LETTER

REPLY TO ROERINK ET AL.:

Metabolomics of chronic fatigue syndrome

Robert K. Naviaux[a,b,c,d,1] and Eric Gordon[e]

We thank Roerink et al. (1) for their comments. We respond to their two points in order. Their first point asked about the effect of physical activity on sphingolipids. The sphingolipid response to exercise is complex. It differs in healthy trained and untrained individuals and has not yet been studied using methods that can distinguish the classes of sphingosines, ceramides, sphingomyelins, and glycosphingolipids measured in our analysis of myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) (2). Our study shows specifically that ceramides were decreased in ME/CFS (2). However, the small study (n = 10 per group) by Baranowski et al. (3) that was cited by Roerink et al. (1) showed that ceramides were the same in trained and untrained subjects and did not change with acute exercise (figure 1 of ref. 3). All subjects in our first study of ME/CFS (2) were ambulatory. We did not specifically control for physical activity because the capacity for physical activity is a fundamental difference between patients with ME/CFS and controls. We wished to distinguish "pathological fatigue" that is chronic and prevents activity at baseline in patients with ME/CFS from "physiological fatigue" that is transient and caused by activity in non-CFS subjects and is not present at baseline. Others have also focused on this distinction (4). Metabolomic analysis showed that ME/CFS is characterized by differences in 20 different metabolic pathways (figure 1E of ref. 2), and not just sphingolipids. With regard to the broader metabolic effects of exercise, a recent large metabolomics study (n = 277) found that only 11 of 591 metabolites were significantly correlated with physical activity in healthy subjects (5). Increased physical activity was associated with a decrease in isoleucine, valine, and glucose, whereas sedentary behavior produced an increase (5). In ME/CFS, isoleucine, valine, and glucose were not changed (2). The absence of metabolic abnormalities in ME/CFS known to be associated with decreased physical activity is strong evidence that physical activity alone cannot explain the distinct pattern of metabolic abnormalities found in ME/CFS (2).

Second, Roerink et al. (1) raise questions about the standard statistical methods used in metabolomics studies. We used multivariate analysis by partial least squares discriminate analysis (PLSDA), area under the receiver operator characteristic (AUROC) curve analysis, random forest methods for biomarker discovery, repeated double cross-validation, and permutation analysis. A convenient implementation of these methods is available at www.metaboanalyst.ca, and nice reviews are available (6). Roerink et al. (1) incorrectly state that the method for selecting the top 60 metabolites was "nondescribed" in our paper (2). We state that we used PLSDA in both the *Results* and *Materials and Methods* sections. Roerink et al. (1) then incorrectly implied that we used 60 metabolites in the AUROC analysis. We did not. We used eight metabolites in males and 13 metabolites in females (figure 2 and table 4 of ref. 2). Ultimately, the best way to confirm the scientific accuracy of our metabolic findings in ME/CFS is to repeat the study using fresh samples collected from an independent cohort of patients and controls. This study is currently underway.

1 Roerink ME, Bronkhorst EM, van der Meer JWM (2016) Metabolome of chronic fatigue syndrome. *Proc Natl Acad Sci USA* 114:E910.
2 Naviaux RK, et al. (2016) Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci USA* 113(37): E5472–E5480.
3 Baranowski M, Charmas M, Długołęcka B, Górski J (2011) Exercise increases plasma levels of sphingoid base-1 phosphates in humans. *Acta Physiol (Oxf)* 203(3):373–380.
4 Aoki R, et al. (2016) Human herpesvirus 6 and 7 are biomarkers for fatigue, which distinguish between physiological fatigue and pathological fatigue. *Biochem Biophys Res Commun* 478(1):424–430.

[a]The Mitochondrial and Metabolic Disease Center, University of California, San Diego School of Medicine, San Diego, CA 92103-8467; [b]Department of Medicine, University of California, San Diego School of Medicine, San Diego, CA 92103-8467; [c]Department of Pediatrics, University of California, San Diego School of Medicine, San Diego, CA 92103-8467; [d]Department of Pathology, University of California, San Diego School of Medicine, San Diego, CA 92103-8467; and [e]Gordon Medical Associates, Santa Rosa, CA 95403

Author contributions: R.K.N. and E.G. designed research; R.K.N. performed research; R.K.N. contributed new reagents/analytic tools; R.K.N. analyzed data; R.K.N. wrote the paper; and E.G. critically reviewed the letter.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: rnaviaux@ucsd.edu.

5 Xiao Q, et al. (2016) Objectively measured physical activity and plasma metabolomics in the Shanghai Physical Activity Study. *Int J Epidemiol* 45(5): 1433–1444.
6 Xia J, Broadhurst DI, Wilson M, Wishart DS (2013) Translational biomarker discovery in clinical metabolomics: An introductory tutorial. *Metabolomics* 9(2): 280–299.