Research Article

Metabolic Effects of Healing Touch During Cervical Cancer Treatment: An Exploratory Analysis

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Abstract

Introduction: Cancer treatment with chemotherapy frequently leads to side effects such as fatigue, pain, nausea, and anxiety. Healing Touch is a non-invasive complementary therapy often used by cancer patients to address side effects of treatment. To better inform the use of complementary therapies, there is a need to understand the biological mechanisms underlying the effects of such treatments.

Methods: This study included 44 patients with cervical cancer undergoing chemoradiation randomized into a Healing Touch (HT), a relaxation training (RT) and a usual care (UC) group. An exploratory metabolomics analysis was conducted on plasma samples taken at baseline, 4, and 6 weeks of ongoing treatment (4 sessions per week).

Results: A multivariate data analysis revealed no significant separation in metabolites between the 3 groups. Univariate data analysis revealed changes in metabolites between baseline and week 6 within each group. The main findings were lower levels of acylcarnitines, bile acids and proline in the HT group, higher levels of fatty acids in the HT and RT groups, and lower levels of kynurenine and quinolate in the UC group. The network of correlations between metabolites shows clear differences in correlations between steroids, fatty acids, sphyngomyelins, amino acids, and γ glutamyl peptides between the 3 groups, suggesting a more flexible and resilient metabolism in the HT and RT groups compared with UC.

Conclusion: This first exploratory study investigating metabolic effects of Healing Touch in cancer patients indicated suggestive differences in metabolic signatures which need further investigation in a larger study.

Keywords

metabolomics, healing touch, cervical cancer, integrative oncology, chemotherapy, relaxation training

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Introduction

Integrative medicine approaches such as Healing Touch (HT) and relaxation training (RT) have been frequently observed to have a positive effect on pain, nausea, anxiety, and fatigue in adults with cancer.[1-6] We have previously reported that among patients with cervical cancer, a 6-week HT biobehavioral intervention during chemoradiation preserved natural killer cell cytotoxicity (NKCC),[7] which is a key component of cellular immunity and cancer control.[8,9] We also found that HT decreased depressive symptoms in patients with cervical cancer receiving chemoradiation when compared to RT and usual care (UC).[7] Natural killer cell function is related to the activity of metabolic pathways, such as glycolysis and fatty acid oxidation[10]; however, the molecular mechanism(s)

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underlying these specific beneficial effects of HT is not fully understood.

Chemotherapy and radiation therapies are known to exacerbate the hypoxic tumor environment, thereby worsening deficiencies in glycemic control and inducing diabetes type 2-like phenotypes in cancer patients.[11] Cancer patients exposed to chemotherapy often show elevations in glucose and lactate, as well as in fatty acids (FA) conjugated to carnitine (acylcarnitines) by the mitochondrial membrane carnitine-palmitoyltransferase 1 and 2 (CPT1/2). CPT2 allows for the internalization of FA for their oxidation. CPT1 converts long-chain acyl-CoA species to their corresponding long-chain acyl-carnitines for fatty acid beta-oxidation within mitochondria.[12] Other expected consequences of the metabolic "reprograming" in cancer patients exposed to chemotherapy are elevations in alpha-ketobutyrate and ketone body β-hydroxybutyrate (BHB). These compounds can provide cells with an alternative carbon source to feed mitochondria in conditions of low glucose and respiratory failure or hypoxia.[13] Therefore, hypoxic stress and subsequent prolonged impairments in the use of glucose and pyruvate as mitochondrial fuels can lead to bioenergetic dysfunction and oxidative damage, resulting in inflammation, fatigue, lactic acidosis, and anemia.[14,15] These side effects of chemotherapy can impair the treatment and quality of life of cancer patients and impact the recovery process.

To provide greater insight into the potential cellular pathway(s) underlying the preservation of NKCC associated with HT treatment during chemotherapy, the metabolic composition of plasma samples from patients with cervical cancer collected in the study conducted by Lutgendorf et al[7] was analyzed. Plasma samples were collected prior to chemotherapy in weeks 1, 4, and 6. Plasma samples were subjected to untargeted metabolomics analysis to reveal possible metabolic changes in HT-treated cancer patients compared to those treated with RT or with UC over time.

Methods

Details of the study design, patient recruitment, intervention, and procedures are described in the main publication of the study results.[7] A summary is provided below to understand the essentials of the study. The metabolomics analysis, multivariate data analysis, bioinformatics, and biological interpretation is new and is described in detail below. The clinical trial was registered at ClinicalTrials.gov (NCT04905576). Ethics approval was obtained from the IRB of the University of Iowa (IRB # 200105058) and included approval for the metabolomics measurements.

Participants

Patients over 18 years of age with stage IB1 through IVA cervical squamous or adenocarcinoma were recruited between May 2002 and March 2007 through the Gynecologic Oncology service at a large Midwestern academic medical center. Patients were excluded for conditions affecting the immune system (eg, lupus), use of systemic steroid medication within a month of study entry, receipt of radiation treatment at another medical center, refusal of radiotherapy, and poor English fluency. All participants provided written informed consent. All patients received standard medical treatment consisting of weekly platinum-based chemoradiation, external beam radiation (total dose 45-50.4 Gy), and brachytherapy. In the original study a total of 60 patients were randomized and the study was completed by 17 in each condition. The current study includes 129 available samples from 44 patients.

Intervention

Healing Touch (HT). Healing Touch is a non-invasive therapy used to restore harmony and balance in the energy system of the patient (the biofield) and to improve the self-healing capacity of the patient.[3,16] Healing Touch was provided in 20 to 30-min sessions 4 days per week during the 6-week chemoradiation treatment by 3 nurses who were certified Healing Touch practitioners. Sessions were usually provided by a team of 2 practitioners (63.5% of the sessions) and were provided on non-chemotherapy days following radiation treatment. The following techniques were used: (1) grounding and centering, (2) pain drain, (3) chakra connection, (4) magnetic unruffling, and (5) mind clearing. Techniques 2, 3, and 5 may involve physical touch, 1 and 4 do not. Additional techniques were used depending on patient presentation.

