

Original Research

Saliva Fatigue Biomarker Index in a COVID SampleLeonard A. Jason ^{1,*}, John Kalns ², Lauren Ruesink ¹, Ben Z. Katz ³

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Received: April 19, 2026**Accepted:** August 18, 2026**Published:** October 25, 2026**Abstract**

Following COVID, some individuals have developed Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). We assessed whether a salivary biomarker of fatigue could identify young adults with ME/CFS following COVID. We examined the ratio of the concentrations of 2 peptide fragments in saliva, referred to as the Fatigue Biomarker Index (FBI), in participants from our study diagnosed with severe ME/CFS, moderate ME/CFS, and recovered controls. Participants classified with severe ME/CFS exhibited higher FBI values than participants with moderate ME/CFS and recovered controls. These findings are exploratory and require replication in larger, adequately powered studies.

Keywords

Fatigue biomarker index; chronic fatigue syndrome; myalgic encephalomyelitis; myalgic encephalomyelitis/chronic fatigue syndrome; COVID



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1. Introduction

Salivary biomarkers have increasingly been explored as noninvasive indicators of physiological states, including fatigue. Prior work has identified specific peptide fragments in saliva whose relative concentrations shift under conditions of physical strain and exertion [1]. The ratio of these peptides, termed the Fatigue Biomarker Index (FBI), has shown sensitivity to fatigue-related processes across a range of contexts, including exercise, sleep deprivation, and occupational performance. Evidence from prior studies suggests that this index may reflect underlying biological processes associated with fatigue severity rather than subjective reports alone [2, 3]. Although these findings are promising, relatively few studies have examined whether such biomarkers can distinguish clinical populations characterized by persistent and disabling fatigue.

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), particularly in cases emerging following viral infections such as COVID-19, presents an opportunity to evaluate whether objective salivary markers correspond to illness severity. We have previously shown that in a community-based pediatric sample of individuals with ME/CFS [4], those with severe ME/CFS had significantly lower FBI values than controls. The present study investigates the hypothesis that those with severe ME/CFS would have significantly different FBI scores than the combined moderate ME/CFS and recovered post-COVID groups.

2. Methods

2.1 Procedures

Participants were recruited between 2023 and 2024 through online Long COVID communities and university outreach efforts. Individuals reporting persistent post-COVID symptoms underwent clinical evaluation to confirm prior SARS-CoV-2 infection. Saliva samples were collected during in-person visits using standardized procedures within daytime hours.

We classified patients with Long COVID as meeting either the Fukuda et al. CFS criteria [5], the Institute of Medicine 2015 ME/CFS clinical criteria [6], and/or the ME/CFS Canadian Consensus Criteria [7]. The Fukuda et al. criteria [5] are broader and identify a larger group of patients with less severity than those identified by other criteria [6, 7]. Participants who met only one set of criteria for ME/CFS, primarily the Fukuda et al. [5] criteria, were defined as having moderate ME/CFS. Those who met more than one case definition (i.e., the Fukuda [5] and either Institute of Medicine [6] criteria or the Canadian Consensus Criteria [7]) were defined as having severe ME/CFS. Those who recovered were labeled as recovered controls. The final sample sizes with collected saliva available for analysis were 26 participants with Severe ME/CFS following COVID, 11 participants with moderate ME/CFS following COVID, and 72 recovered controls (C). All samples were coded and analyzed in a blinded fashion. This study was approved by all relevant Institutional Review Boards before data collection began and informed consent was obtained from all participants.

2.2 Determination of Fatigue Biomarker Index in Oral Fluid (OF)

The oral fluid collection method was designed to make collection at home or in clinic easy and reliable. The Salivette device by Sarstedt was used. Briefly, subjects were required to place the cotton sponge part of the device in their mouths for at least 1-2 minutes setting a timer to ensure

compliance. After collection, the sponge was placed into a tube containing 0.5 ml of stabilizing buffer. The stabilizing buffer preserves concentrations of fatigue peptides for at least 7 days at temperatures as high as 40°C; without stabilization, fatigue peptide concentrations will vary significantly unless frozen within 15 minutes of collection.

The Fatigue Biomarker Index was derived from the relative concentrations of two salivary peptides (ESPSLIA and GGHPPPP) and quantified using liquid chromatography coupled with tandem mass spectrometry. Samples were processed blinded as to their disease classification using established protocols including centrifugation, stabilization, and filtration prior to analysis. Internal standards were used to ensure measurement reliability, and assay validation procedures included assessments of precision, stability, and linearity.

In some samples measured levels of peptides were below quantification limits and thus concentrations had a somewhat higher degree of uncertainty than samples with peptide levels above the limits. However, in all samples reported peptide associated peaks passed quality checks for retention time and abundance of quantifying fragments.

2.3 Statistics

We examined the differences among participants' salivary fatigue biomarker indexes. Based on our previous community-based investigation of the Fatigue Biomarker Index [4], we specified a priori that our primary hypothesis would compare participants with severe ME/CFS to the combined moderate ME/CFS and recovered control groups. Our previous study demonstrated lower FBI values among participants with severe ME/CFS, but in the current paper, the FBI sign convention has been reversed, so higher current scores correspond directionally to lower scores under the earlier paper.

