

# Serum Metabolites as Predictors of Symptom Burden in Patients with Breast Cancer Undergoing Chemotherapy

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## Research Article

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# Abstract

## Background

Patients with breast cancer undergoing chemotherapy experience multiple symptoms that cause decrements in quality of life. A deeper understanding of serum metabolites as biomarkers may guide in predicting symptom occurrence and severity. This study evaluated change in serum metabolite levels from before chemotherapy (T1) to after the initial first or second cycle of chemotherapy (T2), after the third cycle of chemotherapy (T3) and finally after a month of chemotherapy completion (T4). Change in metabolite levels were analyzed in association with symptom occurrence.

## Methods

Patients with breast cancer ( $n = 10$ ) completed study measures at four timepoints. Neuropsychological and gastrointestinal (GI) symptoms were evaluated using the Memorial Symptom Assessment Scale. Dietary intake was assessed using the NHANES Dietary Screener Questionnaire. Serum metabolites were analyzed using gas chromatography-mass spectrophotometry.

## Results

Five patients experienced  $> 3$  GI symptoms at T2 and T3. At all 4 timepoints,  $\geq 6$  patients experienced moderate to severe neuropsychological symptoms severity. Daily mean intake of fiber, whole grain, and calcium were less than the recommended guidelines. Compared to T1, long chain fatty acid and amino acid derivative levels decreased at T2 and T3 ( $p < 0.05$ ). Increase in metabolite levels of glucose-6-phosphate ( $p = 0.02$ ) and 6-phosphogluconate ( $p = 0.05$ ) at T2 were associated with fatigue occurrence.

## Conclusions

Patients experienced several gastrointestinal and neuropsychological symptoms during chemotherapy and early survivorship. Chemotherapy-induced changes in metabolites involved in cellular energy metabolism may explain patient symptom experience. Metabolite level changes overtime may reflect inadequate dietary intake. Larger sample size studies may guide in building metabolic prediction models for symptom burden.

## INTRODUCTION

Patients with breast cancer undergoing chemotherapy experience several debilitating symptoms causing decrements in quality of life [1–4]. Many of these symptoms can persist for years after treatment completion [2, 5]. Gastrointestinal (GI) toxicity symptoms, for example, nausea, diarrhea, dry mouth, and taste change are common during initial treatment and generally dissipate over time [6–8]. In contrast,

neuropsychological symptoms, for example, fatigue, pain, sleep disturbance, depression and anxiety, experienced during initial treatment, may persist into survivorship [9–11]. Importantly, GI and neuropsychological symptoms often co-occur, compounding overall symptom burden [4, 12]. Increase in severity and persistent occurrence of these symptoms are associated with clinically meaningful consequences, including treatment discontinuation, nutritional deficits, and sustained impairments in quality of life [7, 8].

Common demographic and clinical risk factors for these symptoms in breast cancer include age < 60 years, administration of moderate to high emetogenic chemotherapy, and lower physical function [8, 13]. However, these risk factors do not fully explain the inter-individual variability in symptom experience. Biological mechanisms for some cancer and cancer treatment related symptoms are well documented in the literature [8]; nevertheless, metabolic mechanisms driving majority of individual and co-occurring symptoms remain poorly understood and warrant further investigation to inform targeted interventions. For example, despite the advances in anti-emetic regimens and improved understanding of serotonin and neurokinin mediated pathways for chemotherapy-induced vomiting, 12% to 20% of patients still experience vomiting [8]. In contrast, over 90% of the patients experience fatigue [14], up to 75% experience cognitive impairment [15], and approximately 50% experience GI symptoms such as dry mouth, nausea and diarrhea during chemotherapy [16].

Recent studies on chemotherapy-induced symptoms have increasingly focused on alterations in the gut-brain axis functions to explain multiple co-occurring symptoms in oncology patients [17, 18]. Co-occurrence of two or more symptoms referred to as symptom cluster, is commonly observed in patients undergoing chemotherapy and may reflect a shared etiology [5, 19]. Perturbations in liver function, inflammatory pathways, apoptosis, cell proliferation processes, and microbiome composition following chemotherapy have been associated with fatigue [20, 21], cognitive dysfunction [15], nausea [18], and dry mouth [22]. These biological perturbations may be impacted by changes in metabolite levels after chemotherapy to explain patient symptomatology [23]. In addition, the cumulative symptom burden may influence dietary intake and in turn, further alter metabolic processes.

A recent multi-omics pilot study investigated changes in metabolite levels before (T1) and after the first or second cycle of chemotherapy (T2) in patients with breast cancer [24]. In this study, 94% experienced fatigue and 64% experienced chemotherapy-induced nausea (CIN). Metabolomic analysis of the serum samples identified perturbations in lipid and amino acid metabolism in the fatigue and CIN groups from T1 to T2. Lipid and amino acid metabolism primarily occur in the liver. Perturbation in metabolite levels within these pathways suggest that fatigue and CIN may be partially explained by hepatotoxicity-related mechanisms [25, 26].

The occurrence of fatigue, sleep disturbance, CIN in the initial cycles of chemotherapy is associated with an increased risk of greater symptom severity with receipt of subsequent chemotherapy cycles [11, 27]. Given the limited research examining associations between chemotherapy-induced perturbations in metabolic processes and patient symptom experience across the entire course of chemotherapy, further

investigation is warranted. Therefore, the purpose of the current study is to evaluate symptom characteristics, dietary intake, and associated changes in metabolite levels from before chemotherapy (T1) to after the first or second cycle (T2), after the third cycle (T3) and one month following completion of chemotherapy (T4) in patients with breast cancer.