Relaxation Training (RT). Relaxation training was provided in 20 to 25-min sessions 4 days per week during the 6-week chemoradiation treatment on non-chemotherapy days immediately following radiation, by 1 of 3 trained research assistants or graduate students. The therapists used manualized scripts adapted from existing protocols[17] to guide the relaxation process. The scripts included: (1) passive progressive relaxation, (2) autogenic relaxation, (3) relaxation with nature imagery, and (4) relaxation including imagery of a special place selected by the patient.

Usual Care (UC). Patients in the Usual Care group received no intervention in addition to standard chemoradiation treatment.

Procedure

The study was conducted as a 3-arm randomized single-blinded clinical trial. Patients were randomized to 1 of 3 conditions by permuted block randomization performed by the Holden Comprehensive Cancer Center statistical core. Blood samples were collected prior to chemotherapy in

weeks 1, 4, and 6 in heparinized tubes (BD Biosciences, San Jose, CA). Plasma was frozen at −80°C until analysis, which is a standard storage procedure ensuring good metabolite stability for at least 16 years.[18] All samples were coded. The study interventions started following radiation treatment the day after the baseline blood samples were taken. Psychosocial surveys were completed before each blood draw. Laboratory personnel and health care providers were blind to randomization status.

Blood pressure measurements were taken to assess the extent of relaxation during study interventions. A series of 3 blood pressures was taken before and after the second RT or HT session in weeks 1, 3, and 5 using a Dinamap Pro 100 (Critikon, Tampa, FL) vital signs monitor applied to the non-dominant arm. For UC patients, similar blood pressure measurements were taken before and after a 20 to 25 min neutral video at equivalent timepoints.

Clinical information was collected from medical records. Demographic information, health behavior information and psychosocial surveys were collected before each blood draw. Data from these surveys and blood pressure assessments is reported elsewhere.[7]

Metabolomics Measurements

Plasma samples were sent to Metabolon, Inc. (Durham, NC) for metabolomics analyses in 2017. The analysis of plasma samples for metabolomics was done blinded. Sample preparation consisted of protein precipitation with methanol under vigorous shaking for 2 min followed by centrifugation. A pooled sample consisting of a small volume of all samples was used as a quality control (QC) sample.[19] Water samples were used as blanks. All samples were spiked with a cocktail of QC standards. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections.

All analyses were conducted using a Waters ACQUITY ultra-performance liquid chromatograph (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization source (HESI-II) and Orbitrap mass analyzer operated as 35 000 mass resolution. One aliquot was analyzed using a C18 column (Waters UPLC BEH C18-2.1 × 100 mm, 1.7 µm) using a water methanol gradient containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA) in positive ion mode. For the second aliquot, the same C18 column was used with a methanol, acetonitrile, water gradient, containing 0.05% PFPA and 0.01% FA in positive ion mode. For the third aliquot a separate C18 column was used with a methanol water gradient, with 6.5 mM ammonium bicarbonate at pH 8 in negative ion mode. For the fourth aliquot a HILIC column (Waters UPLC BEH Amide 2.1 × 150 mm, 1.7 µm) was used with a water acetonitrile gradient with 10 mM ammonium formate at pH 10.8 in negative ion mode. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slighted between methods but covered 70 to 1000 m/z.

Metabolomics Data Analysis

Data Processing. Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Biochemical identifications are based on 3 criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library ±10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. For metabolite quantification, peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step (median centering) was performed to correct variation resulting from instrument inter-day tuning differences.

Data were log transformed, variables with ≥20% missing values were removed, and remaining missing values were imputed by the minimum observed value for each variable.[20] Fold changes between week 6 versus week 1 were calculated (week 6-week 1/week 1).

Multivariate Data Analysis. Principal Component Analysis[21] was applied on auto-scaled data and the fold change data to explore differences in metabolite profiles between the HT, RT, and UC groups at week 1 and week 6. Scree plots were used to determine the number of relevant components. Partial Least Squares Discriminant Analysis with cross validation and permutation testing[22] using the Kodama R-package[23] was conducted per time point to construct models to confirm and predict differences between the groups.

Univariate Data Analysis. To further explore the data, *T*-tests[24] were conducted to test differences between groups on the fold change data (comparing week 1 with 6). *T*-tests were also conducted per variable to assess differences between week 1 and 6 for each of the 3 groups. The Benjamini-Hochberg procedure was used to correct for multiple testing as this procedure is deemed suitable for exploratory multivariate analyses in which correlated metabolites are expected.[25,26] Significance levels are presented as *q*-values.[27] Our focus was on differences between week 1 and 6 to examine overall changes over time; thus, data on differences between week 1 and 4 and between 4 and 6 are not shown. Standard mean differences according to Cohen's *d* are calculated to present effect sizes.

Network Analysis. Network analysis is an increasingly popular approach which is often conducted in addition to multivariate or univariate data analysis.[28] A change between metabolic states doesn't necessarily result in changes of average levels of metabolites but can also result in changes in pairwise correlations between metabolites.[29] The correlation structure of a list of metabolites therefore provides a global fingerprint of a physiological state and can provide insights regarding systemic changes between conditions.[30] The 100 metabolites with the largest variance accounted for (VAF) in the dataset were selected. Spearman correlations[31] were calculated per time point per intervention group. The correlations $>.70$ and $<-.70$ were converted to an edge list per time point and group. The edge lists were imported into CytoScape 3.9.0.[32] to visualize the correlation structures per time point and group. A perfuse force directed layout was used to draw a first network, the layout of the nodes was then fixed for the subsequent networks. Edge color and edge thickness was used to visualize differences in the strength of the correlations between the intervention groups and time points.

Results

Demographic characteristics of the participating patients are presented in Table 1. Significant differences were found in FIGO stage between HT and UC and between RT and UC, not between HT and RT. No other significant differences in clinical variables or in demographics were found between the groups.

The number of included blood samples per time point is presented in Table 2. Since this was an exploratory analysis and the number of samples per group was limited, all available samples were included in data analyses. A total number of 884 metabolites were measured and identified; 221 of these were removed due to >20% missing values across the samples leaving 663 metabolites for data analysis.