3. Results

There were no significant age or gender sociodemographic differences between the patient groups. 61% of the sample was female, most were 19-20 years of age, and 68% were White (see Table 1).

Table 1 Demographic and biomarker characteristics by ME/CFS group.

Characteristic	Severe ME/CFS (n = 26)	Moderate ME/CFS plus controls (n = 83)	p
Age in years	20.27 (2.63)	19.45 (1.48)	0.139
Gender			0.899
Male	8 (30.8%)	27 (32.5%)	
Female	17 (65.4%)	50 (60.2%)	
Other	1 (3.8%)	5 (6.0%)	
Prefer not to answer	0 (0.0%)	1 (1.2%)	
Race			0.627
Asian	2 (7.7%)	13 (15.7%)	
Black	1 (3.8%)	6 (7.2%)	
White	19 (73.1%)	55 (66.3%)	
Other	4 (15.4%)	9 (10.8%)	
Fatigue Biomarker Index (FBI)	2.28 (0.57)	2.05 (0.78)	0.030*
P700 (nM)	3.89 (4.58)	11.21 (29.65)	0.033*
Log P700	0.34 (0.48)	0.52 (0.63)	0.120

Note. Values are M (SD) or n (%). The comparison group includes 11 moderate ME/CFS participants and 72 recovered controls. All 109 participants have data for the variables shown.

*FBI uses a one-tailed Welch t test for the prespecified hypothesis that the severe ME/CFS group had significantly higher scores than the moderate ME/CFS plus controls. P700 was significant using a two-tailed Welch t test.

As predicted, an overall three-group ANOVA comparing participants with severe ME/CFS, moderate ME/CFS, and recovered controls was not statistically significant, indicating no overall group differences in the Fatigue Biomarker Index [$F(2,106) = 2.14$, $p = 0.08$]. Because a comparison between the severe ME/CFS group and the combined moderate ME/CFS/recovered control groups had been specified a priori based on previous work, we also conducted this planned comparison. When we compared the severe ME/CFS group ($M = 2.28$, $SD = 0.57$) to the combined moderate ME/CFS and control groups ($M = 2.05$, $SD = 0.78$), using one-sided p , there was a significant difference [$t(56.03) = -1.92$, $p = 0.03$].

4. Discussion

Consistent with our a priori hypothesis derived from our previous community-based study [4], the planned comparison between the severe ME/CFS group and the combined moderate ME/CFS/and recovered control groups reached statistical significance. These findings are consistent with prior work suggesting that the FBI may differentiate individuals with more severe illness, although they do not establish that the FBI directly reflects the continuum of clinical disease severity.

One interpretation of our findings is that biomarker signals become more detectable as physiological dysregulation intensifies. This may help explain why differences are more apparent when focusing on more severely affected individuals both in our current and previous [4] study. From a measurement perspective, this suggests that biomarker research in ME/CFS may benefit from stratifying samples by severity rather than treating the condition as homogeneous.

These findings are consistent with broader work indicating that post-infectious fatigue syndromes may share underlying biological features regardless of the specific triggering event. The similarity between post-COVID ME/CFS and previously studied ME/CFS populations supports the possibility of common mechanisms.

These preliminary findings do not establish the Fatigue Biomarker Index as a biomarker of disease severity. Rather, they provide hypothesis-generating evidence that the FBI may be associated with more severe post-COVID ME/CFS, a possibility that requires confirmation in larger, prospectively designed studies with comprehensive clinical characterization. Replication in larger samples using prospectively specified analytic plans will be important for establishing the clinical utility of the Fatigue Biomarker Index.

Our study has several limitations. First, the moderate ME/CFS group was relatively small, and to evaluate our primary hypothesis, we combined the moderate ME/CFS and recovered control groups for the planned comparison with the severe ME/CFS group. Second, although this study was designed as a preliminary evaluation of the salivary FBI, we did not include comprehensive measures of clinical illness severity, such as symptom duration, post-exertional malaise severity, physical functioning, or other validated measures of symptom burden. Consequently, we did not examine the relationship between the FBI and these important clinical characteristics. Future biomarker studies should continue to examine patients across the spectrum of illness severity while ensuring adequate representation of those with more severe disease.

In conclusion, a prespecified comparison was consistent with our hypothesis and prior findings. These results should be considered exploratory because of the modest sample size, and limited clinical characterization. Larger, prospectively designed studies are needed before conclusions can be drawn regarding the FBI's clinical utility as a biomarker for post-COVID ME/CFS.

Author Contributions

Conceptualization: LAJ, BZK, JK; Data analysis: LAJ, LR; Write up Paper: LAJ, BZK; editing: LAJ, BZK, JK, LR.

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Competing Interests

The authors report no conflicts of interest. John Kalns is the owner and founder of Hyperion and is a co-inventor of the fatigue biomarker technology used here.

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