## METHODS

### Study design and procedures

This study is a descriptive, longitudinal, cohort pilot study examining serum metabolite biomarkers in association with symptom burden in patients with breast cancer during chemotherapy from July 2024 to March 2025. The analyses presented here use serum metabolomic and symptom experience data from adult women receiving chemotherapy for breast cancer. Study procedures were approved by the Institutional Review Boards at Mayo Clinic (Phoenix, AZ and Rochester, MN) and University of Iowa (Iowa City, IA, USA). Informed consent was obtained from all participants. Patients across all the sites completed study questionnaires at four timepoints: 5–7 days before chemotherapy (T1), after the first or second cycle of chemotherapy (T2), after the third cycle of chemotherapy (T3) and finally a month following chemotherapy completion (T4) (Fig. 1).

### Patients and settings

Participants were eligible if they were: an adult woman ( $\geq 21$  years of age), newly diagnosed with stage I–III breast cancer, chemotherapy naïve and scheduled to begin chemotherapy. Patients were excluded if they were unable to read and write in English, had metastatic disease (stage IV), were currently or had recently received radiation therapy, had cognitive impairment precluding completion of study questionnaires, had GI co-morbidities. Patients were recruited from Mayo Clinic Comprehensive Cancer Center (AZ and MN) and at the University of Iowa Holden Comprehensive Cancer Center (IA). Of the 32 patients approached, 11 provided informed consent and 1 withdrew the study. The primary reason for refusal to participate was feeling overwhelmed by cancer diagnoses and treatment planning.

### Instruments

**Demographic and clinical characteristics** – A phenotype questionnaire was completed to collect demographic characteristics that included age, race, ethnicity, education level, employment status, marital status, and income. Clinical characteristics were abstracted from electronic health records and included body mass index (BMI), cancer diagnosis and stage, chemotherapy regimen and dose. Alcohol intake was assessed using the Alcohol Use Disorders Identification Test (AUDIT) [28].

**Symptom assessment** – Memorial Symptom Assessment Scale (MSAS) [29] was used to evaluate the occurrence, severity and distress of 12 GI (i.e., dry mouth, nausea, feeling bloated, vomiting, diarrhea,

lack of appetite, feeling full early, difficulty swallowing, mouth sores, weight loss, constipation, change in the way food tastes) and 11 neuropsychological symptoms (i.e., difficulty concentrating, pain, lack of energy, feeling nervous, feeling drowsy, difficulty sleeping, feeling sad, worrying, dizziness, feeling irritable, numbness/tingling in hands and feet) in the past week. The “lack of energy” item on MSAS was used for fatigue assessment.

Dietary intake assessment – A 25-item NHANES 2009–2010 Dietary Screener Questionnaire (DSQ) [30] was used to assess intake of fruits and vegetables, whole grains, added sugars, dairy, fiber, and calcium at T1, T2, T3, and T4 based on 24-hour recall.

## **Blood sample collection and processing**

Sample collection – Blood samples were collected in a 3.5mL serum separator tube during routine clinic visits at timepoints corresponding to T1, T2, T3, and T4. Samples were processed for serum isolation and stored at -80°C until analysis.

Sample processing for metabolomics – Metabolomic processing of serum samples was conducted at University of Iowa Core Laboratory. Processed serum samples were analyzed by gas chromatography-mass spectrophotometry (GC-MS). Raw data were processed using TraceFinder 5.1 (ThermoFisher, CA, USA). Metabolite identification and annotation required at least two ions (target + confirming) and a unique retention time that corresponded to the ions and retention time of a reference standard. NOREVA corrected data were then normalized to the total signal per sample to control for extraction, derivatization, and/or loading effects [31]. Sample processing details are highlighted in the Appendix.

## **Data analyses**

Phenotype characteristics – Descriptive statistics and frequency distributions were performed for phenotype characteristics using SPSS, version 28 (IBM, Armonk, NY). Patients were grouped based on symptom occurrence, severity and distress as measured on the MSAS (Fig. 1A-F).

Dietary intake data analysis - Quantitative estimates for intake of each food group were calculated from the DSQ using the publicly available scoring algorithms derived from the NHANES 2009–2010 data provided by National Cancer Institute [32] at all four timepoints.

Metabolomics data analysis - Metabolite analyses were conducted using R version 4.2.1. Metabolite data were log-transformed due to skewness. Distribution across timepoints were visualized using boxplots and fold changes from baseline were assessed. Non-parametric Wilcoxon signed-rank tests were used to identify metabolites with significant median log fold changes at each timepoint. Wilcoxon rank-sum tests were used to compare metabolite levels between participants with and without each symptom occurrence as well as between with or without two co-occurring symptoms. Given the

exploratory nature of this pilot study, metabolites with unadjusted p-values < 0.05 are highlighted in Figs. 2 and 3.

Additional Analysis and Supplementary Data Visualization - Data processing, statistical analysis, and visualization were performed using R Statistical Software (version 3.3.1) to evaluate GI symptom mean severity score change from T1 to T2 and to evaluate association between GI symptom severity and distress scores. Analysis procedures are described in the Appendix. Briefly, raw and MetaboAnalyst-adjusted datasets were imported and wrangled using the readxl and tidyverse (version 2.0.0). All data visualizations were generated using the ggplot2 package. Analysis details are described in the Appendix.

## RESULTS

Participant characteristics – Mean age of the patients was 61 years and the mean body mass index was 25.9. Patients were predominantly white, non-Hispanic. Patients received one of four types of chemotherapy regimens (taxotere/cyclophosphamide, adriamycin/cytosan, carboplatin-based regimen, or paclitaxel +/- trastuzumab) (Table 1).

