UNIVERSITAT ROVIRA i VIRGILI

IISPV
INSTITUT D'INVESTIGACIÓ SANITÀRIA PERE VIRGILI

METABOLOMIC PROFILING OF BREAST CANCER PATIENTS AFTER TREATMENTS: INSIGHTS INTO THERAPEUTIC RESPONSE

JUAN MANUEL JIMÉNEZ AGUILAR

Bachelor's Degree Final Project

Biochemistry and Molecular Biology

Directed by:

Dr. Anna Hernández

Prof. Jorge Joven

Andrea Jiménez

Tarragona, June 2023

To my grandmother, your strength and your personal battle have fueled my determination to contribute to the fight against cancer.

INDEX

ABBREVIATIONS

ATP: Adenosine triphosphate

AUC: Area under the curve

BC: Breast Cancer

BMI: Body mass index

DCIS: Ductal carcinoma in situ

ECM: Extracellular matrix

EDTA: Ethylenediaminetetraacetic acid

ER: Estrogen receptor

ESTRO: European Society for Radiotherapy and Oncology

HER 2: Human epidermal growth factor receptor 2

IDC: Invasive ductal carcinoma

ILC: Invasive lobular carcinoma

KB: Ketone body

LCIS: Lobular carcinoma in situ

MSTFA: N-Methyl-N-trimethylsilyl-trifluoroacetamide

mtDNA: Mitochondrial deoxyribonucleic acid

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

PLS-DA: Partial Least Squares - Discriminant Analysis

PR: Progesterone receptor

QTA: Adjuvant chemotherapy

QTNA: Neoadjuvant Chemotherapy

ROC: Receiver operating characteristics (ROC) curves

ROS: Radical oxygen species

RT: Radiotherapy

RU: Relative units

SCAD: Short chain acyl-CoA dehydrogenase

SLNB: Sentinel lymph node biopsy

TAG: Triglycerides

TCA: Tricarboxylic Acid cycle

TMCS: Trimethylchlorosilane

TME: Tumor microenvironment

TNBC: Triple-negative breast cancer

TNM: Tumor node metastasis (system)

VIP: Variable importance in projection (VIP) score

ABSTRACT

Background and aims: Breast cancer (BC) is the most frequent type of solid tumor and the leading cause of cancer mortality among women worldwide. One of the key hallmarks of cancerous cells is their altered and reprogrammed energetic metabolism throughout the progression of the disease, which leads to distinct metabolic profiles. This study aims to investigate the actual effects and implications that surgery procedure, chemotherapy, and radiotherapy have on BC patients by analyzing their metabolome, looking for metabolic biomarkers which can describe patient's response to the treatment.

Methods: A cohort of 48 female patients diagnosed with BC at the Hospital Universitari Sant Joan de Reus were recruited along with a group of 50 healthy women, who presented no evidence of carcinogenesis, to serve as a control group for comparison purposes. Plasma samples were collected after each treatment in BC patients. Targeted metabolomics was performed in spite to explore metabolites associated with various metabolic pathways, including carbohydrates, amino acids, and others.

Results: Employing multivariate analysis, we found dysregulated metabolism in BC patients. Carbohydrate, nucleotide, lipid, and amino acid metabolism showed alterations. Post-treatment samples also exhibited altered metabolism. Across the different treatments, a progressive normalization of various metabolites was seen (hypoxanthine, maltose, and 3-phosphoglyceric acid), corresponding to the normalization of nucleotide and carbohydrate metabolism. Furthermore, when classifying BC patients based on their clinicopathological characteristics, distinct metabolic profiles were found at the end of the treatment strategy.

Conclusion: Significant alterations in the metabolome of BC patients were seen when compared to the control group. Moreover, both surgery and radiotherapy exhibited a significant influence in BC patients' metabolome, causing alterations which could be potentially related with eradication of cancer cells. Consequently, a list of metabolites emerged, which holds promise as potential biomarkers for studying and assessing patients' response to treatments. Finally, altered metabolic profiles have been observed with regard to clinicopathological characteristics of patients.

Key words: biomarkers, chemotherapy, disease management, metabolic alterations, radiotherapy, surgery, targeted metabolomics

1. Introduction

1.1. Breast cancer (BC) & Prevalence

Breast cancer (BC) is the most frequent type of solid tumor and the leading cause of cancer mortality among women worldwide [1,2]. In 2020, BC was the most diagnosed cancer worldwide, with an estimated 2.3 million new cases, supposing 11.7% of all types of cancer [3], and it was responsible for almost 685,000 deaths. While BC is a significant global health issue, its impact varies widely by region, as seen in **Figure 1**. Moreover, in developed areas, overall 5-year survival from BC is well over 80%, whereas it is reportedly less than 50% in other less developed regions such as South Africa [1].

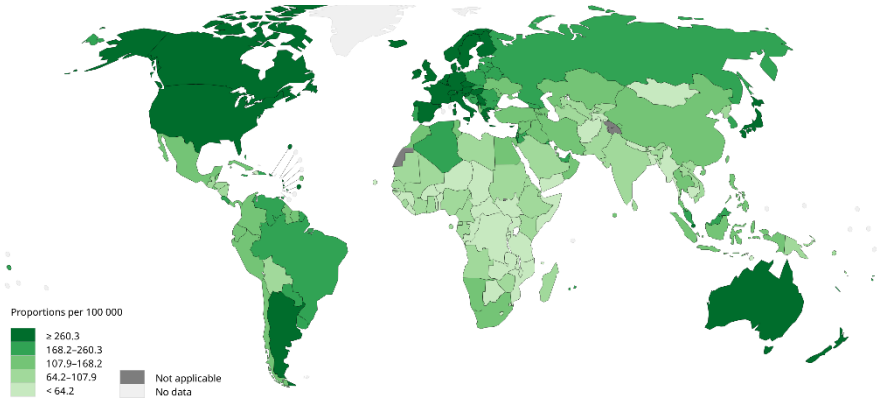

Figure 1. **Estimated number of prevalent cases of BC in women (5-year) as a proportion in 2020, all ages.** Extracted from GCO (2020) [4].

