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Preliminary differences in peripheral immune markers and brain metabolites between fatigued and non-fatigued breast cancer survivors: a pilot study

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Abstract

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments described in this manuscript comply with the current laws of the country in which they were performed.

Persistent cancer-related fatigue (PCRF) is one of the most troubling side-effects of breast cancer (BC) treatment. One explanatory model for PCRF is sickness behavior, which is a set of adaptive responses including sleepiness and depressed mood in reaction to an inflammatory trigger. Prior research has investigated differences in inflammatory cytokines between fatigued and non-fatigued BC survivors, but no study has examined differences in brain metabolites. Differences in inflammatory markers, and brain metabolites using proton magnetic resonance spectroscopy were evaluated within 16 fatigued and 13 non-fatigued BC survivors. Fatigued BC survivors had significantly higher ratios of two markers derived from brain metabolites; namely (a) creatine, normalized to total creatine (creatine + phosphocreatine (Cr/tCr)) ratio ($P = 0.03$) and (b) glutamate + glutamine (Glx) to N-acetyl-aspartate (NAA) ratio ($P = 0.01$) in the posterior insula compared to non-fatigued breast cancer survivor. Further, serum IL-6 was increased in fatigued women compared to non-fatigued women ($P = 0.03$). Using receiver operator curves (ROC) we determined that the posterior insula Glx/NAA ratio was the best predictor of fatigue with an overall area under the receiver operating characteristic curve (AUROC) of 79 %, with a sensitivity of 81 % and a specificity of 69 %. However, posterior insula Glx/NAA, Cr/tCr and serum IL-6 were not significantly correlated with one another implying the possibility of independent biological mechanisms for PCRF rather than an interrelated mechanism as represented by the sickness behavior model. This study provides novel preliminary evidence of several distinct neurobiological changes in the posterior insula associated with PCRF in BC survivors. Future, longitudinal studies are needed to explore these distinct biological phenomena where changes through time in peripheral immune markers and brain metabolites are examined to determine if they correlate with changes in fatigue.

Keywords

Magnetic resonance spectroscopy; Persistent fatigue; Breast cancer survivors; Creatine; Glutamate; Cytokines; C-reactive protein; Posterior insula; Anterior insula; Anterior cingulate; Occipital cortex

Introduction

There are nearly 3 million women in the United States living as breast cancer (BC) survivors (American Cancer Society 2012). Persistent cancer-related fatigue (PCRF) is one of the most troubling long-term side-effects of cancer treatment (Montazeri 2008) and continues to affect around 33 % of BC survivors even 10 years after completion of cancer treatments (Bower et al. 2011). PCRF is associated with decreased quality of life, (Alexander et al. 2009; Kim et al. 2008; Reid-Arndt et al. 2010) decreased sleep quality, (Alexander et al. 2009; Kim et al. 2008) depression (Bower 2005) and impaired cognition (Bower 2008). Currently, limited treatments exist for PCRF. This may in part be due to our limited understanding of the mechanisms underlying this complex subjective symptom.

One possible mechanism to explain PCRF is maladaptive sickness behavior (Myers 2008). Sickness behavior is a set of adaptive behaviors such as lethargy, sleepiness, hyperalgesia and depressed mood, in response to an illness (Hart 1988). Sickness behavior is thought to be initiated by multiple pro-inflammatory perturbations during both cancer and cancer

treatment (Lee et al. 2004). It is known that these perturbations trigger the immune system to produce pro-inflammatory cytokines such as interleukin-1 β and -6 (IL-1 β & IL-6), and tumor necrosis factor- α (TNF- α) (Dantzer and Kelley 2007). These peripherally released cytokines then act on the brain through the vagus nerve, the choroid plexus, and circumventricular organs or they may also directly cross the blood-brain barrier causing neuroinflammation (Dantzer 2001; Maier 2003). In maladaptive sickness behavior these behavioral and physiological changes become chronic, leading to long term symptoms such as fatigue (Dantzer and Kelley 2007).

Support for the sickness behavior model of PCRF in BC survivors, comes from findings of sustained elevations in pro-inflammatory cytokines following the end of cancer treatment, even when the person is apparently cancer free (Bower et al. 2002, 2003; Collado-Hidalgo et al. 2006). In particular, BC survivors with persistent fatigue have significantly higher levels of soluble inflammatory markers and cellular immune responses such as IL-1 β , IL-6 and soluble TNF- α receptors as compared to non-fatigued BC survivors (Bower et al. 2002, 2003, 2011; Collado-Hidalgo et al. 2006; Schubert et al. 2007), although these associations are not consistently observed. (Gelinass and Fillion 2004) These inflammatory immune changes remain after controlling for age, BMI, depressed mood, cognitive problems, length of time since diagnosis, and treatment type (Bower et al. 2002, 2003). Inflammatory marker, C reactive protein (CRP), has also been shown to be elevated in breast cancer survivors (Liu et al. 2012; Starkweather et al. 2011).