Multivariate Data Analysis

A PCA analysis was conducted on the fold change data (week 6-week 1/week 1). A scree plot indicated 2 relevant principal components. No separation between the 3 intervention groups was visible in the PCA score plot. A PLS-DA model on the same data was not significant ($P=.191$), indicating that no significant model could be constructed to accurately predict differences in fold changes between the 3 groups.

Principal component analyses were then performed on (a) the full data set, (b) per intervention group, and (c) per time point. Scree plots indicated the number of relevant components per PCA analysis. A separation between intervention groups or time points was not visible in any of the score plots. Since the main research aim was to explore whether there were differences between intervention groups over time, partial least squares discriminant analyses (PLS-DA) were conducted on the data at week 1 and week 6. Neither of the PLS-DA models was significant (week 1: $P=.92$, week 6: $P=.63$) indicating that no significant models could be constructed that could accurately predict differences in the metabolome between the 3 groups at baseline and week 6.

Univariate Data Analysis

T-tests were performed for the calculated fold changes between week 1 and week 6 for all metabolites. None of the fold changes were significantly different between the intervention groups after Benjamini-Hochberg correction. The list of metabolites with the largest fold change differences (week 1 vs week 6) between the HT and UC groups (n = 36 with uncorrected $P<.10$) and between the HT and RT groups (n = 56 with uncorrected $P<.10$) are shown in Supplemental Data Tables S1 and S2.

T-tests were then performed on all 663 metabolites between time points 1 and 6, within each intervention group separately. No significant differences were found between the groups at baseline. Significant differences between week 1 and week 6 after Benjamini-Hochberg correction were found for 14 metabolites in the UC group, 26 metabolites in the RT group and 24 metabolites in the HT group. These 54 significant differences represent 49 unique metabolites, which are presented in Table 3 with the corresponding *q*-values and effect size (Standard Mean Difference or Cohen's *d*).

Several metabolites showed significantly different abundance at week 6 compared with baseline in the HT group, and not in the RT or UC groups (see Table 3). These metabolites are: 1,2-dipalmitoyl-GPC (16:0/16:0), 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4), arachidonoylcarnitine (C20:4), dihomo-linolenoylcarnitine (20:3n3 or 6), dihomo-linoleoylcarnitine (C20:2), eicosenoylcarnitine (C20:1), ergothioneine, linoleoylcarnitine (C18:2), proline, taurodeoxycholate, and taurolithocholate 3-sulfate. The majority of these metabolites showed significant decreases over time. Metabolites specifically different over time within the RT group are: 1-methylhistidine, 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2), 1-palmitoyl-2-oleoyl-GPC (16:0/18:1), 1-palmitoyl-2-oleoyl-GPE (16:0/18:1), 5-bromotryptophan, androstenediol (3alpha, 17alpha) monosulfate (2), cerotoylcarnitine (C26), citrulline, epiandrosterone sulfate, gamma-carboxyethyl hydroxychroman, guanidinoacetate, homoarginine, imidazole lactate, oleoyl-arachidonoyl-glycerol (18:1/20:4) [2], and stearoylcarnitine (C18). Significant changes found only in the UC group included the following: 7-methylguanine, kynurenine, N6-carbamoylthreonyladenosine, N-acetylalanine, N-acetylthreonine, O-sulfo-L-tyrosine, phenyllactate (PLA), pseudouridine, and quinolinate. All of

Table 1. Demographics of the Participants.

Measure	Healing touch (HT)	Relaxation (RT)	Usual care (UC)
Age, years (standard deviation) (n = 14,17,13)	49.57 (13.62) Range 29-73	42.00 (9.70) Range 24-60	46.62 (14.21) Range 26-77
Education (n = 12,17,12) (%)			
Less than high school	0.0	5.9	0.0
Some high school	8.3	0.0	8.3
High School Graduate	41.7	35.3	25.0
Trade School	0.0	5.9	0.0
Some college	25.0	29.4	33.3
College graduate	16.7	17.6	33.3
Post-graduate	8.3	5.9	0.0
Annual Income (n = 14,17,11) (%)			
$10 000 or less	28.6	29.4	18.2
$10 001-$20 000	7.1	23.5	18.2
$20 001-$30 000	28.6	17.6	18.2
$30 001-$40 000	21.4	0.0	18.2
$40 001-$50 000	7.1	23.5	27.3
> $50 000	7.1	5.9	0.0
Race (n = 14,17,13) (%)			
American Indian/Alaskan Native	0.0	0.0	7.7
Asian/Pacific Islander	0.0	0.0	0.0
African American (non-Hispanic)	0.0	0.0	0.0
Caucasian (non-Hispanic)	100.0	100.0	92.3
Ethnicity (n = 14,17,13) (%)			
Hispanic	7.1	0.0	0.0
Non-Hispanic	92.9	100.0	100.0
Relationship status (n = 14,17,12) (%)			
Married/Living with partner	64.3	70.6	50.0
Single/divorced/widowed	35.7	29.4	50.0
FIGO stage (n = 14,17,13) (%)			
IBI	42.9	11.8	15.4
IBII	7.1	17.6	28.5
IIA	0.0	17.6	0.0
IIB	35.7	41.2	7.7
IIIA	0.0	0.0	0.0
IIIB	14.3	11.8	30.8
IVA	0.0	0.0	7.7
Body Mass Index (kg/m^2; n = 14,17,13) (%)			
Underweight (<18.5)	7.1	17.6	23.1
Normal weight (18.5-24.9)	23.7	47.2	38.5
Overweight (25.0-29.9)	42.9	17.6	7.7
Obese (≥30)	14.3	17.6	30.7
Sleep in past week, hours/nite, mean (SD; n = 14,17,13)	6.83 (1.47)	6.97 (1.91)	6.33 (1.87)
Cigarettes, packs/day, mean (SD; n = 13,17,12)	0.17 (0.31)	0.24 (0.47)	0.25 (0.45)
Caffeine, cups/day, mean (SD; n = 12,17,12)	1.67 (2.27)	1.29 (1.99)	1.83 (2.72)
Alcohol, drinks/day, mean (SD; n = 13,16,11)	0.23 (0.60)	0.19 (0.54)	0.18 (0.60)
Cycles of chemotherapy before final blood draw (n = 14,17,12)	4.36 (0.74)	4.12 (0.78)	4.21 (0.71)

Table 2. Number of Blood Samples Analyzed for Metabolomics.