Table 1  
Demographic and Clinical Characteristics of Breast Cancer Patients at Baseline (n = 10)

| <b>Demographic Characteristics</b>                                                                                                          |             |
|---------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Age (years; mean (SD))                                                                                                                      | 61.0 (7.3)  |
| Body Mass Index (kg/m <sup>2</sup> ; mean (SD))                                                                                             | 25.9 (3.7)  |
| Married or partnered (% yes (n))                                                                                                            | 60.0 (6)    |
| Race (% (n))                                                                                                                                | 90.0 (9)    |
| White                                                                                                                                       | 10.0 (1)    |
| Black                                                                                                                                       | 0.0 (0)     |
| Unknown                                                                                                                                     |             |
| Ethnicity (% (n))                                                                                                                           | 90.0 (9)    |
| Non-Hispanic or Latina                                                                                                                      | 10.0 (1)    |
| Unknown                                                                                                                                     |             |
| Education (% (n))                                                                                                                           | 20.0 (2)    |
| Highschool/GED                                                                                                                              | 40.0 (4)    |
| Associate degree or bachelor's degree                                                                                                       | 30.0 (3)    |
| Master's degree                                                                                                                             | 10.0 (3)    |
| Professional degree                                                                                                                         |             |
| Income (% (n))                                                                                                                              | 10.0 (1)    |
| \$20,000-\$40,000/year                                                                                                                      | 0.0 (0)     |
| \$41,000-\$80,000/year                                                                                                                      | 80.0 (8)    |
| >\$80,000/year                                                                                                                              | 10.0 (1)    |
| Missing                                                                                                                                     |             |
| Exercise, minutes per week (mean (SD))                                                                                                      | 77.8 (94.4) |
| Currently employed (% yes (n))                                                                                                              | 50.0 (5)    |
| Clinical Characteristics                                                                                                                    |             |
| AUDIT score (mean (SD))                                                                                                                     | 1.3 (1.4)   |
| Body mass index (kg/m <sup>2</sup> ; mean (SD))                                                                                             | 25.9 (3.7)  |
| Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, kg/m <sup>2</sup> = kilograms per meters squared, SD = standard deviation |             |

| Demographic Characteristics                                                                                                                 |          |
|---------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Chemotherapy regimen (%(n))                                                                                                                 | 50.0 (5) |
| Taxotere/Cyclophosphamide                                                                                                                   | 10.0 (1) |
| Adriamycin/Cytosan                                                                                                                          | 20.0 (2) |
| Carboplatin based regimen                                                                                                                   | 20.0 (2) |
| Paclitaxel +/- Trastuzumab                                                                                                                  |          |
| Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, kg/m <sup>2</sup> = kilograms per meters squared, SD = standard deviation |          |

Symptom occurrence, severity, and distress - Fig. 1A-F highlights the occurrence, mean severity and mean distress scores of the 12 GI symptoms and 11 neuropsychological over time as assessed on the MSAS. GI symptom occurrence was highest at T2 and T3 with five patients reporting > 3 symptoms at those timepoints. While number of patients reporting multiple gastrointestinal symptom occurrence decreased at T4, symptoms did not return to baseline (T1). Between three to five patients reported > 3 neuropsychological symptoms between T1 to T4, respectively (Fig. 1A-B).

Average severity scores for GI symptoms changed from T1 to T2 (0.14 vs 0.43,  $p = 0.055$ ) (Supplementary Fig. 1(a)) and did not return to baseline (T1) severity at T4 (Fig. 1C-D). In terms of distress rating, six patients rated GI symptom distress as “somewhat” to “very much” at T3, and seven patients rated neuropsychological symptom distress as “somewhat” to “very much” at T1 (Fig. 1E-F). Average GI symptom severity increased from T1 to T2 (Supplementary Fig. 1(a)). Average distress scores of GI symptoms correlated with severity ( $\rho = 0.96$ ,  $p < 0.001$ , Supplementary Fig. 1(b)). For five to seven patients, neuropsychological symptom severity scores were between moderate to severe and distress scores between somewhat to very much, across the four timepoints. Supplementary table 1 highlights the percentage occurrence, mean severity, and mean distress scores of all the GI symptoms and neuropsychological symptoms over time.

Dietary intake – While the daily mean intake of fiber, calcium, whole grain and dairy were below the national dietary intake recommendations at all four timepoints, daily mean intake of added sugar was higher than national dietary intake recommendations (Table 2).

Table 2  
Dietary Intake Recall Before Chemotherapy for Patients with Breast Cancer