While peripheral pro-inflammatory cytokines are elevated in fatigued BC survivors (Bower et al. 2002, 2003; Collado-Hidalgo et al. 2006; Segerstrom and Miller 2004), there is a significant gap in our knowledge of how peripheral immune dysfunction may subsequently affect the central nervous system (CNS) and cause PCRF. While neuroimaging studies have explored cognitive function in breast cancer patients and survivors (reviewed in Reuter-Lorenz and Cimprich 2013), no studies have explored changes in brain metabolic function of chronically fatigued breast cancer survivors. However, emerging data in people with chronic fatigue syndrome, which like PCRF is also characterized by increased peripheral pro-inflammatory cytokines (Fletcher et al. 2009) and long term debilitating fatigue, have shown changes in various brain metabolites. These include elevations in lactate within the cerebral spinal fluid (Mathew et al. 2009); a significant increase of choline-containing compounds (Cho) in the basal ganglia (Chaudhuri et al. 2003) and occipital cortex (Puri et al. 2002); and decreased concentrations of NAA in the hippocampus (Brooks et al. 2000) compared to age and gender matched healthy controls. Also, a study in lung cancer patients, prior to treatment, found a significant negative association between the inflammatory cytokine TNF- α and NAA in the occipital cortex compared to age-matched controls (Benveniste et al. 2012). In aggregate, these data suggest a possible interplay between the development of PCRF, and alterations in immune signaling and brain metabolites.

To specifically explore the interplay between brain-immune signaling and the presence of PCRF, differences in CRP, peripheral inflammatory cytokines, and brain metabolites were evaluated between age-matched fatigued and non-fatigued BC survivors within a cross sectional pilot study. The areas of the brain focused on in this study were chosen because they have been shown to have alterations in brain metabolites in various fatigued groups and

they have functions that are conceivably involved in the awareness, conceptualization and interpretation of fatigue: the occipital cortex (Benveniste et al. 2012; Puri et al. 2002), the insula (Boksem and Tops 2008; Lopez Zunini et al. 2012), and the anterior cingulate cortex (Boksem and Tops 2008; Lopez Zunini et al. 2012). The study hypothesized that pro-inflammatory peripheral cytokines, CRP, and key brain metabolites involved in excitotoxicity, i.e., glutamate and glutamine (Mehta et al. 2013) would be increased in the fatigued BC survivors as compared to non-fatigued BC survivors; and that brain metabolites associated with energy metabolism (creatine and phosphocreatine) (Maddock and Buonocore 2012), neuronal loss of function (NAA) (Moffett et al. 2007), a marker of glial cells (myo-inositol) (Kesler et al. 2013), and cell membrane integrity (choline containing compounds) (Kesler et al. 2013) would be decreased in the fatigued BC survivors as compared to non-fatigued BC survivors; and that increased levels of inflammatory markers would be significantly associated with differences in brain metabolites. To test these hypotheses the following aims were investigated: 1) to compare the peripheral inflammatory immune markers (serum cytokines concentrations of IL-1 β , IL-6, and TNF- α), a systemic inflammatory marker (serum CRP levels) and brain metabolites (Creatine {Cr}, creatine plus phosphocreatine {Cr/tCr}, glutamine plus glutamate {Glx}, choline {Cho}, myo-Inositol {Ins}, NAA, and the ratio of Glx to NAA {Glx/NAA}) between fatigued and non-fatigued BC survivors. The secondary aims included: 2) To investigate the correlation between peripheral inflammatory immune markers, CRP, and brain metabolites in fatigued, and non-fatigued BC survivors; and 3) To determine predictors of fatigue in breast cancer survivors.

Research design and methods

This was a cross-sectional pilot study comparing peripheral inflammatory immune markers (IL-1 β , IL-6, TNF- α , CRP) and the following brain metabolites: Cr, the ratio of creatine to total creatine, which is Cr/tCr, Glx, Cho, Ins, NAA, Glx/ NAA comparing fatigued to non-fatigued BC survivors. The study was approved by the University of Michigan Medical School Institutional Review Board and participants provided written informed consent.

Participants and eligibility

Eligible participants were women, 18 years of age and older who have a diagnosis of breast cancer (stage 0 to IIIa); have completed all cancer-related treatments (i.e., surgery, chemotherapy, radiotherapy, immunotherapy, etc.) except for hormonal therapy and/or Herceptin at least 12 weeks previously; have no contraindications to magnetic resonance imaging (MRI) and without evidence of cancer recurrence.