In Spain, there have been reported 34.088 new cases and 6.606 deaths in 2020 [5], and its incidence has been reported to be increased with high body mass index (BMI), late menopause, unbalanced diet, smoking, etc. [6].

Additionally, global cancer incidence is projected to increase by 47% from 2020 to reach 28.4 million cases in 2040 [3]. This being shown, and knowing that the five-year survival rate in metastatic BC is less than 30% [6], it is acknowledged that more investigation into BC biomarkers and treatments should be performed.

1.2. Subtypes of BC tumors and classification

1.2.1. Molecular classification and clinical utility

BC is a heterogeneous disease encompassing various subtypes, characterized by different molecular markers, gene expression profiles, and clinical behaviors. These

subtypes include luminal estrogen receptor (ER) positive (luminal A and luminal B), human epidermal growth factor 2 (HER2) positive, and basal-like (also called triple-negative BC or TNBC) [7, 8], based on the expression of the following hormone receptors: estrogen, progesterone and human epidermal growth factor, as it can be seen in **Table 1**. Different subtypes can be identified with techniques such as immunohistochemistry and gene expression profiling [7].

Table 1. **Characteristics of molecular subtypes of BC.**

	Luminal A	**Luminal B**	**HER2**	**TNBC**
Frequency (%)	50	15	20	15
ER	+	+	+/-	-
PR	+	+/-	+/-	-
HER	-	-	+	-
Ki67 %	Low (<14)	High (>14)	High (>14)	High (>14)
Therapy used	Hormonal	Hormonal/Chemo	Hormonal/Chemo/ Herceptin	Chemo/Experimental

ER: estrogen receptor; PR: progesterone receptor; HER: human epidermal growth factor; TNBC: triple negative breast cancer. Adapted from Orrantia-Borunda, E. *et al*. (2022) [8].

ER and progesterone receptor (PR) are hormone receptors located on the surface of BC cells. When estrogen and progesterone bind to these receptors, they can stimulate the growth and proliferation of BC cells. The use of endocrine agents to downregulate ER signaling is the primary systemic therapy for ER-positive or PR-positive BCs [9] (for example, tamoxifen [7]).

On the other side, HER2 is overexpressed in approximately 20% of BCs [9] and is a robust prognostic biomarker for an aggressive clinical course [7]. These receptors lead to increased overactivation of proto-oncogenic signaling pathways, leading to uncontrolled cancer cell growth and worse clinical outcomes [8]. These subtypes are normally treated with agents which disrupt HER2 signaling (such as Herceptin) [10].

Coupled with classification of molecular subtypes, Ki67 is a nuclear proliferation marker expressed in all phases of the cell except G0 [7]. In general, Ki67 in BC is related to the worst outcomes, as the Ki67 index reflects the aggressiveness of the cancer [8, 11].

Additionally, patients who do not express these biomarkers (triple-negative BC, TNBC), which comprise approximately 15% of all breast tumors [9], may require more aggressive treatment options, such as chemotherapy, as they will not respond to endocrine therapies.

Overall, the status of these biomarkers is an essential factor in determining the prognosis and treatment options for patients with BC.

In the context of this classification, in terms of severity, the National Cancer Institute reports that the 5-year relative survival percentage is a valuable metric. Among the various subtypes of BC, the luminal A subtype exhibited the highest survival rate of 94.8%, followed by the luminal B subtype at 91.0%, the HER2 subtype at 85.6%, and the TNBC subtype at 77.6% [12]. However, it is essential to consider not just the subtype but also the stage of cancer at the time of diagnosis, as this can significantly impact the prognosis and treatment options for the patient. Therefore, a comprehensive assessment of the cancer subtype, stage, and other clinical factors is necessary to determine each patient's most appropriate treatment strategy.

1.2.2. Histological classification

In addition to the previous subdivision, BC tumors can be further categorized based on their anatomical location [9]. Specifically, BC can be ductal carcinomas and lobular carcinomas. Ductal carcinomas are within milk ducts, while lobular carcinomas arise in breast lobes [11]. At the same time, those two subtypes can also be cataloged into invasive and non-invasive carcinomas, depending on if tumoral cells have spread to surrounding breast tissue (invasive ductal/lobular carcinoma) or not (ductal/lobular carcinoma *in situ*), as it can be seen in **Figure 2**.

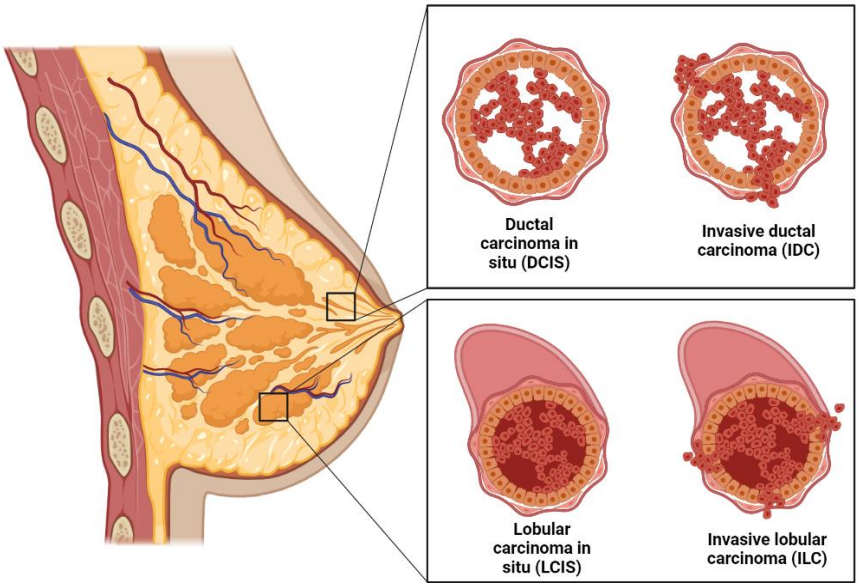

Figure 2. **Representation of different types of BC tumors based on their location and invasiveness.** Adapted from Moccia, C. *et al.* (2021) [13].