| Dietary component                                                                      | Timepoint<br>1 (n = 9) | Timepoint<br>2 (n = 9) | Timepoint<br>3 (n = 9) | Timepoint<br>4 (n = 9) | USDA<br>Recommended<br>daily intake for<br>women |
|----------------------------------------------------------------------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------------------------------|
|                                                                                        | Mean (SD)              | Mean (SD)              | Mean (SD)              | Mean (SD)              |                                                  |
| Fiber intake (g/day)                                                                   | 15.5 (2.4)             |                        | 15.4 (2.2)             | 15.3 (2.4)             | 21–25 g/day                                      |
| Low intake, %                                                                          | 24.9%                  |                        | 25.5%                  | 27.1%                  |                                                  |
| High intake, %                                                                         | 21.2%                  |                        | 20.0%                  | 19.5%                  |                                                  |
| Calcium intake (mg/day)                                                                | 796.6 (123.9)          |                        | 884.9 (177.5)          | 870.3 (158.5)          | 1200 mg/day                                      |
| Low intake, %                                                                          | 53.6%                  |                        | 42.2%                  | 44.2%                  |                                                  |
| High intake, %                                                                         | 11.8%                  |                        | 20.8%                  | 18.3%                  |                                                  |
| Whole grain intake (oz. equivalents/day)                                               | 0.5 (0.2)              | 0.7 (0.3)              | 0.7 (0.3)              | 0.7 (0.3)              | 3–3.5 oz. equivalents/day                        |
|                                                                                        | 32.7%                  | 26.3%                  | 28.2%                  | 27.0%                  |                                                  |
| Low intake, %                                                                          | 13.0%                  | 21.7%                  | 21.5%                  | 21.8%                  |                                                  |
| High intake, %                                                                         |                        |                        |                        |                        |                                                  |
| Added sugar intake (tsp equivalents/day)                                               | 11.0 (1.3)             | 12.4 (2.9)             | 12.4 (2.2)             | 12.2 (2.6)             | No more than                                     |
|                                                                                        | 48.6%                  | 42.3%                  | 41.7%                  | 43.6%                  | 10 tsp equivalents/day                           |
| Low intake, %                                                                          | 4.4%                   | 6.8%                   | 6.3%                   | 6.0%                   |                                                  |
| High intake, %                                                                         |                        |                        |                        |                        |                                                  |
| Dairy intake (cup equivalents/day)                                                     | 1.2 (0.4)              |                        | 1.4 (0.6)              | 1.4 (0.5)              | 3 cup equivalents/day                            |
|                                                                                        | 52.9%                  |                        | 42.8%                  | 42.6%                  |                                                  |
| Low intake, %                                                                          | 4.8%                   |                        | 11.1%                  | 8.5%                   |                                                  |
| High intake, %                                                                         |                        |                        |                        |                        |                                                  |
| Fruits and vegetables intake (cup equivalents/day)                                     | 2.7 (0.6)              | 2.4 (0.3)              | 2.4 (0.4)              | 2.3 (0.4)              | Fruits: 1.5-2 cup equivalents/day;               |
|                                                                                        | 19.5%                  | 23.9%                  | 26.4%                  | 27.6%                  | Vegetables: 2–3 cup equivalents/day              |
|                                                                                        | 29.3%                  | 18.2%                  | 18.5%                  | 16.1%                  |                                                  |
| Low intake, %                                                                          |                        |                        |                        |                        |                                                  |
| High intake, %                                                                         |                        |                        |                        |                        |                                                  |
| Vegetables intake (cup equivalents/day)                                                | 1.8 (0.6)              | 1.6 (0.2)              | 1.5 (0.4)              | 1.4 (0.2)              |                                                  |
|                                                                                        | 16.4%                  | 18.9%                  | 22.9%                  | 26.0%                  |                                                  |
| Low intake, %                                                                          | 43.5%                  | 31.5%                  | 31.0%                  | 23.9%                  |                                                  |
| High intake, %                                                                         |                        |                        |                        |                        |                                                  |
| Abbreviations: SD = standard deviation; USDA = United States Department of Agriculture |                        |                        |                        |                        |                                                  |

| Dietary component                                                                       | Timepoint<br>1 (n = 9) | Timepoint<br>2 (n = 9) | Timepoint<br>3 (n = 9) | Timepoint<br>4 (n = 9) | USDA<br>Recommended<br>daily intake for<br>women |
|-----------------------------------------------------------------------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------------------------------|
|                                                                                         | Mean (SD)              | Mean (SD)              | Mean (SD)              | Mean (SD)              |                                                  |
| High intake, %                                                                          |                        |                        |                        |                        |                                                  |
| Fruits and<br>vegetables<br>(excluding French<br>fries) intake (cup<br>equivalents/day) | 2.6 (0.7)              | 2.3 (0.3)              | 2.3 (0.5)              | 2.2 (0.4)              |                                                  |
|                                                                                         | 24.1%                  | 29.0%                  | 32.1%                  | 34.0%                  |                                                  |
|                                                                                         | 26.9%                  | 15.7%                  | 16.1%                  | 13.4%                  |                                                  |
| Low intake, %                                                                           |                        |                        |                        |                        |                                                  |
| High intake, %                                                                          |                        |                        |                        |                        |                                                  |
| Vegetables<br>(excluding French<br>fries) intake (cup<br>equivalents/day)               | 1.8 (0.6)              | 1.8 (0.6)              | 1.8 (0.6)              | 1.8 (0.6)              |                                                  |
|                                                                                         | 20.9%                  | 24.5%                  | 29.6%                  | 32.9%                  |                                                  |
|                                                                                         | 41.4%                  | 27.6%                  | 27.6%                  | 20.2%                  |                                                  |
| Low intake, %                                                                           |                        |                        |                        |                        |                                                  |
| High intake, %                                                                          |                        |                        |                        |                        |                                                  |
| Fruits intake (cup<br>equivalents/day)                                                  | 0.8 (0.3)              | 0.8 (0.2)              | 0.8 (0.1)              | 0.8 (0.1)              |                                                  |
|                                                                                         | 34.8%                  | 32.6%                  | 30.7%                  | 30.1%                  |                                                  |
| Low intake, %                                                                           |                        |                        |                        |                        |                                                  |
|                                                                                         | 11.2%                  | 10.2%                  | 10.3%                  | 10.7%                  |                                                  |
| High intake, %                                                                          |                        |                        |                        |                        |                                                  |
| Added sugar from<br>sugar-sweetened<br>beverages intake<br>(tsp equivalents/day)        | 3.7 (0.2)              | 3.9 (0.5)              | 3.9 (0.6)              | 3.7 (0.3)              |                                                  |
|                                                                                         | 53.1%                  | 50.7%                  | 51.1%                  | 52.4%                  |                                                  |
|                                                                                         | 6.7%                   | 7.3%                   | 7.2%                   | 6.9%                   |                                                  |
| Low intake, %                                                                           |                        |                        |                        |                        |                                                  |
| High intake, %                                                                          |                        |                        |                        |                        |                                                  |
| Abbreviations: SD = standard deviation; USDA = United States Department of Agriculture  |                        |                        |                        |                        |                                                  |

Chemotherapy associated perturbation in serum metabolite levels – Fig. 2 highlights significant mean fold change in levels of various metabolites from baseline (T1) to T2 and T3.

*Fatty acids* – Compared to levels at T1, a decrease in levels of laurate ( $p = 0.012$ (T2);  $p = 0.008$ (T3)), myristate ( $p = 0.020$ (T2);  $p = 0.016$ (T3)), oleate ( $p = 0.027$ (T2);  $p = 0.008$ (T3)), linolenate ( $p = 0.020$ (T2);  $p = 0.016$ (T3)), and linoleate ( $p = 0.020$ (T2);  $p = 0.008$ (T3)) were observed at T2 and at T3. Furthermore, a decrease in palmitate ( $p = 0.039$ ) was observed at T2, and a decrease in heptanoate ( $p = 0.016$ ) and pentadecanoate ( $p = 0.023$ ) was observed at T3.