Women were ineligible if they were pregnant or lactating; diagnosed with anemia [defined as hemoglobin levels <12 g/ dl] or receiving treatment for anemia; diagnosed with any unstable or untreated comorbidities likely to cause significant fatigue (i.e., moderate to severe heart failure, hypothyroidism); have a diagnosis of untreated DSM-IV-TR Axis-I or Axis-II disorders; have an initiation, a cessation or change of dose (up to 3 weeks prior to the study's start) of any chronic medications, or dietary supplements; and have implanted (e.g., surgical clips or staples) metal objects or other contraindications with magnetic resonance imaging.

Participants were identified through the University of Michigan Breast Cancer Clinics and from participants in former pilot clinical trial conducted in BC survivors between March 2011 and March of 2012. Interested women were invited to a screening visit where socio-demographics, concomitant medications and supplements; medical history, brief physical including vitals; a blood draw for a complete blood count; height and weight (used to calculate BMI); and a urine pregnancy test, if indicated, were conducted. Menopausal status at time of breast cancer diagnosis was determined through the women's medical chart where women who had experienced at least 12 continuous months without a menstrual cycle were deemed post-menopausal. Women also answered a battery of questionnaires: Brief Fatigue Inventory (BFI) (Mendoza et al. 1999), Hospital Anxiety and Depression Scale (HADS) (Bjelland et al. 2002), Pittsburgh Sleep Quality Index (PSQI) (Berger et al. 2009), and the Brief Pain Inventory (BPI) (Tittle et al. 2003). For the 2 weeks after the screening visit women were contacted once per week and a BFI was administered. Approximately 4 weeks after the screening visit women came for their final study visit wherein a blood draw was performed for CRP and cytokines; proton magnetic resonance spectroscopy (^1H -MRS) was done for assessment of brain metabolites; and an identical set of questionnaires to their screening visit were administered.

To be designated a fatigued BC survivor, women needed to have an average BFI ≥ 4.0 based upon three BFIs administered approximately 1 week apart at their screening visit and via phone contacts on the following 2 weeks. All three BFI scores were added together and divided by three to obtain our average BFI. An average BFI ≥ 4.0 indicates clinically relevant and at least moderate levels of fatigue severity (Chang et al. 2007). Non-fatigued BC survivors needed an average BFI < 4.0 administered on the same timeframe as fatigued survivors; as well as an average pain score of < 4 on the BPI, a PSQI total score of < 7 , and a HADS < 11 for both anxiety and depression sub-scales. Non-fatigued BC survivors were age-matched ± 2 years to the fatigued BC survivors.

Laboratory methods

Blood was collected by venipuncture after a 12 h fast in the morning just prior to ^1H -MRS. Then the following assays were performed.

Serum analysis of inflammatory biomarkers—Blood was collected into ethylene diamine tetra acetic acid (EDTA) and was allowed to clot for 2 h, centrifuged at 8,000 rpm in a microcentrifuge and the serum removed, aliquoted and immediately stored at -80°C prior to analysis. Serum levels of TNF- α , IL-1 β , and IL-6 were measured in triplicate using a bead-based immunofluorescence assay (Luminex Inc., Austin, TX, USA). Human cytokine/ chemokine assay kits were purchased from Millipore Corp. (Cat # HSCYTO-60SPMX13). Assays were performed according to the protocol supplied by the manufacturer. Data were collected and analyzed using the Luminex-100 system Version IS (Luminex, Austin, TX, USA). A four or five-parameter regression formula was used to calculate the sample concentrations from the standard curves. The intra- and inter-assay coefficients of variation (CV) are respectively for TNF- α , IL-1 β , and IL-6 are: 3.49, 3.78; 3.11, 2.16; and 3.51, 4.48. Lower level of quantification was 0.13 pg/mL and all cytokine levels are reported in pg/mL.

Analysis of serum C - reactive protein—Blood was collected into a serum separating tube (SST), centrifuged at 8,000 rpm in a microcentrifuge and the serum removed. CRP levels were determined using an immunoturbidimetric method carried out by the central pathology laboratories at the University of Michigan.