Samples	Healing touch (HT)	Relaxation (RT)	Usual care (UC)
Week 1	14	17	13
Week 4	13	17	13
Week 6	14	17	11

these represented decreases over time. Several metabolites showed significantly different abundance at week 6 compared with week 1 in both the RT and HT group, and not in the UC group. These metabolites are: 1,5-anhydroglucitol (1,5-AG), 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4), citrate, glycosyl ceramide (d18:2/24:1, d18:1/24:2), glycosyl-N-behenoyl-sphingadienine (d18:2/22:0), myo-inositol, and taurine. The majority of these metabolites showed significant decreases over time.

Network Analysis

A network was constructed with a total of 654 metabolite correlations (edges) representing the most important metabolites for the variations in the dataset. Figure 1 illustrates the differences in correlation structure between the HT, RT and UC group at week 6. The correlation networks show that the metabolites are grouped into 5 major clusters, representing sphingomyelins, amino acids, γ-glutamyl peptides, fatty acids, and steroids. Differences in the color and width of the edges between conditions represent general changes in correlation strength between the metabolites related to these clusters. The UC group has a higher number of strong correlations (thicker edges and more red and gray edges) than the RT and HT groups at week 6. The correlations between the steroids and fatty acids are much stronger in the HT group than in the RT group, and in the UC group these are mostly negatively correlated. In the UC group, the correlations between the fatty acids and sphingomyelins are much stronger than in the RT and HT groups. Furthermore, the γ-glutamyl peptides are more strongly correlated in the UC group.

In Figure 2, the differences in the correlations in the HT group are illustrated over time. The structure of the networks is kept the same as in Figure 1 to provide easy comparisons between the figures. The edges of the networks are colored according to the strength of correlation in the HT condition. The red arrows in Figure 2 point to areas with the more pronounced differences in correlation structure between week 1 and week 6. The strength of the correlations between the steroids and fatty acids seem to increase over time (more red and thicker lines). The negative correlations (gray) between the γ-glutamyl peptides and fatty acids in week 1 are much less present in week 6. Additionally, the γ-glutamyl peptides are much less strongly correlated with each other as a group at week 6 compared to week 1. These latter changes in γ-glutamyl peptides and fatty acids suggest more metabolic flexibility in these areas, while the stronger correlations between steroids and fatty acids suggest more metabolic inflexibility in those areas. Metabolic flexibility refers to the ability of the organism to efficiently adapt its metabolic state to conditions of stress, such as physical exercise, mental stress, and infections.[33]

Discussion

This is the first investigation of which we are aware to explore possible effects of Healing Touch on metabolites measured in blood using state of the art analytical chemistry methods. Participants were patients with cervical cancer undergoing chemoradiation. Because of the small number of subjects in each group, we consider these analyses to be primarily exploratory. Principal component analysis did not reveal differences either between the groups, between time points, or between the changes in the groups over time, indicating that there were no overarching metabolic variations between the groups, time points, or between the groups over time. However, univariate analysis revealed a list of metabolites with significant within group changes over time (week 1 vs week 6). This list contained 11 metabolites specifically changed in the HT group over time and not in the RT and UC groups. It also contained 15 metabolites significantly changed in the RT group only, and 8 metabolites significantly changed in the UC group only. These will be discussed below.

Metabolic Effects of HT

Five long chain acyl carnitines were found to be significantly decreased in the HT group over time (arachidonoylcarnitine (C20:4), dihomo-linolenoylcarnitine (20:36n3 or), dihomo-linoleoylcarnitine (C20:2), eicosenoylcarnitine (C20:1), and linoleoylcarnitine (C18:2)), whereas no significant changes in acyl carnitines were found in the RT or UC groups over time. The main function of long chain acylcarnitines is the transportation of long chain fatty acids into mitochondria.[34] Mitochondria are multifunctional organelles that contribute to organismal health by receiving, integrating, and producing various molecular and non-molecular signals, including metabolites.[35] The carnitine systems may be involved in the metabolic flexibility of cancer cells and the switch between glucose and fatty acid metabolism.[36,37]

Table 3. Metabolites with Significant Differences Between Week 1 and Week 6 (After Benjamini-Hochberg Correction).

	Usual care (UC)		Relaxation (RT)		Healing Touch (HT)	
Metabolite name	SMD	*q*-Value	SMD	*q*-Value	SMD	*q*-Value
Significant changes in HT group only						
1,2-dipalmitoyl-GPC (16:0/16:0)	1.43	0.062	0.81	0.160	1.72	**0.014**
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	0.80	0.312	1.15	0.051	1.52	**0.036**
arachidonoylcarnitine (C20:4)	−0.93	0.223	−1.07	0.067	−1.43	**0.042**
dihomo-linolenoylcarnitine (20:3n3 or 6)	−0.88	0.245	−1.01	0.086	−1.32	**0.046**
dihomo-linoleoylcarnitine (C20:2)	−1.22	0.137	−0.95	0.102	−1.61	**0.016**
eicosenoylcarnitine (C20:1)	−0.67	0.402	−0.79	0.166	−1.53	**0.032**
ergothioneine	1.47	0.052	1.03	0.080	1.40	**0.044**
linoleoylcarnitine (C18:2)	−1.33	0.110	−0.80	0.164	−1.35	**0.043**
proline	−0.85	0.289	−0.51	0.394	−1.37	**0.038**
taurodeoxycholate	−1.29	0.127	−0.95	0.101	−1.34	**0.041**
taurolithocholate 3-sulfate	−1.43	0.103	−0.68	0.238	−1.31	**0.045**
Significant changes in RT group only						
1-methylhistidine	−1.35	0.112	−1.47	**0.012**	−1.13	0.077
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.54	0.521	1.35	**0.019**	0.94	0.201
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	1.07	0.169	1.28	**0.024**	0.79	0.293
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.71	0.391	1.46	**0.011**	1.04	0.154
5-bromotryptophan	−0.87	0.279	−1.53	**0.009**	−1.26	0.070
androstenediol (3alpha. 17alpha) monosulfate	−0.92	0.237	−1.23	**0.034**	−1.17	0.083
cerotoylcarnitine (C26)	−0.46	0.581	−1.31	**0.021**	−0.66	0.353
citrulline	−0.97	0.225	−1.20	**0.039**	−0.85	0.235
epiandrosterone sulfate	−0.83	0.306	−1.39	**0.016**	−0.73	0.269
gamma-CEHC	−0.87	0.286	−1.53	**0.008**	−0.46	0.525
guanidinoacetate	−0.12	0.892	−1.25	**0.029**	−0.60	0.394
homoarginine	−0.62	0.466	−1.16	**0.048**	−1.15	0.071
imidazole lactate	−1.18	0.151	−1.34	**0.019**	−0.74	0.262
oleoyl-arachidonoyl-glycerol (18:1/20:4)	0.63	0.436	1.17	**0.050**	0.68	0.363
stearoylcarnitine (C18)	−0.26	0.758	−1.15	**0.050**	−0.77	0.267