*Carbohydrates* – Compared to levels at T1, a decrease in level of glycerol was observed at T2 ( $p = 0.008$ ) and T3 ( $p = 0.039$ ). In addition, an increase in level of glucose-6-phosphate ( $p = 0.020$ ) and 6-

phosphogluconate ( $p = 0.012$ ) as well as a decrease in inositol ( $p = 0.012$ ) was observed at T2.

*Amino acid derivatives* – Compared to level at T1,  $\beta$ -hydroxy-beta-methyl-butyric acid (HMB) decreased at T2 ( $p = 0.008$ ) and T3 ( $p = 0.039$ ). A decrease in level of  $\alpha$ -ketoisocaproate ( $p = 0.008$ ),  $\alpha$ -ketoisovalerate ( $p = 0.020$ ), dihydroxyphenylalanine (DOPA) ( $p = 0.012$ ) were observed at T2 and a decrease in mandelate ( $p = 0.008$ ) was observed at T3.

*Ketone body* – Compared to level at T1,  $\beta$ -hydroxybutyrate decreased at T2 ( $p = 0.008$ ) and T3 ( $p = 0.016$ ).

*Organic acids* – Compared to level at T1, pyruvate ( $p = 0.008$ ) and 2-hydroxybutyrate ( $p = 0.027$ ) decreased at T2. In addition, 3-hydroxypropionate ( $p = 0.016$ ) increased at T2.

*Pyrimidine derivatives* – Cytosine ( $p = 0.016$ ) and orotate ( $p = 0.039$ ) levels increased at T2.

Supplementary Fig. 2(a and b) highlights the change in levels of metabolites over time and their mean fold change at T2 and T3 compared to baseline (T1). Mean fold change of metabolites detected at T4 were not significantly different from T1. The levels of all 104 metabolites at all timepoints are highlighted in a heatmap in Supplementary Fig. 3.

Associations of perturbations in serum metabolite levels with symptoms – As illustrated in Fig. 3, compared to level at T1, perturbation in 3-hydroxypropionate (mean log fold change =  $-0.312$ ,  $p = 0.02$ ) at T2 and heptanoate (mean log fold change =  $-0.536$ ,  $p = 0.05$ ) at T2 was associated with dry mouth. Perturbations in level of mandelate at T3 was associated with difficulty concentrating (mean log fold change =  $0.546$ ,  $p = 0.04$ ). Perturbation in glucose 6-phosphate (mean log fold change =  $-0.329$ ;  $p = 0.02$ ) at T1, 3-hydroxypropionate (mean log fold change =  $-0.244$ ,  $p = 0.04$ ) at T3, and 6-phosphogluconate (mean log fold change =  $0.242$ ,  $p = 0.05$ ) at T2 was associated with fatigue. Perturbation in pyruvate (mean log fold change =  $0.946$ ;  $p = 0.03$ ) at T1 and in 3-hydroxypropionate (mean log fold change =  $-0.244$ ,  $p = 0.04$ ) at T3 levels was associated with the co-occurring symptoms of fatigue and difficulty concentrating.

## DISCUSSION

Findings from this pilot study highlight changes in levels of metabolites from before chemotherapy (T1) to post-chemotherapy (T2 and T3) and up to one month after completion of chemotherapy (T4). Consistent with reports, despite evidence-based interventions over 50% patients in the current study experience multiple GI and neuropsychological symptoms over time post-chemotherapy (Fig. 1A-B). While several metabolite levels were perturbed post-chemotherapy (Fig. 2), some metabolite levels recovered after the first cycle of chemotherapy, others, for example, long chain fatty acids decreased consistently with additional chemotherapy cycle. The following paragraphs will highlight chemotherapy-induced changes in metabolites (Fig. 2), and how these changes alter gut environment that may explain patient symptom experience (Fig. 3).

## Chemotherapy-induced change in serum metabolite levels

Compared to T1,  $\beta$ -hydroxybutyrate showed a  $< 1.5$  log mean log fold change at T2 and a  $< 1.0$  log mean log fold change at T3. A decrease in serum  $\beta$ -hydroxybutyrate correlates with impaired fatty acid  $\beta$ -oxidation and liver inflammation [33]. In a state of starvation or carbohydrate restriction diet, the liver uses  $\beta$ -hydroxybutyrate as an alternative energy source to glucose [34]. Acetyl-CoA, a product of fatty acid  $\beta$ -oxidation is needed for tricarboxylic acid cycle to synthesize adenosine triphosphate (ATP) or to generate ketone bodies like  $\beta$ -hydroxybutyrate to reduce oxidative stress [33, 35]. Future studies need to investigate change in substrate for cellular energy synthesis during chemotherapy treatment as a precursor to change in symptom experience.

Chemotherapy-induced decrease in levels of fatty acids identified in this study (Fig. 2) highlight a decrease in lipogenesis and potentially a decrease in dietary intake of these fatty acids [36]. A decrease in fatty acid levels negatively impacts intracellular energy levels synthesis. This finding is consistent with findings from a pilot study on a different cohort of patients with breast cancer highlighting chemotherapy-induced perturbations in tricarboxylic acid cycle [24]. In addition, changes in fatty acid levels influence inflammatory processes that may contribute to patient symptom experience [37, 38].