Proton magnetic resonance spectroscopy (¹H-MRS)—All subjects were imaged while at rest on a Philips Achieva 3 T system (Best, Netherlands) using an 8 channel receive head coil. For localization of spectroscopy voxels, we performed T1-weighted 3D-MPRAGE imaging with (0.9 mm)³ isotropic voxel resolution. MR spectra were acquired from using 3.0 cm×2.0 cm×3.0 cm volumes from the anterior cingulate, right anterior insula, right posterior insula, and occipital cortex (Fig. 1). Single-voxel point resolved spectroscopy (PRESS) spectra (TR/TE=2,000/ 35 ms) were acquired using ‘VAPOR’ water suppression with 96 averages for each voxel. The spectroscopy data were analyzed using LCModel (Stephen Provencher, Oakville, Ontario, Canada). Metabolite levels were only used for statistical analysis from LCModel if the Cramér-Rao bounds were less than 20 %. The metabolite level analyses utilized two approaches: 1) estimation of metabolite levels expressed as ratios to total creatine (tCr; combination of creatine and phosphocreatine) and 2) estimation of metabolite levels expressed as concentrations in institutional units (IU) adjusted for cerebral spinal fluid content. The metabolites included: Glx, NAA, Cho, Cr, and Ins. Combined glutamate and glutamine, i.e., Glx, instead of the individual compounds, was used because glutamate and glutamine have overlapping peaks on the spectroscopy, which cannot be reliably separated. For the estimate of metabolite concentrations, we calculated absolute concentrations, expresses in IU, using the water signal for normalization (Provencher 1993). Since our voxels incorporated cerebrospinal fluid (CSF) and the volume of CSF dilutes ¹H-MRS derived metabolite values, we corrected our metabolite levels for CSF volume for each participant. To adjust for CSF volume Voxel Based Morphometry, a toolbox which operates within the image analysis program Statistical Parametric Mapping (SPM; <http://www.fil.ion.ucl.ac.uk/spm/> software) was used. Corrected metabolite concentrations were entered into SPSS v.20 (Chicago, IL) for calculation of differences between fatigued and non-fatigued groups and correlational analyses with clinical outcomes.

Statistical analysis plan

Baseline socio-demographic and clinical characteristics are reported, stratified by fatigue status, using means and standard deviations (SD) for continuous variables, and counts and percentages for categorical variables. Comparison between fatigued and non-fatigued participants on baseline characteristics was tested using independent sample t-tests for continuous variables and Pearson Chi-square tests for categorical variables.

To examine our primary aim we report means and SD comparing group differences between brain metabolites, serum CRP and serum cytokines by calculating independent sample t-tests. Association between cytokines, metabolites, and CRP that were found to be significantly different between groups ($p < 0.05$) was assessed using partial correlation coefficient. The variables found to be significantly different between groups ($p < 0.05$) using t-tests, were assessed for predictive power of fatigue in a regression model with the continuous fatigue level as response. Further Receiver Operator Characteristic curves were

constructed for brain metabolites and peripheral inflammatory markers as predictors of fatigue status (fatigued vs. non-fatigued) Markers were investigated both individually and as a combined panel including all significant markers.

It is important to note, that this study was not powered to correct for multiple testing of the numerous markers examined. As this was a pilot study the findings should be viewed as exploratory and thus geared to provide valuable guidance to future studies.

Data were entered into and analyzed with IBM SPSS, Windows version v 20 (SPSS, Chicago, ILL). SAS version 9.3 (SAS Institute Inc., Cary, NC) was used for the ROC analysis. For all hypothesis tests, two-sided tests and significance levels of 0.05 was used. No adjustments were made for multiple hypotheses testing as the secondary outcomes were viewed as hypothesis generating.

Power analysis—To detect a difference in brain metabolite levels in the posterior and anterior insula similar to the one obtained in Harris et al. (Harris et al. 2009) with 15 subjects per group, we have 86 % power based on a two-group satterthwaite t-test at 5 % level of significance.

With measures available on 29 subjects, a correlation of 0.5 can be detected against a baseline of 0.1 with more than 80 % power. The power will be almost 90 % to detect a correlation of 0.6 to be significant against a null hypothesized correlation of 0.2. Thus, a difference in correlations of (0.4 and 0.9) or (0.5 and 0.92) can be detected between two groups of size 15 each with 80 % power.

Results

Screening, enrollments and withdrawals

Fifty-three BC survivors were screened of which 20 were found to be ineligible; 11 fatigued and nine non-fatigued. Four screened eligible women, two fatigued and two non-fatigued chose not to complete the study. Figure 2 documents reasons for exclusions and withdrawals. Twenty-nine women completed the study, 16 fatigued and 13 non-fatigued.

Sociodemographic and clinical characteristics

The sociodemographic and clinical characteristics by fatigue status are presented in Table 1. Fatigued women scored significantly higher on the BFI ($p < 0.01$), HADS depression scale ($p < 0.01$), BPI severity ($p < 0.01$). There were no other significant differences in other sociodemographic and clinical characteristics.