(continued)

Table 3. (continued)

Metabolite name	Usual care (UC)		Relaxation (RT)		Healing Touch (HT)	
	SMD	*q*-Value	SMD	*q*-Value	SMD	*q*-Value
Significant changes in UC group only						
kynurenine	−1.56	**0.048**	−0.64	0.275	−0.48	0.463
N6-carbamoylthreonyladenosine	−1.75	**0.032**	−0.42	0.484	−0.61	0.379
N-acetylalanine	−1.87	**0.026**	−0.84	0.146	−1.21	0.072
N-acetylthreonine	−1.83	**0.022**	−0.79	0.167	−0.89	0.198
O-sulfo-L-tyrosine	−1.86	**0.023**	−0.96	0.102	−1.02	0.137
phenyllactate (PLA)	−1.54	**0.050**	−0.38	0.537	−0.66	0.318
pseudouridine	−1.62	**0.049**	−0.40	0.509	−0.51	0.453
quinolinate	−1.76	**0.034**	−0.43	0.474	−0.76	0.273
Significant changes in both RT and HT groups						
1.5-anhydroglucitol (1.5-AG)	−1.18	0.145	−1.39	**0.016**	−1.91	**0.005**
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	0.75	0.361	1.29	**0.024**	1.42	**0.040**
citrate	−0.88	0.271	−1.54	**0.009**	−1.58	**0.030**
glycosyl ceramide (d18:2/24:1. d18:1/24:2)	−0.94	0.225	−1.57	**0.011**	−2.93	**0.000**
glycosyl-N-behenoyl-sphingadienine (d18:2/22:0)	−0.51	0.548	−1.40	**0.017**	−1.42	**0.042**
myo-inositol	−1.03	0.178	−1.33	**0.019**	−1.56	**0.019**
taurine	−0.79	0.305	−1.54	**0.011**	−1.86	**0.007**
Significant changes in all 3 groups or in HT and UC						
deoxycarnitine	−1.69	**0.030**	−1.37	**0.016**	−1.80	**0.006**
glycosyl-N-nervonoyl-sphingosine (d18:1/24:1)	−1.66	**0.037**	−1.81	**0.002**	−2.84	**0.000**
lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)	−3.12	**0.000**	−1.83	**0.002**	−2.87	**0.000**
lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	−4.18	**0.000**	−2.04	**0.001**	−2.90	**0.000**
7-methylguanine	−2.49	**0.001**	−0.59	0.314	−1.42	**0.048**
ceramide (d18:1/14:0. d16:1/16:0)*	1.83	**0.025**	0.84	0.145	1.40	**0.040**

Abbreviations: SMD, standard mean difference according to Cohen's d. Significant q-values* (<0.05) are printed in bold type.
*Q-values are calculated as the p-value times the number of tests (664) divided by the rank of the p-value (all p-values are ranked from lowest to highest) according to Hochberg and Benjamini.[27]