1.3. *Metabolic alterations and oxidative stress in cancer*

Altered metabolism is considered a key hallmark of tumorigenesis, as it plays a critical role in regulating essential processes associated with proliferation, migration, and invasion [14]. The remodeling of cellular metabolism gives rise to distinct metabolic phenotypes that hold potential applications in early cancer diagnosis, patient selection for clinical trials, and the identification of treatment response biomarkers [15].

One of the earliest and widely acknowledged metabolic alterations observed in cancer cells is heightened glucose uptake [16]. In 1924, Otto Warburg first described the phenomenon of altered metabolism in cancer cells, which involves the aerobic metabolism of glucose, even in the presence of oxygen [14]. Pyruvate is the end product of glycolysis and is metabolized via oxidative phosphorylation to generate energy. Still, in cancer cells, most of the pyruvate is converted into lactic acid in the presence of oxygen [17], which appears to be an essential mechanism by which cancer cells maintain an appropriate balance of redox cofactors to support biosynthetic functions [15]. This altered metabolism results in an increase in lactate production but, moreover, increased output of glycolytic intermediates that can funnel to other metabolites, such as lipids, proteins, and nucleic acids, which are essential building blocks required for cell growth and division [18], which promotes cancer cell survival and development [19].

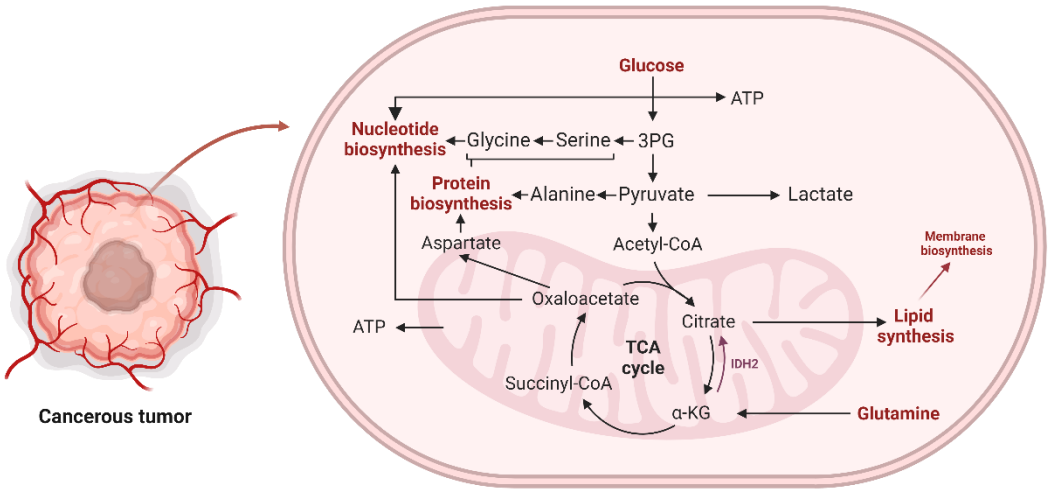

Figure 3. **Metabolic alterations in BC cells.** Adapted from Schmidt, D. R. *et al.* (2021) [15].

In addition to glucose-dependent metabolism, it has been postulated that tumors take advantage of alternative oxidizable substrates, such as glutamine, serine, and fatty acids, suggesting that tumor cells possess a metabolic plasticity that enables them to use various nutrients to support their growth and proliferation [14]. Indeed, fatty acid

profiles and the levels of triacylglycerides (TAGs) seem to change in the tissues and plasma of patients with various cancers. In these cancer cells, the bulk of their lipids are obtained from *de novo* synthesis [20].

Numerous studies have emphasized the crucial role of the glutaminolysis pathway in converting glutamine to glutamate and α-ketoglutarate for entry into the TCA cycle. These enzymatic modifications enable glutamate to activate multiple biochemical pathways that promote tumor development, including protein and nucleic acid synthesis, epigenetic changes, metabolite exchange between the mitochondria and the cytosol, and the stimulation of antioxidant defense mechanisms [16].

Another crucial key factor of cancer metabolism is mitochondria. Mitochondria are a convergence point for glucose, glutamine, and lipid metabolism [19]. Metabolic reprogramming also has an impact on mitochondrial functions in cancer cells. These include altering the production of ATP and NADPH (bioenergetics), converting various nutrients into fundamental building blocks required for cell growth (biosynthesis), as well as alterations in the redox status by radical oxygen species (ROS) production [14]. In fact, this metabolic reprogramming disrupts mitochondrial function, leading to the accumulation of ROS and the subsequent onset of oxidative stress [21, 22].

This oxidative stress stimulates cell proliferation, facilitates cell migration, enhances the production of proangiogenic factors by tumor cells, induces genomic instability, and promotes their metastatic potential [23]. The dysregulated cellular energetics associated with mitochondrial dysfunction can be attributed to different underlying mechanisms, including mtDNA mutations, mitochondrial enzyme defects, and alterations in tumor suppressors [24]. Oncogenes can also alter mitochondria's normal function. A potent and widely known oncogene, c-MYC, regulates the expression of numerous genes involved in mitochondrial biogenesis, thereby upregulating mitochondrial metabolism to drive tumor progression [23].

Another critical point to consider is that BC's development, its metastatic potential, and its response to therapy are influenced by the intrinsic characteristics of cancer cells and their interactions with the surrounding stroma [25]. This dynamic entity, known as the tumor microenvironment (TME), consists of cancerous and non-cancerous cells, including fibroblasts, adipocytes, endothelial cells, and various immune cells such as macrophages, lymphocytes, natural killer cells, soluble factors, and extracellular matrix

(ECM) components. TME plays a significant role in influencing the behavior of cancer cells by providing energy substrates, thereby contributing to the metabolic demand of cancer cells [26].