Differences in cytokines and brain metabolites by fatigue status

Serum cytokine, CRP and brain metabolites by fatigue status are presented in Table 2. There was no significant difference in TNF- α , IL-1- β or CRP between the groups. There was, however, significantly more serum IL-6 ($p = 0.03$) in fatigued breast cancer survivors compared to non-fatigued survivors.

There was no difference in any of the brain metabolites (NAA, Glx, Ins, and Cho) or their ratios (Glx/NAA, Cr/tCr) in the anterior insula, occipital cortex, and the anterior cingulate. In contrast, in the posterior insula, a significant difference between Cr/tCr ($p=0.03$) and Glx/NAA ($p=0.01$) was found compared to non-fatigued women, with the fatigued women showing higher ratios.

Correlations between brain metabolites and IL-6

There were no significant correlations between the serum cytokine IL-6, posterior insula Cr/tCr and posterior insula Glx/NAA.

Predictors of fatigue severity in BC survivors

In Table 3 results for area under the curve (AUC), and values needed of brain metabolites and IL-6 to predict fatigued versus non-fatigued women with a given sensitivity and specificity and cutoff are presented. All three of our fatigue predictor candidates (PI Glx/NAA, PI Cr/tCr and IL-6) had AUC of 70 % or better with a sensitivity of 81 %. However, PI Glx/NAA and IL-6 had specificities (69 and 62 %, respectively) that were higher than PI Cr/tCr with a specificity of 31 % (Fig. 3). When all three markers were combined the AUC was improved to 90 %, but sensitivity was decreased to 50 %.

Discussion

Our data does not support the maladaptive cytokine induced sickness behavior hypothesis as the brain metabolites investigated were not significantly associated with the inflammatory cytokines. Alternatively, our small sample size may not have provided enough power to detect differences in markers that have been previously implicated in cancer fatigue. The study's results of increased levels of inflammatory serum cytokines are, however, consistent with numerous other studies in fatigued BC survivors, which have observed increased levels of both the pro-inflammatory cytokines (IL-1 β , IL-6, neopterin, and TNF- α) and their soluble receptors (sTNF-RII and sIL-1ra) when compared to both non-fatigued BC survivors and healthy age-matched controls. (Bower et al. 2002, 2003, 2011; Collado-Hidalgo et al. 2006; Schubert et al. 2007) Two studies, however, found contradictory results with no significant correlation between fatigue in BC survivors and circulating cytokines (Gelinas and Fillion 2004; Cameron et al. 2012). Unlike some other studies, (Liu et al. 2012) we did not find a significant difference in serum CRP between groups.

Although brain metabolites from multiple regions were examined, differences were only observed in the posterior insula. This finding is unique in that prior work in breast cancer patients has not investigated the insula. The posterior insula may be involved in fatigue expression as this area of the brain is known to be a key structure in the perception and modulation of sensory stimuli such as pain and physical weariness. Higher order processing in this region is known to be involved in the mapping of visceral states that are associated with emotional experience, giving rise to conscious feelings such as fatigue and other sensations (Craig 2010, 2011).

Of note, of the three fatigue predictor variables, the ratio of Glx/NAA in the posterior insula was the best single predictor of fatigue. Previous work in other brain disorders has shown

that decreased levels of NAA as detected by ^1H -MRS are indicators of neuronal/axonal loss, or compromised neuronal metabolism (Moffett et al. 2007). This study's results is also consistent with previous work showing lower NAA in fatigued populations (Brooks et al. 2000). Glutamate, on the other hand, is the brain's main excitatory neurotransmitter and is indicative of heightened neural activity, which may even be toxic in some conditions (Mehta et al. 2013). Glutamate has several key roles in the development and function of normal brain activities, including regulation of the communication process between neurons, development of plasticity in the CNS, and serving as an energy reserve (Filosa et al. 2009; Hawkins 2009; Hedberg and Stanton 1996; Matsui et al. 2005; Nakanishi 1992). As such, alterations in its concentration may be indicative of imbalance in neurotransmission and disruption of any, or all, of these activities could conceivably lead to fatigue (Ronnback and Hansson 2004). Also, higher Glx/ NAA levels have been previously reported in epilepsy patients where these higher levels have been interpreted as a marker of elevated neural activity, although the exact pathophysiology is unclear (Castillo 2007; Savic et al. 2000). Consequently, it is possible that the increased Glx/NAA ratio may be related to overburdened or heightened sensory processing of bodily sensations leading to fatigue, although further research is needed to better understand these findings.