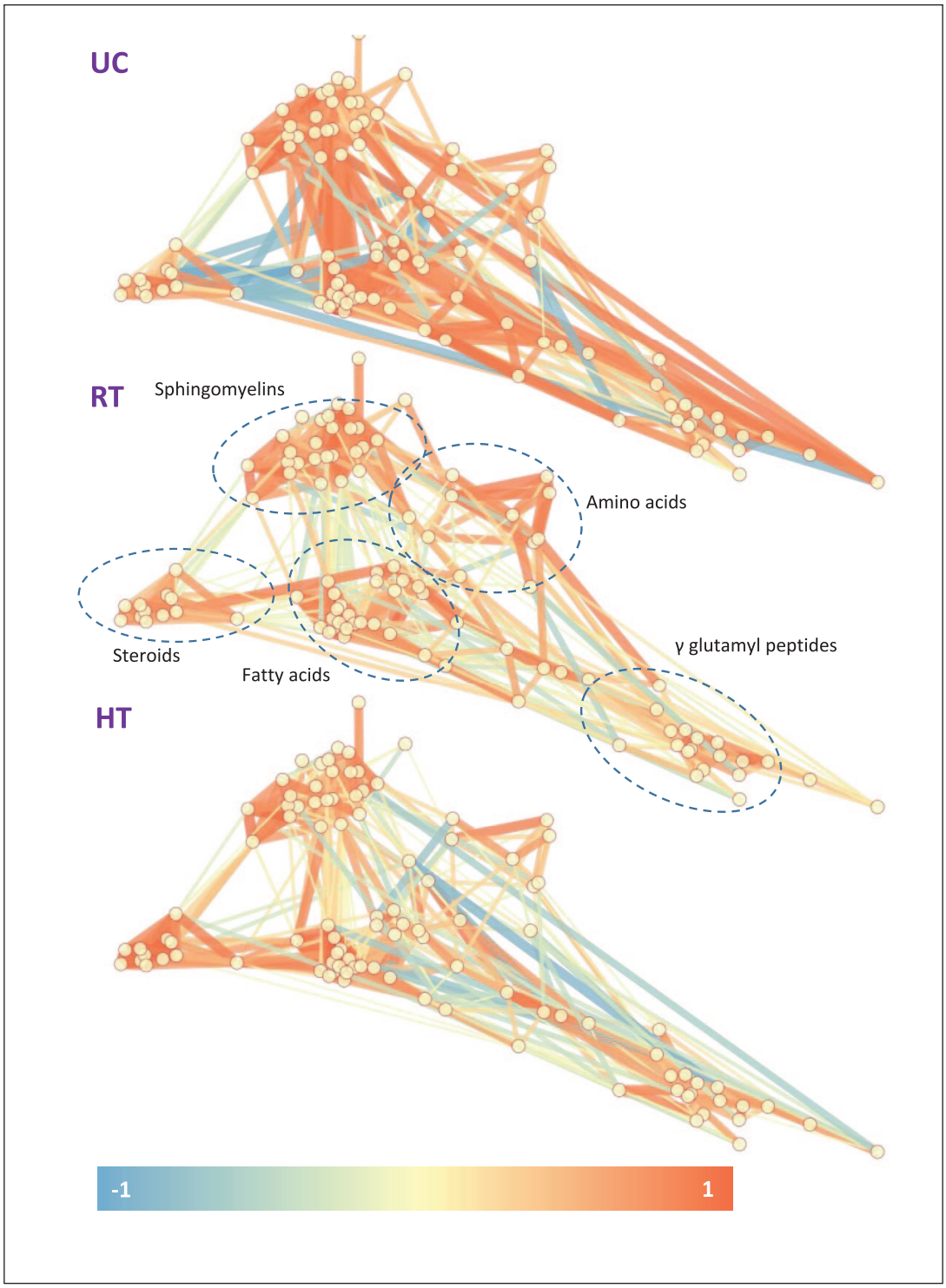

Figure 1. Differences in correlations between HT, RT, and UC groups at week 6. Correlations $>.70$ and $<-.70$ are visualized. Correlations are indicated on a scale from red (positive) to blue (negative) and thick (stronger) to thin (weaker).

An increase in long chain acyl carnitines is associated with metabolic changes in several types of cancer.[38] Changes in mitochondrial metabolism are also found to be associated with platinum-based chemotherapy, which can increase the concentration of long chain acylcarnitines in blood and is associated with cancer related fatigue.[39] This effect appears to be reduced in the HT group.

Psychological stress is characterized by changes in energy balance and metabolism, aimed at priming the organism for a rapid response to a threatening situation.[40]

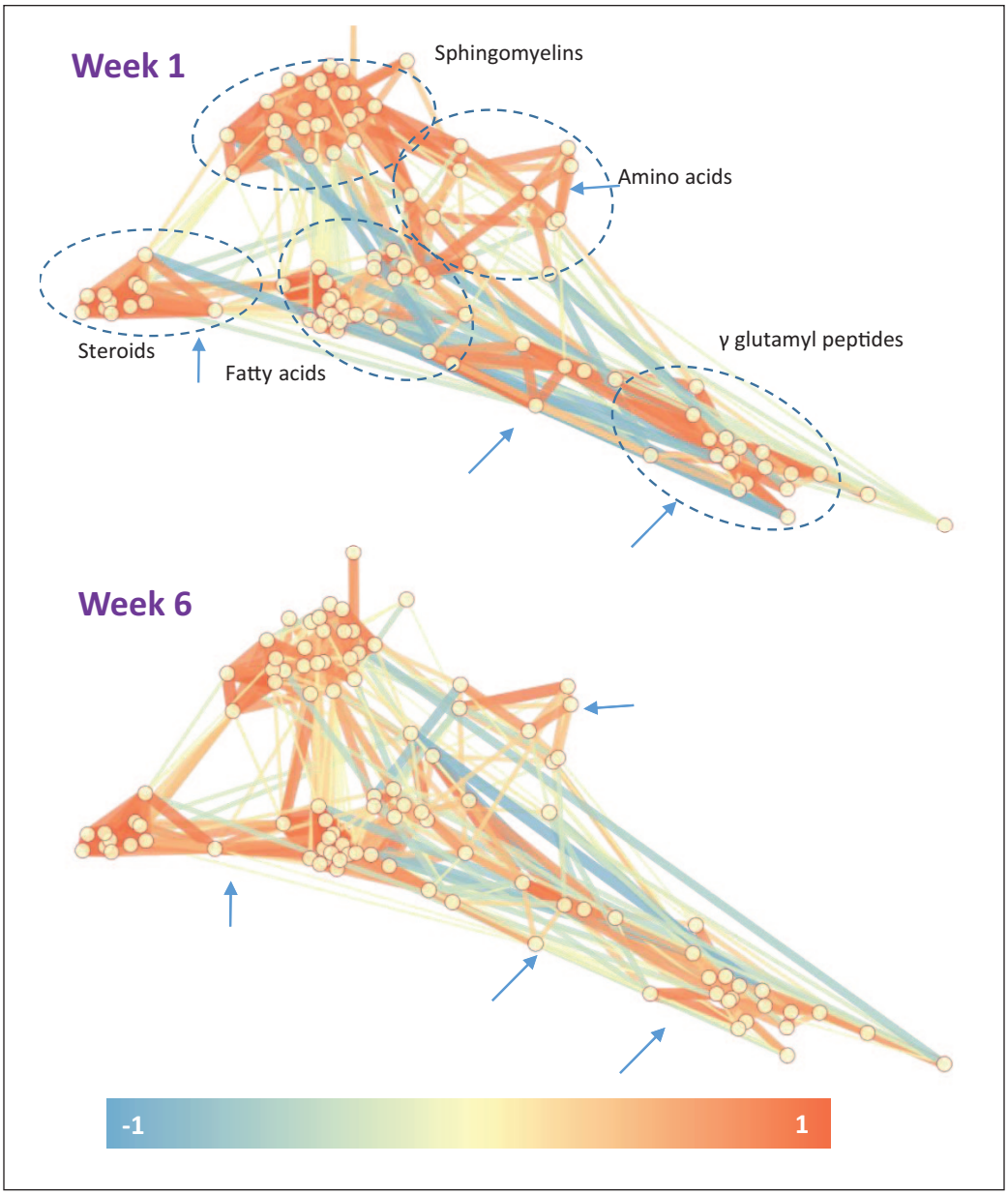

Figure 2. Differences in correlations in HT group over time. Correlations >.70 and <−.70 are visualized. Correlations are indicated on a scale from red (positive) to gray (negative) and thick (stronger) to thin (weaker). The red arrows point to areas with differences between week 1 and week 6.