According to all these metabolic disturbances, previous studies performed by our group found that women with BC had significant alterations in plasma concentrations of metabolites related to glycolysis, TCA cycle, and amino acid metabolism, and that radiotherapy (RT) partially rectified these disturbances [2].

1.4. Treatments used in BC

As previously reported, BC is a complex and heterogeneous disease that requires personalized treatment based on individual patient characteristics and tumor biology. The most common treatments for BC include surgery, radiotherapy, chemotherapy, targeted therapy, and hormone therapy. Depending on the cancer's stage, type, and aggressiveness, these treatments can be used alone or in combination. BC treatment aims to remove or destroy the cancerous cells, prevent the spread of the disease, and improve overall survival and quality of life.

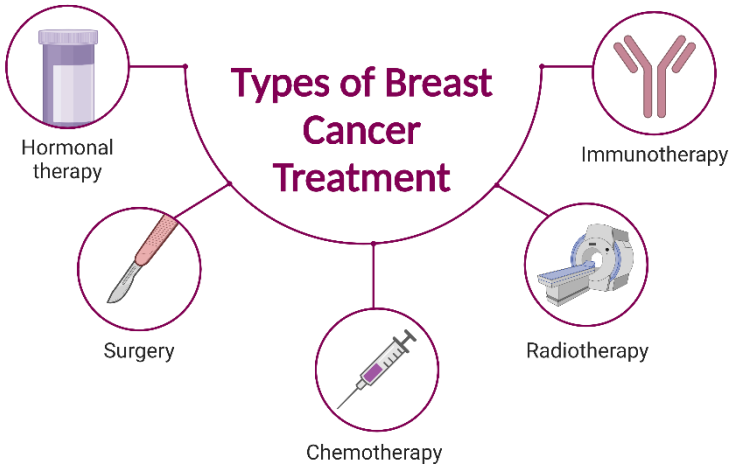

Figure 4. **Types of BC treatments available.**

1.4.1. Tailored treatment strategies

Frequently, in cases where BC is diagnosed in the early stages, surgical resection is considered the primary treatment modality to remove the majority of the lesion. However, surgery may not be suitable as the initial treatment for all patients, even those with early-stage disease. For those cases, **neoadjuvant chemotherapy** (**QTNA**) was

developed to convert inoperable or locally advanced BC into an operable illness [27], and in primarily operable tumors, downstaging results in a modest increase (7% to 12%) in breast conservation rates [28]. Recent studies have reported that QTNA can avoid surgery in up to 25% of patients [29].

After undergoing QTNA or in the case of an operable tumor, as previously stated, the subsequent treatment for almost 90% of diagnosed women is **surgery** [30]. This procedure involves surgical resection of cancer and sampling or removal of axillary lymph nodes, a sentinel lymph node biopsy (SLNB), which will be examined to determine the presence of cancer cells in the lymphatic system [31] to assess the extent of the tumor and the need for future treatments such as postoperative radiation [9].

The selection of the appropriate surgery type depends on the tumor's characteristics. Mastectomy, involving the complete removal of the breast tissue, is recommended for larger multicentric tumors, a history of chest wall radiation, or during pregnancy when radiation is contraindicated. On the other hand, lumpectomy involves the removal of the tumor's surrounding area and is restricted to smaller tumors, with mandatory whole breast radiation [30, 32].

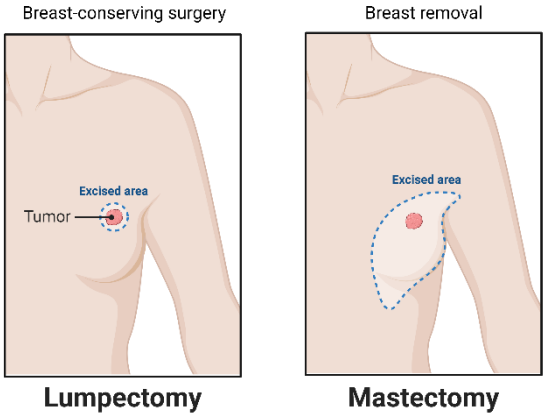

Figure 4. **Subtypes of surgery performed on BC patients.**

Following surgery, patients may be considered for **adjuvant chemotherapy** (**QTA**). The origins of QTA can be traced back to the early 1980s, when it was introduced as a treatment modality to address the issue of distant relapse, a leading cause of mortality for many patients with early-stage BC [33]. As previously noted, BC cells can migrate from the tumor site and settle in other tissues, forming micrometastases. To mitigate the risk of relapse, metastasis, and mortality from BC, oncologists evaluate factors such as tumor size and hormone receptor status to determine which patients are suitable candidates for QTA [34].

Finally, BC treatment ends with **radiotherapy** (**RT**), which is an integral part of the multidisciplinary management of BC [35]. RT is a local treatment to ensure that all cancerous cells remain destroyed, minimizing the possibility of BC recurrence [36]. RT

planning is guided by disease stage, risk of recurrence, the correct definition of the target volumes, and treatment objectives. Currently, there are guidelines endorsed by the European Society for Radiotherapy and Oncology (ESTRO) for target volume delineation for BC and elective nodal volumes, as this, along with meticulous radiation planning, total dose and fractionation, dose homogeneity, and organs at risk doses are significant for reducing radiation-induced toxicity [37].

Recently, there has been increasing interest in administering preoperative radiotherapy, which can reduce the stage of tumors, improve the accuracy of targeting cancer, and minimize soft tissue toxicity through smaller treatment volumes [38].

Additional treatments commonly used include **endocrine therapy** and **immunotherapy**. Endocrine therapy is used to slow or stop the growth of hormone-sensitive tumors by blocking the body's ability to produce hormones or interfering with the effects of hormones on cancerous cells [39]. In contrast, cancer immunotherapy represents one of the most significant advances in oncology in recent years. The most famous example of this kind of treatment is Trastuzumab, a monoclonal antibody that binds to an extracellular domain of this receptor and inhibits HER2 homodimerization, thereby preventing HER2-mediated signaling [40].