Another possible predictor of PCRf was the increase in the Cr/tCr ratio in the posterior insula of fatigued women. These results must be interpreted cautiously as the concentrations of creatine and phosphocreatine by themselves cannot be reliably measured by ^1H -MRS, but requires additional measurements with phosphorous MRS (^{31}P -MRS) neuroimaging (Maddock and Buonocore 2012). Thus, an increased Cr/tCr ratio could be caused by an increase in creatine levels, a decrease in phosphocreatine concentrations, or both. In any of these scenarios, the study's findings suggest that there is more creatine compared to phosphocreatine in the posterior insula of fatigued women in contrast to non-fatigued BC survivors. Phosphocreatine is made when the enzyme creatine phosphokinase transfers phosphorous (Pi) from adenosine triphosphate (ATP) to creatine, creating adenosine diphosphate (ADP) and phosphocreatine (Reyngoudt et al. 2012). Also, phosphocreatine can donate a phosphate group to regenerate ATP. In tissues and cells that consume ATP rapidly, such as skeletal muscle and the brain, phosphocreatine serves as an energy reservoir for the rapid buffering and regeneration of ATP from ADP (Wallimann et al. 1992). This energy reserve is especially important in high energy consuming tissues, as the normal aerobic production of ATP is slow, and relatively inefficient, making it difficult to supply cerebral energy demands. Currently, there is a lack of research examining concentrations of creatine or phosphocreatine in the brain of chronically fatigued populations. Interestingly, several MRS studies in fatigued patients with hepatitis C, (Bokemeyer et al. 2011) HIV-infected individuals (Schifitto et al. 2011), and patients with various mitochondrial diseases (Friedman et al. 2010) have found significantly decreased levels of creatine and/or phosphocreatine in various areas of the brain compared to age-matched controls. Also, decreased or disrupted levels of creatine and phosphocreatine have been found in several areas of the brain in people with a variety of psychiatric disorders such as major depressive disorder (Allen 2012), which is highly comorbid with fatigue; and numerous randomized clinical trials have shown that daily creatine supplementation in both young and elderly adults can improve both cognitive functioning and enhance fatigue resistance (Rawson and

Venezia 2011). Consequently, increased Cr/tCr in the posterior insula could indicate an association between fatigue and disrupted cerebral energy metabolism. The addition of ^{31}P -MRS imaging to future studies is needed to confirm our results.

Despite contradicting the cytokine induced sickness behavior model of PCRF, several promising fatigue predictors were identified: serum IL-6, brain Glx/NAA and Cr/tCr. From our ROC curves the individual markers have greater than 80 % specificity with varying sensitivity. Of these markers, Glx/NAA performed the best with identifying true positives and excluding false negatives. In contrast, while the panels of all three markers give a greater AUC of 90 % than any individual marker, the sensitivity was poor at 50 %. The higher sensitivity but low specificity could indicate that our markers were not associated with one another and that different PCRF mechanisms may be operative in different women. This is supported by the lack of significant correlations across the three markers.

This study had several limitations including a small sample size of relatively young and racially homogenous (Caucasian) sample of BC survivors, a cross-sectional design, the ability to examine only a handful of regions in the brain for alterations in levels of brain metabolites; a limited power to investigate the effect different types of chemotherapy, and hormonal therapies on the associations between fatigued and non-fatigued women; and inability to explore comorbid pain and depression in the fatigued cohort due to our limited sample size. All of these limitations make the study results preliminary in nature. While the impacts of hormonal therapies on fatigue were not investigated, the majority of the study participants were 5 years or further from the end of their cancer treatment. As such, they were no longer taking these therapies. Similarly, the long time since receiving cancer treatments also likely limits the impact of different chemotherapies on fatigue.

Fatigue symptoms are often associated with other comorbid symptoms such as pain and mood disorders (Montazeri 2008). We did not attempt to control for these other symptoms due to the small sample size in our trial. Also, given the cross-sectional design we are also not able to determine any causal relationships between fatigue and either brain metabolites or peripheral immune markers. The results do however, point to the need for future longitudinal study designs where changes through time in peripheral immune markers and brain metabolites are examined to determine if they correlate with changes in fatigue. In addition, studies in larger diverse BC survivors, and other fatigued cancer populations, would be needed to examine the generalizability and robustness of our results.

In conclusion, this study does not provide evidence in support of a model of cytokine-induced sickness behavior in women who are BC survivors with PCRF. However, our data does indicate that persistent fatigue in BC survivors is significantly associated with several neurobiological markers in the posterior insula, which are associated with increased heightened sensory processing of bodily perceptions and inadequate energy reserves. These neurobiological markers are independent of one another as well as the inflammatory cytokine IL-6. Consequently, persistent cancer related fatigue in BC survivors appears to be associated with several distinct biological phenomena. These changes in brain metabolites should be investigated in larger studies using longitudinal designs to determine their utility as meaningful predictors of fatigue and as possible clues to the underlying mechanisms that

underpin persistent fatigue in BC survivors. Ultimately, this could help us design better more targeted treatments for these patients.