Studies in animal models of chronic unpredictable stress show substantial changes in metabolomics, including altered levels of amino acids, fatty acids, carnitines, and phospholipids.[41] This is consistent with changes in several acylcarninites we observed in the HT group over time. Multiple metabolomics studies have been conducted in the area of mental health conditions such as depression and anxiety.[42,43] In a systematic review of 27 studies on lipidomics and genomics in mental health conditions, changes are reported in lipid signatures including triglycerides (TG), ceramides, fatty acids, and phosphatidylcholine between healthy controls and individuals with mental health conditions indicating common metabolic pathways underlying these mental health conditions.[42]

Lower concentrations of long chain acylcarnitines have been found in plasma samples of subjects with anxious depression, depression and neurovegetative symptoms of melancholia.[44] In depressed patients the relevant long chain acylcarnitines had a carbon chain length between C14 and C18, while in our study 4 significant acylcarnitines with

length C20 and 1 of C18 decreased over time. Various patterns of changes in short, medium and long chain acylcarnitines have been found in patients with anxious depression, depression and melancholia as a result of 8 weeks treatment with an SSRI.[44,45] Interestingly, the C18:2 acylcarnitine decreased after treatment with an SSRI and was also found to decrease after HT treatment in our study.

Proline was found to be significantly reduced in the HT group at week 6. This metabolite is known as an overall risk factor for cancer and has a possible role in tumor growth.[46] Quinolinate and kynurenine were significantly reduced at week 6 compared to week 1 in the UC group, but not in the RT and HT group. These 2 metabolites are part of the kynurenine pathway which is related to the production of immunosuppressive metabolites and is reported to be related to cancer cell motility and migration.[47] The mitochondria-related kynurenine pathway is the only pathway responsible for the synthesis of the metabolic cofactor nicotinamide adenine dinucleotide (NAD^+, of which kynurenine is a precursor), which decreases with aging and whose supplementation improves resilience to age-related deterioration and mitochondrial respiratory capacity in animal studies (reviewed in Castro-Portuguez and Sutphin,[48] Mitchell et al.,[49] and Miwa et al.[50]). Furthermore, reduced plasma kynurenine levels are associated with major depressive disorder, indicating a reduced availability of tryptophan.[51] The decline of these metabolites in participants receiving chemotherapy and UC compared to their preserved levels in the HT and RT groups, could reflect some protection from these age-related effects and possible depressive symptoms by both treatments.

The univariate analysis indicated different patterns between the HT and UC groups in fatty acid synthesis. Fatty acid oxidation has been shown to be essential for appropriate natural killer cell response in cancer[52] and the exposure of natural killer cells to fatty acids might affect their function.[53] We found a significant increase in 1,2-dipalmitoyl-GPC (16:0/16:0) and 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) in the HT group over time. In the RT group three fatty acids showed a significant increase over time: 1-palmytoyl-2-linoleoyl-GPE (16:0/18:2), 1-palmytoyl-2-oleoyl-GPC (16:0/18:1), 1-palmytoyl-2-oleoyl-GPE (16:0/18:1). One fatty acid (1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)) was significantly increased in both the RT and HT groups over time. Phosphatidylcholine (1,2-dipalmitoyl-GPC (16:0/16:0)) is increased over time in the HT group and is the most abundant phospholipid component of mitochondria, where its abundance relative to other lipids influences several aspects of mitochondrial biology.[54] Phosphatidylcholine metabolism is known to be deregulated in multiple cancer types[55] and mediates cancer cell growth and survival.[56] Phosphatidylcholine catabolism generates lysophosphatidylcholines, which play an important role in tumor invasion, metastasis, and prognosis[56] and also have a more general role in immune function, such as a chemotactic effect on NK cells.[57] Furthermore, low levels of phosphatidylcholine and lysophosphatidylcholine are reported biomarkers for heterogeneous cancers[46] and cervical cancer specifically.[58] The increase in phosphatidylcholine over time that we found in the HT group therefore suggests a positive response. In contrast, in a study of patients with major depressive disorder an upregulation of lysophospoholipids was found, although these were different lipids from the ones in our study.[45]

Two bile acids, taurodeoxycholate (TDCA) and taurolithocholate 3-sulfate, were found to be significantly decreased in the HT group over time, but not in the UC and RT groups. Taurine, an important molecule needed for bile acid synthesis and involved in energy metabolism and positively associated with a longer health span and longer life span,[59] was found to be significantly decreased in both the HT and RT groups over time, and not in the UC group. These results seem contrary to our predictions but might indicate that bile acid metabolism is affected by the HT intervention. Bile acids are increasingly associated with anti-cancer properties in several cancer types[60] and changed bile acid ratios associated with altered gut microbiome are found in people with anxiety and depressive disorder.[61]

Network Analysis

Network analysis is currently considered as an important part of the -omics data analysis strategy.[62] It provides an additional view on the data in terms of relationships between metabolites and provides complementary information in addition to individual metabolite abundance changes over time. For instance, in Park et al[63] metabolite networks were used to interpret the molecular mechanisms related to changes in BMI. However, apart from exceptions like this the relationship between network changes and health outcomes has been largely uncharacterized. In our study network analyses were conducted to further explore differences between the intervention groups and differences in their changes over time. The networks reveal stronger correlations between the steroids and fatty acids in the HT group, compared to the RT and UC groups. Both steroid and fatty acid oxidation occur in mitochondria[35] and the synthesis of all steroid hormones is regulated by mitochondria.[64] Steroids as modulators of the stress response have an important modulatory effect on immune function.[65] In both the RT and HT groups, the correlations between steroids and fatty acids were less strong overall than in the UC group. Strong correlations between subsystems are known to indicate less resilience[66] and may indicate less metabolic flexibility in the case of strong cross-correlations in metabolic network structures. Metabolic flexibility is described as a key indicator for health, which is commonly assessed by challenge tests such as the oral glucose tolerance test or

immunological challenge tests (eg, a lipopolysaccharide test).[33] A less flexible system, which can occur during a disease, is less able to cope with nutritional, mental, inflammatory, or other stressors from the environment. Our data suggest a more flexible and resilient metabolism in the HT and RT groups compared to the UC group.