To conclude this section, BC treatment is a pathological process that requires a thorough assessment of multiple factors, such as disease stage, patient health status, and tumor characteristics. The variety of available treatments allows for a tailored approach to each patient's needs. Ongoing research and development in this field hold promise for further improving the effectiveness and outcomes of BC treatment.

1.5. Targeted Metabolomics & Cancer

Recently, there has been growing interest in understanding the relationship between environmental factors, metabolic small molecules, host genes, and diseases, with metabolism emerging as a critical player in this complex interplay [41].

In cancer cells, metabolism is dysregulated to support the demands of uncontrolled proliferation. This rewiring of cellular metabolism leads to characteristic metabolic phenotypes that can be used for earlier cancer diagnosis, patient selection strategies for clinical trials, and/or as biomarkers of treatment response [15].

In this context, metabolomics is a valuable tool for studying the metabolomic profile of patients submitted to different conditions at a given time, such as therapies [41]. This powerful technique involves the comparative study of metabolite biomolecules, such as carbohydrates, lipids, amino acids, and nucleic acids, that are present in biological samples [17].

Unlike untargeted metabolomics, whose objective is to comprehensively analyze the entire metabolome of the patient, targeted metabolomics, which was used in this project, focuses on a selected group of metabolites of interest previously determined.

1.6. Hypothesis and objectives

Currently, several studies are focused on identifying metabolic biomarkers that can effectively differentiate between BC patients and healthy individuals. However, literature dedicated to examining the metabolic response to BC treatments is remarkably scarce. In light of this circumstance, **we think that differences found in the metabolomic profile of BC patients can be useful to evaluate the effect of the different treatments applied**.

The objectives proposed to test this hypothesis are:

- To determine the differences in the metabolome of BC patients when compared with a control group.
- To identify differentially expressed metabolites after the different treatments, which could be related to patients' progression.
- To investigate if there are differences in treatment response based on the physiopathological characteristics of the patients.

2. Materials and methods

2.1. Participants of the study

The study enrolled 77 female patients diagnosed with BC at the Hospital Universitari Sant Joan de Reus, between September 2020 and October 2021. For comparison purposes, samples from 50 healthy women with no evidence of carcinogenesis were also collected to serve as a control group matched for age and sex. To ensure the study's validity, specific inclusion and exclusion criteria were established. Participants who met the following criteria were included in the study: women ≥ 18 years old with invasive BC. However, patients with a history of cancer, Paget's nipple disease, vascular collagen disease, systemic lupus erythematosus and/or scleroderma, pregnancy or lactation at the time of inclusion, psychiatric disorders, patients with both mamas affected or with 2 or more lesions or any other medical condition were excluded from the study. After applying these criteria, the study cohort was refined to a final analysis of 48 patients, carefully selected from the initial pool of 77 participants.

Participants were divided into 2 modalities of treatment (**Figure 5**); one group was submitted to neoadjuvant chemotherapy, surgery, and radiotherapy, and the other group passed by surgery, adjuvant chemotherapy, and radiotherapy.

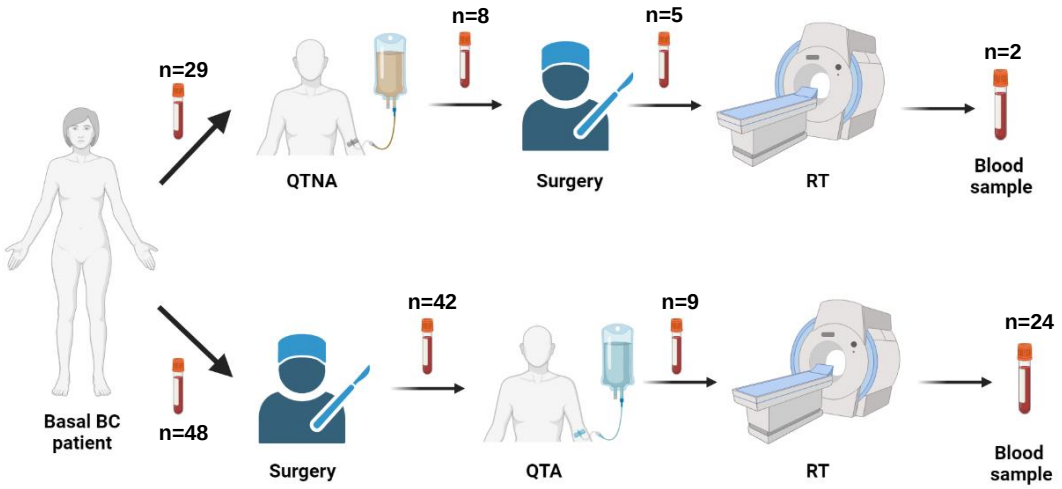

Figure 5. **Modalities of treatments by which patients were divided.** n = number of samples. QTNA: neoadjuvant chemotherapy; RT: radiotherapy; QTA: adjuvant chemotherapy.

All participants signed a written informed consent according to the Helsinki declaration. The study was approved by Ethics Committee of the Hospital Universitari Sant Joan de Reus.

2.2. *Biological samples collection & preparation*

At the time of diagnosis, two blood samples were collected from each participant, each tube containing 10ml of blood. One sample was collected in a tube without anticoagulant, and the other was collected in a tube containing EDTA. Samples were then processed using centrifugation (2.500xg, 15min, 4ºC) to separate serum and plasma aliquots. These aliquots were stored at -80ºC until further analysis. The same sample processing protocol was performed on the control group.

For chromatograph analysis, samples (50 µL plasma) were aliquoted to a 1.5 ml Eppendorf tube and mixed with 200 µL of methanol:water (8:2, v/v) containing internal standards. Samples were vortexed and centrifuged for 5 minutes at 15000 rpm and 4ºC. Supernatants (200 µL) were transferred to a new tube and evaporated in a SpeedVac at 45 ºC. Samples were reconstituted with 30 µL of methoxyamine and incubated for 90 min at 37ºC, after were silylated with 45 µL of MSTFA + 1 % TMCS at room temperature for 60 min.