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Fig. 1.

Voxel Placement and Representative Spectrum. **a** Axial T1-weighted image showing single voxel placements for right anterior (aI), right posterior (pI) insula, anterior cingulate (ACC) and occipital cortex (OCC). **b** Representative ^1H -MRS spectrum from the posterior insula fit with LCMoDel (red trace; * = resonance of glutamate and glutamine {Glx}). LCMoDel uses a linear combination of individual spectra obtained from pure molecular species to fit the experimental spectra. Metabolites were expressed as a ratio to total creatine (tCr)

Fig. 2.
Flow of participants through the study and reasons for exclusion

Fig. 3.

Receiver Operator Curves of the Three Fatigue Predictors: Average_IL_6= IL-6; Glx/NAA = ratio of posterior insula glutamate and glutamine (Glx)/N-acetyl-aspartate (NAA); Cr/tCr = ratio of posterior insula creatine (Cr) to total creatine (creatinine + phosphocreatine {tCr})

Table 1

Sociodemographic and clinical characteristics by disease status

	Non-fatigued (N = 13)	Fatigued (n = 16)	P-value
Age, mean, SD	57±9.5	57±8.4	0.99 ^a
Race, N (%) white	13 (100.0)	16 (100.0)	0.99 ^b
BFI ^c , mean, SD	1.5±1.2	4.8±1.2	<0.01 ^{a,*}
HADS ^c , mean, SD			
Anxiety	4.2±3.7	7.0±4.8	0.10 ^a
Depression	2.2±2.2	6.6±4.9	<0.01 ^{a,*}
BPI ^c (average Pain Intensity), mean, SD	1.5±1.5	3.6±2.2	<0.01 ^{a,*}
PSQI ^c Total Score, mean, SD	6.3±3.0	8.2±4.1	0.13 ^a
Breast Cancer Stage, N (%)			0.22 ^b
0	2 (15.4)	1 (6.3)	
1	6 (46.2)	7 (43.8)	
2	1 (7.7)	6 (37.5)	
3	4 (30.8)	2 (12.5)	
Menopausal Status, N (%)			0.66 ^b
Pre-Menopausal	6 (46.2)	7 (43.8)	
Peri-Menopausal	0 (0.0)	1 (6.3)	
Post-Menopausal	7 (53.8)	8 (50.0)	
Breast cancer treatments, N (%) ³			
Chemotherapy	9 (69.2)	10 (62.5)	0.71 ^b
Radiation	11 (84.6)	12 (75.0)	0.53 ^b
Surgery	13 (100.0)	16 (100.0)	0.99 ^b
Hormone Therapy	7 (53.8)	12 (75.0)	0.23 ^b
Time Since Diagnosis, mean, SD (months)	70±37.4	74±48.2	0.41 ^a

	Non-fatigued (N = 13)	Fatigued (n = 16)	P-value
Receptor status, N (%) ^d			
ER+ ^c	6 (46.2)	13 (81.3)	0.05 ^b
PR+ ^c	4 (30.8)	11 (68.8)	0.11 ^b
HER2/Neu	2 (15.4)	4 (25.0)	0.58 ^b
BMI ^c , mean, SD	28.5±4.2	30.3±7.4	0.52 ^a

^a *p*-value is based on an independent sample t-test

^b *p*-values are based on a Pearson Chi-square

^c *BFI* brief fatigue inventory, *HADS* hospital anxiety depression scale, *BPI* brief pain inventory, *PSQ* Pittsburgh sleep quality index, *ER* + estrogen receptor positive, *PR* + progesterone receptor positive, *BMI*/body mass index

^d Totals add up to greater than 100 % as the same women could have received more than one treatment or had multiple positive receptors statuses

* *p*-value is significant at 0.05

Table 2

Mean and standard deviations of inflammatory serum cytokines, C - reactive protein and brain metabolites by fatigue status