While palmitate (C16:0), oleate (C18:1 n-9), myristate (C14:0), laurate (C12:0) and heptanoate (C7:0) are non-essential fatty acids, linolenate (C18:3 n-3), linoleate (C18:2 n-6) and pentadecanoate (C15:0) are essential fatty acids obtained from diet [39]. Several cell membrane proteins, for example cell adhesion molecules, receptors, and transporters undergo palmitoylation [37] and/or myristoylation [40] to influence cellular signaling processes. For example, cell membrane protein, Adenosine Monophosphate-activated Protein Kinase (AMPK), needed for ATP homeostasis, requires palmitoylation and myristoylation of its protein subunits for proper functioning [41]. A decrease in the levels of fatty acids, palmitate and myristate may negatively affect cellular function. Additionally, pentadecanoate signaling can increase AMPK activity [39]. Perturbation in the AMPK pathway is shown to be associated with breast cancer and chemotherapy related fatigue [42].

A decrease in palmitate level can decrease oleate level in case of inadequate dietary intake of both fatty acids [43]. A decrease in oleic acid levels is associated with decrease in diacylglycerol and increase in triacylglycerol [43]. This change is a risk factor for increased cardiovascular disease [44]. Lauric acid has shown to attenuate hepatic inflammation, a decrease in its level may increase total cholesterol and liver enzymes - serum alanine aminotransferase and aspartate aminotransferase [45]. In one study of neoadjuvant chemotherapy for breast cancer, a decrease in plasma  $\alpha$ -linoleic acid was associated with pathologic complete response [46].

Given the complex effects of fatty acids on metabolic pathways, dietary interventions aimed at reducing inflammation during chemotherapy should be approached with caution. A change in the physiological ratio of saturated and monosaturated fatty acids impacts cell growth, cell differentiation, liver dysfunction, and gut inflammation [43]. In addition, based on dietary guidelines proposed by United States Department of Agriculture, patient participants in our study were reporting less than daily intake

of fiber, whole grain, and vegetables. Diet that meets recommended guidelines for daily intake of fiber, whole grain, and vegetables has shown a decrease in liver inflammatory processes [47]. While the effectiveness of dietary interventions in alleviating chemotherapy-related symptoms during treatment and survivorship remains uncertain, they may hold potential if appropriately individualized and monitored.

A decrease in heptanoate, and branched-chain  $\alpha$ -ketoacids,  $\alpha$ -ketoisocaproate and  $\alpha$ -ketosiovalerate, may make them less available for downstream tricarboxylic acid cycle to produce adenosine triphosphate (ATP), needed for cellular energy [48]. A leucine metabolite,  $\beta$ -hydroxy  $\beta$ -methylbutyrate (HMB) is shown to improve muscle mass [49]. A decrease in HMB,  $\alpha$ -ketoisocaproate and  $\alpha$ -ketosiovalerate serum levels (Fig. 2) from baseline indicate a decrease in intake of essential amino acids, leucine and/or valine or a perturbation in their metabolism [50]. In one study of patients with breast cancer [24], perturbation in essential amino acid metabolism was associated with chemotherapy-induced nausea. Chemotherapy-related changes in fatty acids and branched-chain  $\alpha$ -ketoacids may partially explain chemotherapy-induced symptom occurrence.

A decrease in serum fatty acids and glycerol at T2 compared to T1 level may indicate a decrease in lipolysis activity, breakdown of triglycerides, and a decrease in glycerol as a substrate for gluconeogenesis in the liver [51]. A decrease in serum inositol can decrease inositol derived phosphorylated-inositol cell receptor signaling needed for lipolysis, cell proliferation, and cellular trafficking processes [52]. Inositol can be obtained from diet, recycled from phosphorylated-inositol moieties or synthesized from glucose-6-phosphate [52]. Chemotherapy-induced oxidative stress can lead to an increase in glucose-6-phosphate and a decrease in inositol [52]. Perturbation of *de novo* synthesis of pyrimidine by orotate in the liver maybe related to decrements in liver function. Perturbation in the level of this substrate may be an indicator of hepatic cell proliferation and of symptoms related to hepatotoxicity [53].

## Symptoms associated with change in serum metabolite levels

*Dry mouth* – Chemotherapy-induced dry mouth is common in patients with breast cancer [16, 54]. Although previous metabolomics studies did not evaluate dry mouth in association with change in metabolites levels during chemotherapy in breast cancer, dry mouth was associated with increase in cytosine and uridine-monophosphate [55], downstream products of orotate [53]. Compared to T1, an increase in serum level of 3-hydroxypropionate at T2 (Fig. 3a) maybe correlated with an increase in orotate, a precursor of uridine-monophosphate, at T2 during oxidative stress [53, 56]. This physiological change, an indirect effect of chemotherapy, may increase activation of P2Y2 receptor on salivary glands to increase inflammation, causing a decrease in saliva secretion [55]. Perturbations in serum level of heptanoate, an odd-chain fatty acid, may increase or decrease 3-hydroxypropionate depending on enzymatic activity during initial chemotherapy required to convert heptanoate to 3-hydroxypropionate [56].

*Difficulty concentrating* – Mandelate is a metabolite derived from gut microbiome [57], and perturbations in the level of this metabolite during chemotherapy may indicate chemotherapy-induced gut microbiome dysbiosis. While this finding highlights the complex interaction of biological systems, confirmatory studies are needed for further understanding of this association with difficulty concentrating (Fig. 3).

*Fatigue* – Association of change in levels of 3-hydroxypropionate, glucose-6-phosphate, 6-phosphogluconate and pyruvate maybe related to the shift in energy metabolism as a result chemotherapy-induced oxidative stress [53, 56] Increase in serum 3-hydroxypropionate may indicate a decrease in tricarboxylic acid cycle activity needed for aerobic adenosine triphosphate (ATP) synthesis, which may partially explain fatigue occurrence [56]. Increase in levels of glucose-6-phosphate and 6-phosphogluconate, components of the oxidative branch of pentose phosphate pathway indicate generation of nicotinamide adenine diphosphate (NADPH) needed for synthesis of fatty acids, cholesterol, deoxyribonucleotides needed for cell proliferation[58]. Future studies need to investigate the rate of change in these metabolites in association with fatigue to evaluate the impact of personalized dietary interventions.