To maintain anonymity, all samples were identified using a unique numerical identifier assigned during the anonymization process.

2.3. *Biological samples analyses*

2.3.1. *Targeted metabolomics*

Targeted metabolomics was employed to assess plasma concentrations of 74 energy balance-associated metabolites. The analyzed metabolic classes included carbohydrates (fructose and mannose, galactose, nucleotide sugar, TCA, sucrose, among others), amino acids (including alanine, aspartate, arginine, proline glycine, serine, tyrosine, etc.), lipids (comprising glycolipids and primary bile acid biosynthesis), cofactors and vitamin metabolism, nucleotides (both purine and pyrimidine), xenobiotic biodegradation (specifically benzoate degradation), and energy metabolism (oxidative phosphorylation).

The methodology utilized a 7890A gas chromatograph coupled to a 7200-quadruple time-of-flight mass spectrometer with an electron impact source (Agilent Technologies, Santa Clara, CA, USA). The system was equipped with a 7693 autosampler module and a J&W Scientific HP-5MS column (30ms 0.25 mm, 0.25µm) (Agilent Technologies, Santa

Clara, CA, USA). The calibration curve was established using standard concentrations plotted against the peak area. All the data obtained was in relative units (RU).

2.4. Statistical analyses

2.4.1. Standard statistics

Comparisons between two independent groups, assumed to exhibit a non-parametric distribution, were conducted using the Mann-Whitney test, and descriptive variables were evaluated with the Chi-squared test. Statistically significant differences were determined at a significance level of $p < 0.05$. Quantitative variables were reported as median (interquartile range), while qualitative variables were presented as frequency (percentage).

2.4.2. Graphic representations

PLS-DA (Partial Least Squares - Discriminant Analysis) allowed for analyzing data with multiple predictor variables and categorical outcome variables. It aimed to maximize the data separation between groups or classes by finding linear combinations of the most predictive variables of the outcome. They were made with MetaboAnalyst 5.0 (www.metaboanalyst.ca).

MetaboAnalyst was also used to generate VIP scores, Volcano plots and ROC curves. Data was normalized by log transformation. Variable importance projection (VIP) score was used to find the variables with the most discriminative capacity. Volcano plots were generated to visualize significantly differentially expressed metabolites by visualizing the relationship between fold change and statistical significance. We conducted receiver operating characteristic (ROC) curve analysis to test potential biomarkers by integrating multiple significant variables.

Bubble plots were generated to determine the concentration changes between metabolites based on their species. For this purpose, RStudio 4.2.2. was used. Package managed were ggplot2, deplyr, and ggrepel. All packages were used at the most recent version available at CRAN (cran.r-project.org) on May 19th, 2023.

Ultimately, GraphPad Prism 9.4.1 (GraphPad Software, San Diego, CA, USA) was used to generate boxplots, enabling assessment of the distribution and asymmetry of the significant metabolites.

3. Results

Firstly, metabolomic profiles of basal patients (who were diagnosed with BC but hadn't undergone any treatment) from both pre-surgery and pre-QTNA subgroups were compared, and they showed no significant difference. Considering this fact and having an insufficient number of samples (*n*) which would not allow a correct interpretation of the results, pre-QTNA patients were excluded from this study, and all results were performed with pre-surgery patients; basal patients as will be referred from now on. The metabolomic comparison of the groups is shown in **Table S1**.

3.1. Characterization of the BC patients

The principal clinical characteristics of BC patients observed in this study can be found in **Table 2**. The major part of the participants presented BC after menopausal state. It can also be marked that they displayed overweight, and some of them also showed comorbidities related to this state, such as arterial hypertension and dyslipidemia. Also, more than half of the subjects had oncologic antecedents in their families. In most cases, patients with BC exhibited a lack of affected lymph nodes or metastases. The prevailing pathological diagnosis was histological grade II ductal carcinoma, while the most commonly observed tumor subtype was luminal B.

Table 2. Characteristics of BC patients.

	BC patients (n=48)
Clinical characteristics	
Age at diagnosis (years)	63.5 (49.0-68.3)
Body mass index (kg/m^2)	27.4 (23.9-32.2)
Smoking, n (%)	8 (16.7)
Alcohol consumption (>20g/day), n (%)	6 (12.5)
Type 2 diabetes *mellitus*, n (%)	7 (14.6)
Arterial hypertension, n (%)	15 (31.2)
Dyslipidemia, n (%)	11 (22.9)
Chronic obstructive pulmonary disease, n (%)	7 (14.6)
Ischemic heart disease, n (%)	5 (10.4)
Family oncologic antecedents, n (%)	27 (56.2)
Intake of oral contraceptives, n (%)	13 (27.1)
Motherhood, n (%)	39 (81.3)
Menopause state, n (%)	
Premenopausal	12 (25.0)
Postmenopausal	36 (75.0)
Cancer related characteristics	
Tumor size (TNM system), n (%)	
T0	-

T1	27.0 (56.3)
T2	17 (35.4)
T3	2 (4.2)
T4	-
Nodes (TNM system), n (%)	
N0	29 (60.4)
N1	12 (25.0)
N2	4 (8.3)
N3	1 (2.1)
Metastases (TNM system), n (%)	
M0	44 (91.7)
M1	1 (2.1)
M2	1 (2.1)
Pathological anatomy of the tumor, n (%)	
Ductal carcinoma	36 (75.0)
Lobular carcinoma	7 (14.6)
Others	4 (8.3)
Histological grade, n (%)	
I	12 (25.0)
II	31 (64.6)
III	4 (8.3)
Positive estrogen receptors	97.5 (90.0 - 100.0)
Positive progesterone receptors	70.0 (7.5-90.0)
Positive HER2 in tumor biopsy, n (%)	2 (4.2)
Ki67 antigen in tumor biopsy, n (%)	
Less than 15%	15 (31.3)
15-50%	28 (58.3)
More than 50%	5 (10.4)
Tumor molecular classification, n (%)	
Luminal A	16 (33.3)
Luminal B	26 (54.2)
Triple negative	3 (6.3)
HER2 positive	2 (4.2)

We reported values as either n (percentage) or median (interquartile range). TNM: tumor node metastasis (system); HER2: human epidermal growth factor receptor 2.