	Non-fatigued (<i>N</i> = 13) Mean ± SD	Fatigued (<i>n</i> = 16) Mean ± SD	<i>P</i> -value ^{<i>a</i>}
Serum cytokines (pg/mL)			
Pro-inflammatory			
IL-1 β	0.47±0.70	0.61±0.78	0.63
IL-6	1.68±1.63	8.63±15.26	0.03 [*]
TNF- α	7.17±4.10	7.21±3.66	0.98
C-reactive protein (mg/dL)	0.22±0.19	0.31±0.52	0.58
Brain metabolites (AIU) ^{<i>b</i>}			
Posterior insula			
Cr/tCr ^{<i>b</i>}	0.14±0.02	0.16±0.03	0.03 [*]
Cr ^{<i>b</i>}	2.42±0.41	2.84±0.73	0.07
Glx ^{<i>b</i>}	7.87±0.94	8.34±1.04	0.22
Glx / NAA ^{<i>b</i>}	UI±0.09	1.24±0.15	0.01 [*]
GPC ^{<i>b</i>}	1.42±0.17	1.38±0.20	0.60
Ins ^{<i>b</i>}	5.30±0.83	5.10±0.70	0.49
NAA ^{<i>b</i>}	7.10±0.54	6.74±0.55	0.08
Anterior insula			
Cr/tCr ^{<i>b</i>}	0.51±0.14	0.44±0.21	0.26
Cr ^{<i>b</i>}	3.64±1.29	2.93±1.26	0.15
Glx ^{<i>b</i>}	10.13±2.97	10.49±2.34	0.71
Glx / NAA ^{<i>b</i>}	1.35±0.30	1.37±0.26	0.80
GPC ^{<i>b</i>}	1.64±0.33	1.61±0.34	0.84
Ins ^{<i>b</i>}	5.45±0.74	5.65±0.92	0.53
NAA ^{<i>b</i>}	7.50±1.00	7.65±1.07	0.70
Occipital cortex			
Cr/tCr ^{<i>b</i>}	0.37±0.10	0.33±0.17	0.42
Cr ^{<i>b</i>}	2.51±0.66	2.13±1.13	0.29
Glx ^{<i>b</i>}	7.90±0.95	7.76±1.19	0.74
Glx / NAA ^{<i>b</i>}	0.92±0.09	0.93±0.11	0.81
GPC ^{<i>b</i>}	0.69±0.26	0.76±0.21	0.46
Ins ^{<i>b</i>}	4.98±0.57	4.75±0.51	0.27
NAA ^{<i>b</i>}	8.61±0.71	8.37±0.88	0.44

	Non-fatigued (<i>N</i> = 13) Mean ± SD	Fatigued (<i>n</i> = 16) Mean ± SD	<i>P</i> -value ^{<i>a</i>}
Anterior cingulate			
Cr/tCr ^{<i>b</i>}	0.44±0.10	0.46±0.10	0.69
Cr ^{<i>b</i>}	2.65±0.66	2.62±0.60	0.91
Glx ^{<i>b</i>}	7.77±0.93	7.50±1.14	0.49
Glx / NAA ^{<i>b</i>}	1.12±0.14	1.11±0.14	0.85
GPC ^{<i>b</i>}	1.38±0.25	1.38±0.20	0.96
Ins ^{<i>b</i>}	4.90±0.79	4.74±0.56	0.56
NAA ^{<i>b</i>}	6.99±0.69	6.80±0.91	0.54

^{*a*}*P*-value is based on an independent sample t-test

^{*b*}*IU* institutional units; *Cr* creatine; *Cr/tCr* creatine standardized by total creatine plus phosphocreatine; *Glx* glutamate + glutamine; *Glx/NAA* levels of glutamate + glutamine standardized to N-Acetyl-Aspartate; *Cho* choline; *Ins* myo-inositol; *NAA* N-acetyl-aspartate

**p*-value is significant at 0.05

Table 3

Performance of key peripheral inflammatory, metabolic and neuronal health markers between fatigued and non-fatigued breast cancer survivors

Variables	Area under the curve	95 % Confidence intervals		Positive for being fatigued if value is	Sensitivity	Specificity	Positive likelihood ratio ^b	Negative likelihood ratio ^b
		Lower bound	Upper bound					
IL-6	0.77	0.59	0.95	1.17pg/mL	0.81	0.62	2.13	0.31
PI Cr/tCr ^a	0.70	0.51	0.90	0.36 IU ^a	0.81	0.31	1.17	0.28
PI Glx/NAA ^a	0.78	0.61	0.95	1.15 IU ^a	0.81	0.69	2.84	0.17
IL-6, PI Cr/tCr ^a , & PI Glx/NAA ^a	0.90	0.79	1.00	1.17 pg/mL, 0.36 IU ^a and 1.15 IU ^a	0.50	1.00	NA ^c	0.50

^a PI Cr/tCr posterior insula creatine/total creatine; PI Glx/NAA posterior insula glutamate + glutamine / n-acetyl-aspartate; IU/institutional units

^b Positive likelihood ratio = sensitivity/(1-specificity); measures how likely it is for the test to pick up true positives in comparison to false positives; Negative likelihood ratio = (1-sensitivity)/specificity; measures how likely it is for the test to pick up false negatives in comparison to true negatives

^cNot applicable as 1-specificity = to “0”