## Limitations

While several metabolites were analyzed and symptoms as well as dietary intake were assessed at multiple timepoints, a small sample size is a limitation of this study. Furthermore, multiple-testing comparison and symptom cluster analysis were not performed, thus our findings are exploratory. These results need to be confirmed in a larger cohort. Additionally, there is variability of chemotherapy and antiemetic regimens which could likely confound results. Future larger sample size studies to account for these confounding variables are planned.

## Conclusions and directions for future research

Cumulative effects of chemotherapy-induced perturbations in metabolite levels affect multiple biological pathways that regulate energy metabolism, cell signaling, and proliferation. Alterations were observed across key pathways involved in cellular energy synthesis, including, the tricarboxylic acid cycle, fatty acid  $\beta$ -oxidation, amino acid metabolism, and pentose phosphate pathway. Specific serum metabolites within these pathways were associated with patient symptom experience. Patients' dietary intake, especially fatty acids, essential amino acids, and glucose metabolite levels, need to be monitored throughout chemotherapy. Future mechanistic studies of these metabolic perturbations may provide insights to inform the development of targeted strategies to predict and manage treatment-related symptoms and guide novel therapeutic approaches, with the potential to improve quality of life and clinical outcomes.

## DECLARATIONS

## Competing interests:

The authors have no relevant financial or non-financial interests to disclose.

## Ethics approval:

The study protocol was reviewed and approved by the Institutional Review Board (IRB) at Mayo Clinic (Date:01/05/2024; IRB approval No. 23-007468) and by the IRB at University of Iowa (Date: 7/11/2022; IRB approval No. 202201374). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. All the participants provided written informed consent prior to taking part in the study.

### **Informed consent:**

Informed consent was obtained from all patient participants included in the study.

### **Consent to publication:**

All patient participants consented to publication of findings with no patient identifiers.

### **Patient centered trial**

This study was not an intervention investigation trial. Clinical trial number is not applicable.

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## Author Contribution

Catherine Cherwin: Conceptualization, data curation, formal analysis, funding acquisition, writing original draft. Emily Roberts: Conceptualization, methodology, formal analysis, writing-review, and editing. Felipe Batalini: Resources, formal analysis, writing-review and editing. Sam M. Sorensen: Formal analysis. Lida Mina: Resources, writing-review, and editing. Kathryn J. Ruddy: Resources, writing-review, and editing. Carolyn Harvey-Mead: Formal analysis. Vijay P. Singh: Conceptualization, writing-review, and editing. Linda Chlan: Resources, writing-review, and editing. Bradley Aouizerat: Writing-review, and editing. Komal P. Singh: Conceptualization, funding acquisition, methodology, data curation, project administration, resources, supervision, writing-review and editing.

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## Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. Any additional data needed will be made available on request.

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## Figures

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### Figure 1

Number of patients experiencing occurrence of gastrointestinal symptoms (a) neuropsychological symptoms (b), mean severity score for gastrointestinal symptoms (c) neuropsychological symptoms (d) and mean distress score for gastrointestinal symptoms (e) neuropsychological symptoms (f) over time

# Direction of Change Over Time

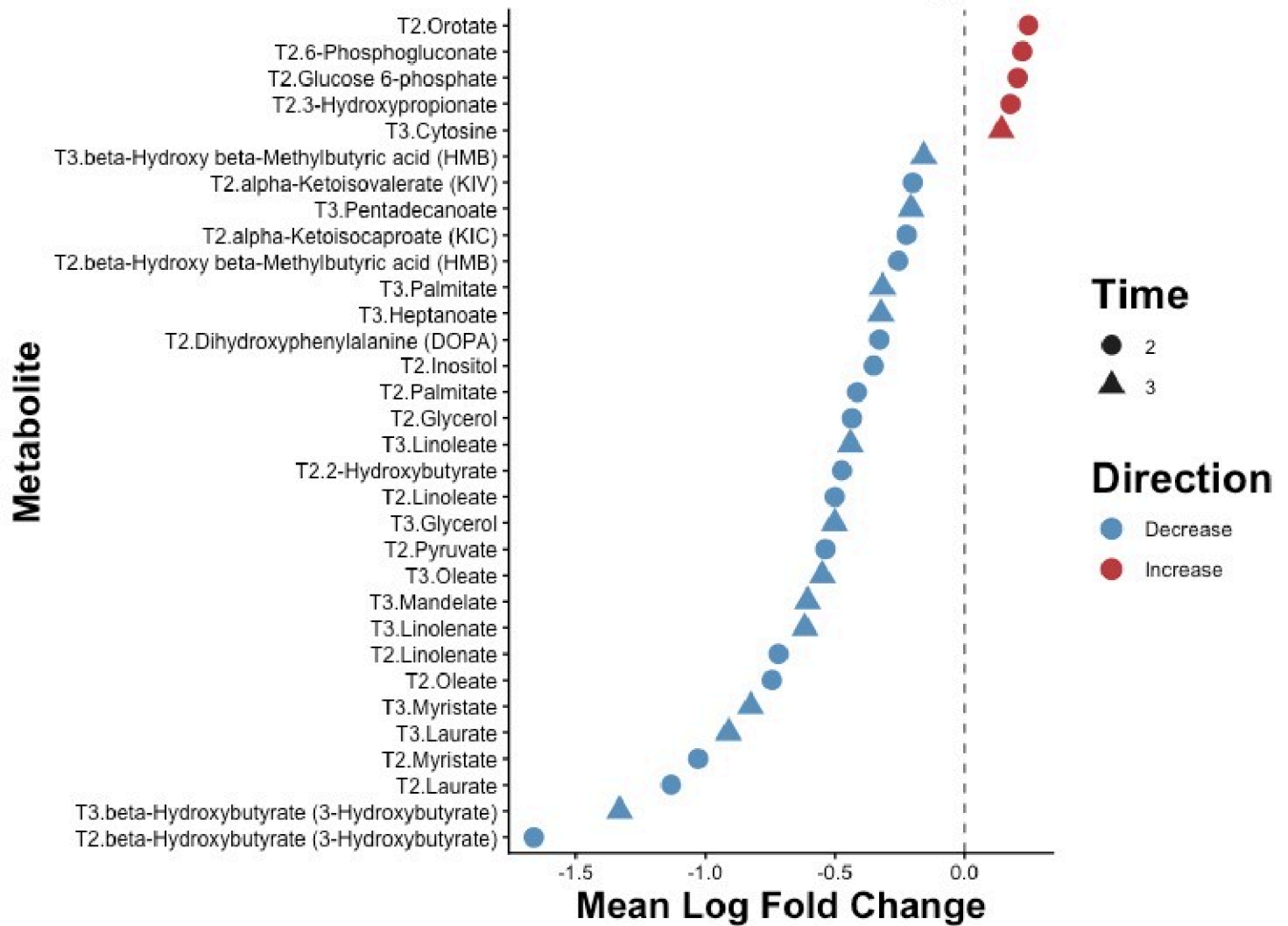
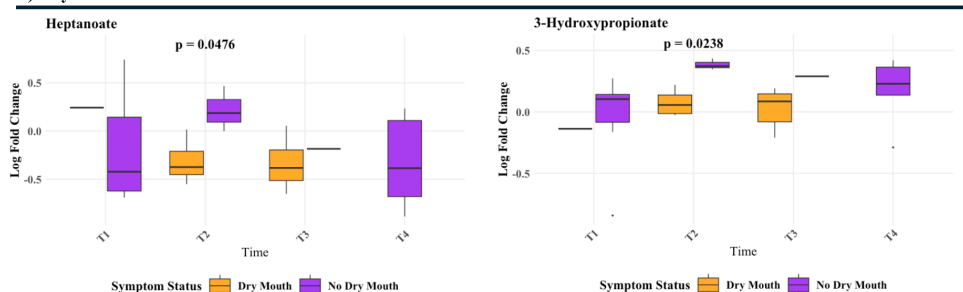