3.2. *Circulatory alterations in BC patients*

Partial Least Squares Discriminant Analysis (PLS-DA) displayed significant distinctions between BC patients and controls, based on their metabolic profile (**Figure 6A**). VIP score analysis was performed to identify the most predictable and statistically significant metabolites: hypoxanthine, maltose, 3-phosphoglyceric acid, xylonic acid, ethylmalonic acid, and glycerol, which showed a score higher than 2 (**Figure 6B**). Boxplots were then made to see how these metabolites' concentrations vary. It was observed that hypoxanthine, maltose, and 3-phosphoglyceric acid showed increased levels in BC patients. In contrast, xylonic acid, ethylmalonic acid, and glycerol were decreased in this

group (**Figure 6C** and **Table S2**). To determine whether those metabolites were powerful predictive variables that could be used to discriminate between BC patients and controls, a ROC curve was generated (AUC = 1) (**Figure 6D**).

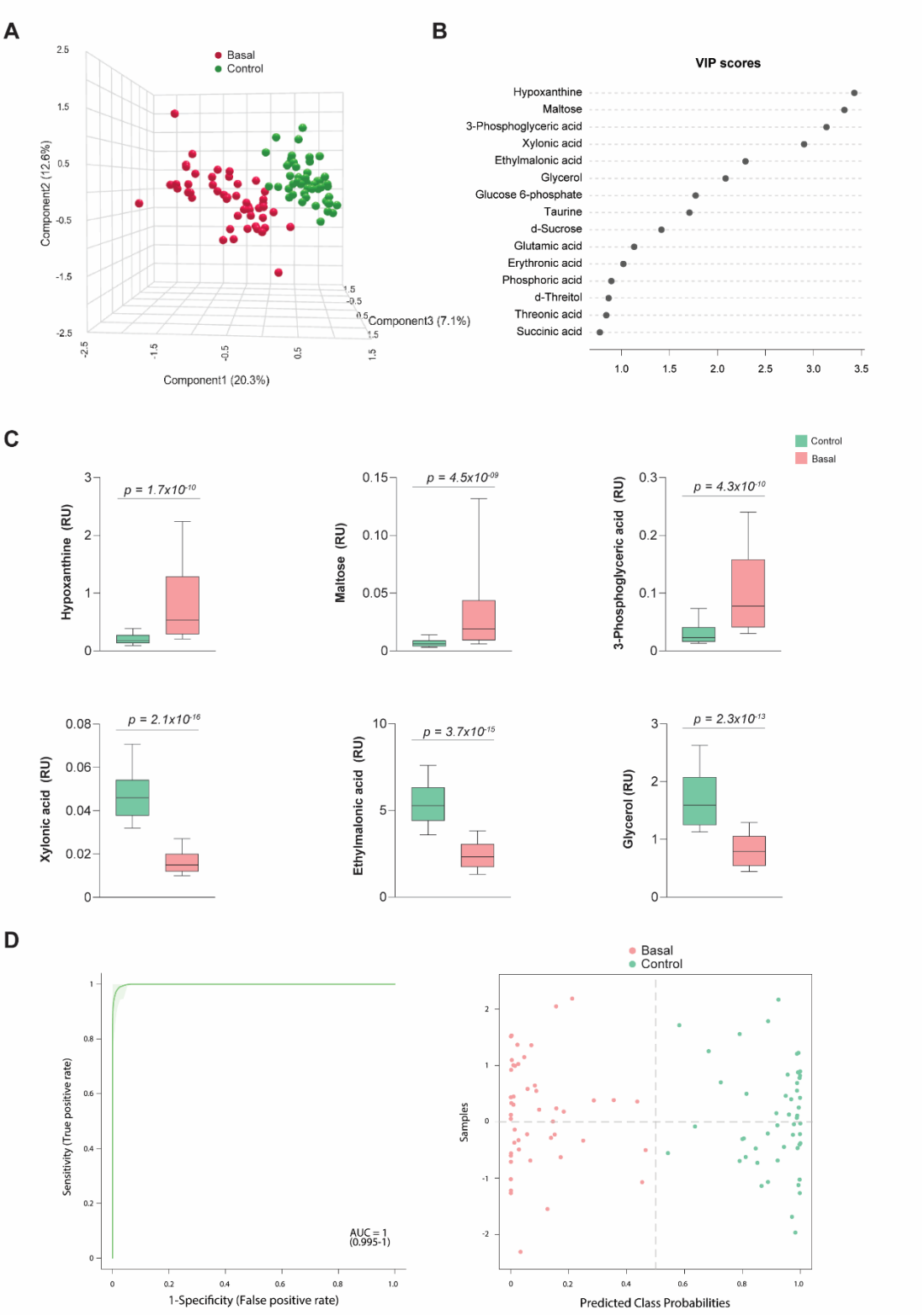

Figure 6. **Identification of overall variations and discriminant biomarkers in BC patients and controls based on metabolomic profiles**. (A) Partial Least Squares Discriminant Analysis (PLS-DA), (B) Variable Importance in Projection (VIP) score of statically significant variables, (C) boxplot for most significant metabolomic variables, (D) Receiver Operating Characteristic (ROC) curve and complexity matrix of hypoxanthine, maltose, 3-phosphoglyceric acid, xylonic acid, ethylmalonic acid, and glycerol. The significance was determined by the Mann-Whitney U-test, $p < 0.05$.