Additionally, there are clear differences in correlations between the fatty acids and sphingomyelins in the UC group compared with the HT and RT groups. Sphingolipids play important roles in mitochondrial biology, including regulation of energy transformation, signaling, and cell death pathways.[67,68] Sphingomyelins have been found to be associated with sensitivity to chemoradiation in cervical cancer patients.[69] The network analysis seems to suggest a more pronounced resilience and metabolic flexibility in the HT condition compared to UC and suggests general effects of HT and RT on steroid, fatty acid, amino acid, sphingomyelins, and γ-glutamyl peptides metabolism. These findings provide overall support to the univariate analysis in which differences in phosphatidylcholine and lysophosphatidylcholine concentrations between the HT, RT, and UC groups were found.

Study Limitations

The primary outcome measures of the original study were natural killer cell activity, depression, and chemoradiation induced toxicities. Therefore, the study was not specifically designed or powered to detect differences in metabolomic profiles of blood samples, and these analyses should be seen as primarily exploratory. Additionally, blood samples were only available for metabolomics analysis for 44 of the 61 patients participating in the original study and in the current analysis, not all patients had complete samples at each timepoint. Because this was an exploratory analysis with limited numbers of samples, we used all available samples and resulting data for the analyses but power to detect between group differences is limited. Samples were stored between 10 and 15 years before the metabolomics analyses were performed, which could have resulted in degradation of certain metabolites; however this storage span is still within generally accepted periods for sample storage.[18]

The results of the PLS-DA analysis didn't indicate a clear separation between the groups and the univariate analysis of the fold changes between week 1 and 6 revealed no significant differences between the groups in changes in metabolites over time. However, significant differences between weeks 1 and 6 were found in each group separately, which were significant even after correcting for multiple testing. Furthermore, multiple changes in groups of metabolites together could each contribute a small part to larger overall effects. Because of this discrepancy between the multivariate and univariate results, all findings are considered exploratory and should be treated with caution until confirmed in a larger sample.

Participants were not aware of their assigned intervention until after the baseline surveys and blood draws had been completed. As both HT and relaxation involved direct interaction with practitioners, it was not possible to blind the participants to their assigned intervention. It is possible that expectations regarding their intervention or non-specific effects from interactions with the practitioners may have affected the results. We have previously reported that there was no difference in expectations prior to the intervention, and no difference in endorsement of benefit between the different conditions following the intervention although there was a possible trend ($P = .06$) for patients in the HT group to report greater reduction of treatment side-effects than patients in RT.[7] Nevertheless, we cannot totally rule out effects of expectations or non-specific factors on these results.

It would have been interesting to examine metabolic differences between the 2 active groups, HT and RT. However, in exploratory analyses with 44 people we were not adequately powered to test the differences between 2 active interventions. We have presented the metabolites with the largest differences in Supplemental Files S1 and S2. The S2 file contains the direct comparisons between RT and HT. For the top 20 metabolites the difference between the change in RT and the change in HT was significant in unadjusted univariate analyses but are non-significant with Benjamini-Hochberg corrections (Q-value). Thus, the question of differences between active interventions such as HT and RT will need to be explored in a larger sample.

In our previous report[7] we indicated that the groups did not differ in treatment delay or in clinically assessed toxicities. However, we do not have a measure of treatment response. Thus, we were not able to examine relationships of metabolites to clinical endpoints, thereby limiting our ability to judge the clinical significance of the observed metabolic shifts. Baseline differences in disease stage were found between the groups which could possibly have influenced the response to the different interventions and and thereby resulted in differences in metabolite profiles between the groups. However, no differences in the metabolite profiles were found at baseline between the groups.

Conclusions

This is the first study investigating potential metabolic effects of the complementary therapy of Healing Touch in cancer patients. Findings indicate that there were not overarching differences in the metabolic profiles of HT versus relaxation or usual care conditions over time. Univariate analyses and network analyses indicated suggestive differences in metabolite signatures potentially consistent with intervention associated differences in acylcarnitines and fatty acid metabolism. We speculate that this could relate to a role of mitochondria operating as a metabolic hub transducing and transforming energy in its different forms.[70]

Future work is needed to assess these relationships in a larger sample, and to establish the clinical significance of any metabolic changes.

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Ethical Considerations

Ethics approval was obtained from the IRB of the University of Iowa (IRB # 200105058).

Consent to Participate

All participants provided written informed consent.

Author Contributions

Herman A van Wietmarschen: Conceptualization, methodology, formal analysis, writing draft, visualization, project administration, funding acquisition. Susan Lutgendorf: Conceptualization, analysis, writing review, project administration and funding acquisition of original project. Estela Area-Gomez: consultation, writing review. Anil Sood: Consultation, writing review. Martin Picard: Conceptualization, initial data analysis, consultation, writing review, funding acquisition for metabolomics. Michael Goodheart: patient acquisition, writing review.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: AKS: Consulting (Merck, Kiyatec, Onxeo, ImmunoGen, GSK, Iylon, AstraZeneca). All other authors declare that there are no conflicts of interest.

Data Availability Statement

The metabolomics data is available here: van Wietmarschen, Herman (2024), "Metabolic effects of Healing Touch during cervical cancer treatment," Mendeley Data, V1, doi: 10.17632/vbp53tz52w.1.

Supplemental Material

Supplemental material for this article is available online.

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