. Peterson, MD, Richard N. Podell, MD, James L. Sepiol, MD, Bruce E. Stein, MD, and Leslie H. Taylor, MD. This research was supported by Hemispherx Biopharma, Philadelphia, PA, U.S.A.

References

1 Bested AC, Saunders PR and Logan AC: Chronic fatigue syndrome: neurological findings may be related to blood-brain barrier permeability. Med Hypotheses *57(2)*: 231-237, 2001.

2 Cleare AJ: Neuroendocrine dysfunction. *In*: Handbook of Chronic Fatigue Syndrome, Jason LA, Fennell PA and Taylor RR (Eds.) J. Wiley and Sons, NJ, 2003, pp 331-360.

3 Cooper C, Davies N, Psychoulis M, Canevari L, Bates T, Dobbie M, Casley C and Sharpe M: Nitric oxide and peroxynitrite cause irreversible increases in the Km for oxygen of mitochondrial cytochrome oxidase: *in vitro* and *in vivo* studies. Biochemica et Biophysica Acta *1607*: 27-34, 2003.

4 DeMeirleir KD, Bisbal C, Campine K, DeBecker P, Salehzada T, Eemettre E and Lebleu B: A 37kDa 2-5A binding protein as a potential biochemical marker for chronic fatigue syndrome. Am J Med *108*: 99-105, 2000.

5 DeMeirleir K, Peterson DL, De Becker P and Englebienne P: From laboratory to patient care. *In*: Englebienne P and DeMeirleir K (Eds.). Chronic Fatigue Syndrome: A Biological Approach. Boca Raton, FL: CRC Press, 2002, pp. 265-284.

6 Engelbienne P, Herst CV, DeSmet K *et al*: Interactions between RNaseL ankyrin-like domain and ABC transporters as a possible origin for pain, ion transport, CS and immune disorders of chronic fatigue immune dysfunction syndrome. J CFS *8(3/4)*: 83-102, 2001.

7 Engelbienne P: RNase L in health and disease – What did we learn recently? J CFS *11(2)*: 97-109, 2003.
8 Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG and Komaroff A: Chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Int Med *121*: 953-959, 1994.
9 Fletcher GF, Balady G, Froelicher VF, Hartley LH, Haskell WL and Pollack ML: Exercise standards: A statement for healthcare professionals from the American Heart Association. Circulation *91*: 580-615, 1995.
10 Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG and Komaroff A: Chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Int Med *121*: 953-959, 1994.
11 Hair JF Jr, Anderson RE, Tatham RL and Black WC: Multivariate Data Analysis with Readings, 3rd Ed. New York: Macmillan, pp. 146-148, 1992.
12 Hobbs AJ, Higgs A and Moncada S: Inhibition of nitric oxide synthetase as a potential therapeutic target. Annu Rev Pharmacol Toxicol *39*: 191-220, 1999.
13 Holmes GP, Kaplan JE and Schonberger LB: Definition of the chronic fatigue syndrome. Ann Int Med *109*: 512-522, 1988.
14 Komaroff AL: The biology of chronic fatigue syndrome. Am J Med *108*: 169-171, 2000.
15 McGregor NR, De Becker P and DeMeirleir K: RNase-L, symptoms, biochemistry of fatigue and co-morbid disease. *In*: Englebienne P and DeMeirleir K (Eds.). Chronic Fatigue Syndrome: A Biological Approach. Boca Raton, FL: CRC Press, pp. 175-200, 2002.
16 Muriel P and Perez-Rojas J: Nitric oxide inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondria membrane fluidity. Comp Biochem Physio Part C 1-7, 2003.
17 Nijs J, Demanet C, McGregor NR, DeBecker P, Verhas M, Engelbienne P and DeMeirleir K: Monitoring a hypothetical channelopathy in chronic fatigue syndrome: preliminary observations. J CFS *11(1)*: 117-133, 2003.
18 Pall ML: Elevated nitric oxide/peryoxinitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome and posttraumatic stress disorder. Ann NY Acad Sci *933*: 323-329, 2001.
19 Patarca R, Klimas NG, Lutgendorf S *et al*: Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome, interrelations with cellular sources and patterns of soluble immune mediator expression. Clin Inf Dis *18*: S147-153, 1994.
20 Riley MS, O'Brien CJ, McCloskey DR, Bell NP and Nicholls DP: Aerobic work capacity in patients with chronic fatigue syndrome. Br Med J *301*: 953-956, 1990.
21 Sargent C, Scroop GC, Nemeth P, Burnet RB and Buckley JD: Maximal oxygen uptake and lactate metabolism are normal in chronic fatigue syndrome. Med Sci Sports Exerc *34*: 51-56, 2002.
22 Snell CR, VanNess JM, Strayer DR and Stevens SR: Physical performance and prediction of 2-5A synthetase/RNaseL antiviral pathway activity in patients with chronic fatigue syndrome. In Vivo *16*: 107-110, 2002.
23 Suhaldonik RJ, Reichenback NL, Hitzges P, Adelson ME, Peterson DL, Cheney P, Salvato P, Thompson C, Loveless M, WEG Muller, Schroder HC, Strayer DR and Carter WA: Changes in the 2-5A synthetase/RNase L antiviral pathway in a controlled clinical trial with poly(I)-poly(C12U) in chronic fatigue syndrome. In Vivo *8*: 599-604, 1994.
24 Suhadolnik RJ, Peterson DL, Cheney PR, Horvath SE, Reichenbach NL, O'Brien K, Lombardi V, Welsch S, Furr EG, Charubala R and Pfleiderer W: Biochemical dysregulation of the 2-5A synthetase / RNase L antiviral defense pathway in chronic fatigue syndrome. J CFS *5*: 223-242, 1999.
25 Szabo C: Multiple pathways of peroxynitrite cytotoxicity. Tox Lett *140*: 105-112, 2003.
26 Thomas JR and Nelson JK: Research Methods in Physical Activity (3rd ed.). Champaign, IL: Human Kinetics, 1996, pp. 159.
27 Urushitani M and Shimohama S: The role of nitric oxide in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord *2*: 71-81, 2001.
28 VanNess JM, Snell CR, Dempsey WL, Strayer DR and Stevens SR: Subclassifying chronic fatigue syndrome through exercise testing. Med Sci Sports Exerc *35(6)*: 908-913, 2003.
29 Vojdani A, Choppa PC and Lapp CW: Downregulation of RNase L inhibitor correlates with upregulation of interferon-induced proteins (2-5A synthetase and RNase L) in patients with chronic fatigue syndrome. J Clin Lab Immun *50*: 1-6. 1998.
30 White RJ and Reynolds IJ: Mitochondrial depolarization in glutamate-stimulated neurons: an early signal specific to excitotoxin exposure. J Neurosci *16*: 5688-5697, 1996.

Received November 29, 2004
Accepted January 11, 2005