REPLY TO VOGT ET AL.:
Metabolomics and chronic fatigue syndrome

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We thank Vogt et al. for their comments (1). We respond to their three points in order. First, we are aware of the need to extend future metabolomics studies to include other disease groups. We stated this fact in the discussion of ref. 2 and are validating the results in independent cohorts. The detailed biochemical phenotype or signature that we found provides a first glimpse at a previously hidden biology. For example, disturbances in sphingolipid metabolism have important implications for immunobiology and neuroendocrine regulation relevant to myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) (3). Sphingolipids are important mediators of the cell danger response (CDR) (4), and the CDR is an important regulator of the behavioral and functional changes produced by infection, and associated with sickness behavior (5). The biochemical phenotype of ME/CFS is distinct from other diseases that Vogt et al. (1) named. For example, in heart failure, metabolomics shows that long chain acyl-carnitines are increased (6), but these long chain acyl-carnitines were not changed in ME/CFS (2). In our view, chemistry and metabolism underlie all aspects of human biology. Our studies show that metabolomics can be used as a new lens to reveal unexpected biology that was invisible before.

The authors' stated belief and supporting citation (7) that the diagnostic criteria for ME/CFS fail to identify a valid clinical syndrome is no longer credible in the face of advancing biochemical and molecular analysis. This view of Vogt et al. (1) conflicts with the recent findings of the Institute of Medicine (8). In addition, if ME/CFS had no valid, unifying biology, then the chances of metabolomics showing the diagnostic accuracy found in our study by receiver operator characteristic curve and permutation analysis can be calculated as 1 in 1,000 ($P = 0.001$). This finding was reported in figure 2 and table 2 of ref. 2. Additional independent studies will be needed to confirm or disprove these results.

Second, Vogt et al. (1) state that "a metabolic correlate to a clinical syndrome does not explain why these abnormalities are there." True, but is not that the objective of science, to find out what is currently unknown? Metabolomics provides a new tool that can be used to design new experiments in the laboratory and new clinical trials to understand how changes in metabolism cause changes in behavior and functional capacity, and how new treatments might change metabolism.

Third, regarding dauer, the chemical details of the metabolic findings in ME/CFS led directly to a survey of other hypometabolic syndromes like dauer, diapause, torpor, hibernation, and caloric restriction. It was the detailed nature and specific pattern of abnormalities affecting six pathways (sphingolipids, phospholipids, purines, cholesterol, polyamines, and redox metabolism), and not the over 50 other biochemical pathways interrogated, that focused our attention on dauer. In dauer, there is a reprioritization of sensory processing that is metabolically controlled (9). This situation bears similarities to ME/CFS. We are hopeful that experiments designed to study dauer and metabolomics will bring more investigators into the field, help to reframe old questions, open fresh directions for research, and shed light on an underlying biology that will ultimately benefit patients with ME/CFS.

1 Vogt H, Ulvestad E, Wyller VB (2016) Metabolic features of chronic fatigue syndrome revisited. *Proc Natl Acad Sci USA*, 10.1073/pnas.1615143113.

2 Naviaux RK, et al. (2016) Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci USA* 113(37):E5472–E5480.

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The authors declare no conflict of interest.

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3 Chakraborty M, Jiang XC (2013) Sphingomyelin and its role in cellular signaling. *Adv Exp Med Biol* 991:1–14.
4 Naviaux RK (2014) Metabolic features of the cell danger response. *Mitochondrion* 16:7–17.
5 McCusker RH, Kelley KW (2013) Immune-neural connections: How the immune system's response to infectious agents influences behavior. *J Exp Biol* 216(Pt 1): 84–98.
6 Hunter WG, et al. (2016) Metabolomic profiling identifies novel circulating biomarkers of mitochondrial dysfunction differentially elevated in heart failure with preserved versus reduced ejection fraction: Evidence for shared metabolic impairments in clinical heart failure. *J Am Heart Assoc* 5(8):e003190.
7 Brurberg KG, Fønhus MS, Larun L, Flottorp S, Malterud K (2014) Case definitions for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): A systematic review. *BMJ Open* 4(2):e003973.
8 Institute of Medicine (2015) *Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an Illness* (National Academies Press, Washington, DC). Available at www.nationalacademies.org/hmd/Reports/2015/ME-CFS.aspx. Accessed January 26, 2016.
9 Chen X, Chalfie M (2014) Modulation of C. elegans touch sensitivity is integrated at multiple levels. *J Neurosci* 34(19):6522–6536.