**Figure 2**

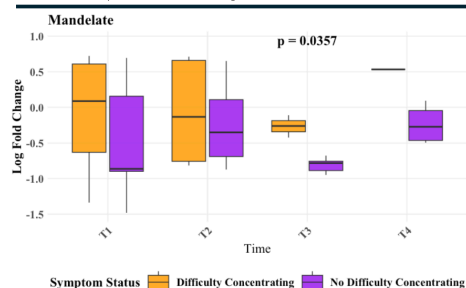
Mean log fold change of metabolites from pre-chemotherapy (T1) to post-chemotherapy (T2 and T3)

**Figure 3**

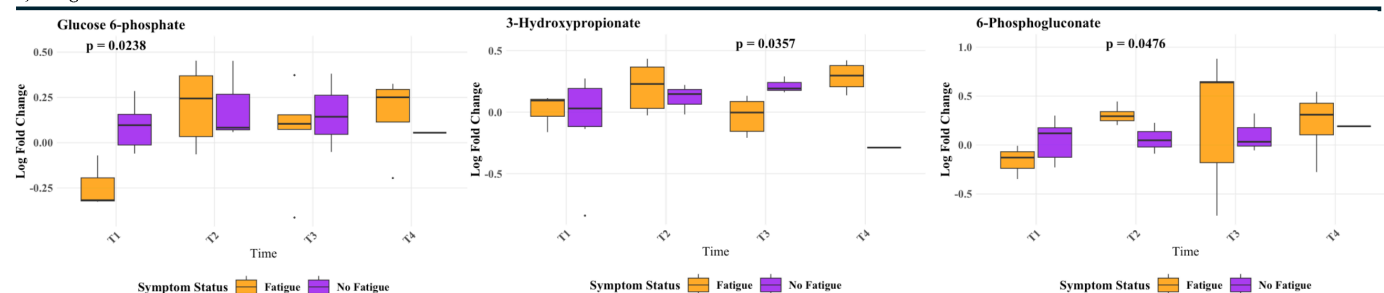
**a) Dry Mouth over Time**



**b) Difficulty Concentrating over Time**



**c) Fatigue over Time**



**d) Fatigue or Difficulty Concentrating over Time**

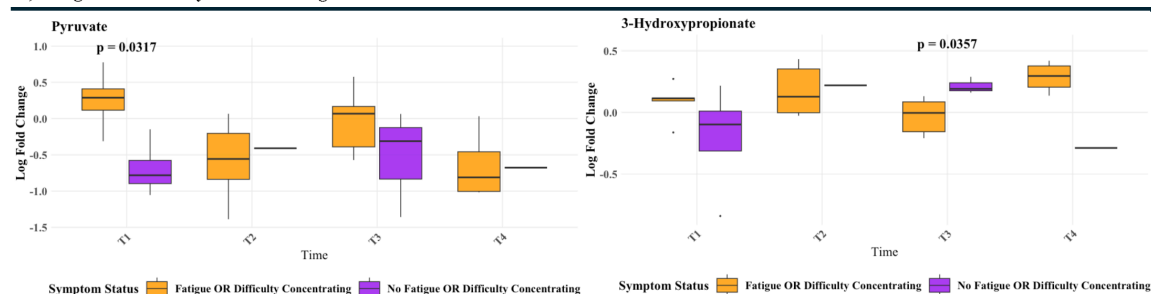


**Figure 3**

Mean log fold change of metabolites in association with dry mouth, difficulty concentrating, fatigue and for the combined symptoms of difficulty concentrating and fatigue

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.pdf](#)
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- [SupplementaryMethodsforsubmission.docx](#)
- [SupplementaryTable1GINeurosymptoms.docx](#)
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