3.3. *Circulatory alterations in BC patients after undergoing surgical intervention*

To elucidate the variations in plasma metabolites among BC patients and assess the impact of surgery, a PLS-DA was conducted on basal and post-surgery samples based

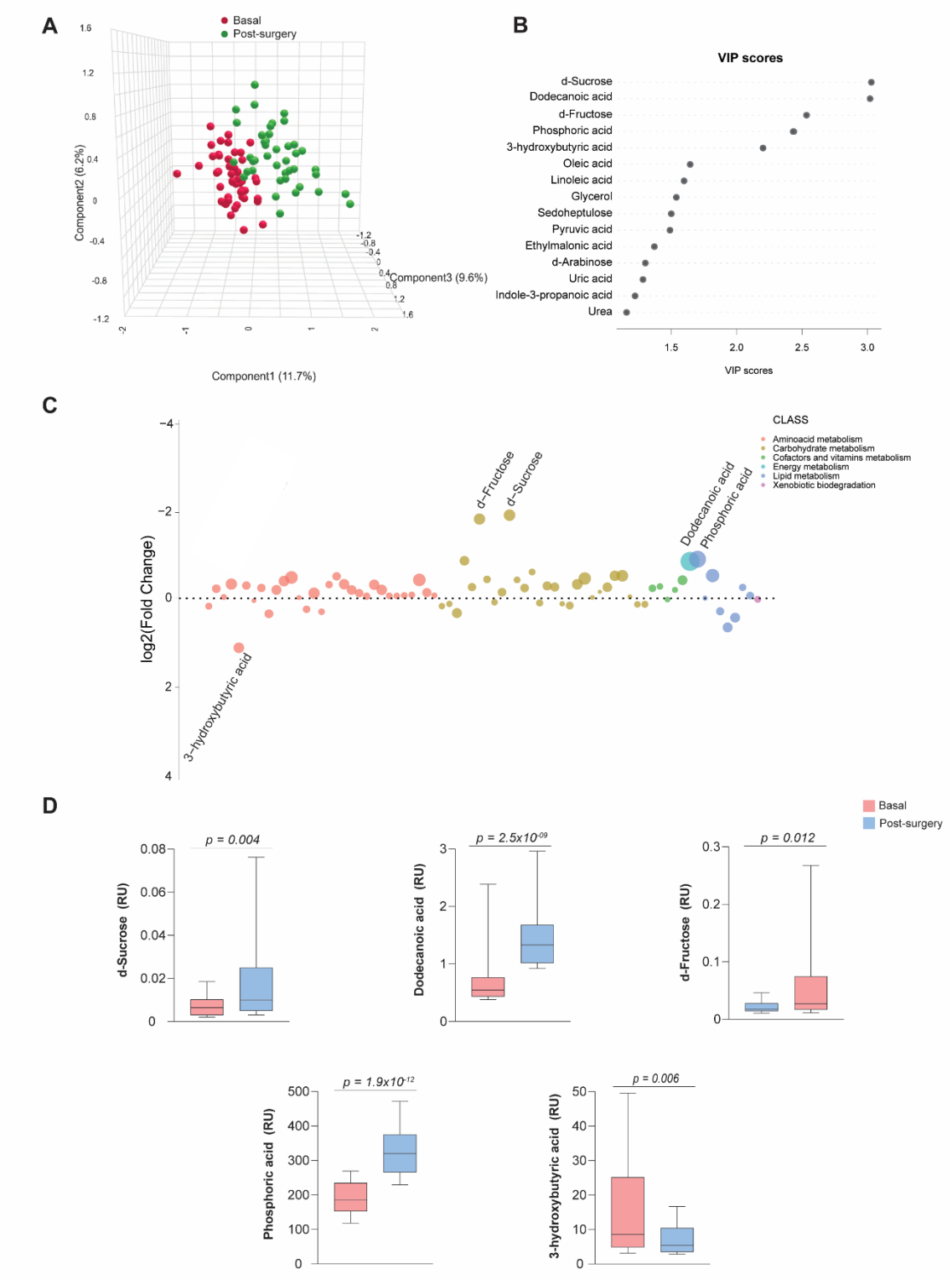

Figure 7. **Identification of overall variations and discriminant biomarkers in basal and post-surgery state based on metabolomic profiles**. (A) Partial Least Squares Discriminant Analysis (PLS-DA), (B) Variable Importance in Projection (VIP) score of statically significant variables, (C) bubble plot with all analyzed metabolites categorized according to their principal pathways and most significant ones highlighted and annotated, (D) boxplot for most significant metabolomic variables. The significance was determined by the Mann-Whitney U-test, $p < 0.05$.

on their metabolomic profiles, and it revealed notable disparities between the groups (**Figure 7A**). VIP score analysis was carried out to exhibit the most predictable and statistically significant metabolites; d-sucrose, dodecanoic acid, d-fructose, phosphoric acid, and 3-hydroxybutyric acid (**Figure 7B**).

Moreover, a bubble plot was generated, representing the expression of all the analyzed metabolites categorized according to their principal pathways (**Figure 7C**). It's important to note that in the bubble plot, a wider bubble size indicates a smaller p-value, signifying higher statistical significance. Conversely, the fold change denotes the degree of differentiation, where a greater deviation from zero signifies a larger difference. In this case, all metabolic pathways seemed to be increased after surgery when comparing them to the basal state of our patients.

From this analysis, it was observed that d-sucrose, dodecanoic acid, d-fructose and phosphoric acid showed increased levels in post-surgery samples. At the same time, 3-hydroxybutyric was decreased in this group (**Figure 7D** and **Table S3**). A ROC curve was made to test the distinction power of those metabolites (AUC = 0.966) (**Figure S1**).

3.4. Circulatory alterations in BC patients after undergoing chemotherapy

To reveal the alterations in plasma metabolites among BC patients and evaluate the impact of chemotherapy, a comprehensive analysis was conducted comparing basal and post-QTA samples. Statistically significant divergence between the groups was seen (**Figure 8A**), and dodecanoic acid, d-fructose, pyruvic acid, and phosphoric acid were the most discriminative metabolites (**Figure 8B**).

Carbohydrate, lipidic, and energy metabolism showed significant alterations in BC patients after undergoing chemotherapy (**Figure 8C**). Within this analytical framework, boxplots were created with the most predictive metabolites: dodecanoic acid, d-fructose, pyruvic acid, and phosphoric acid, which displayed elevated concentration in post-QTA samples (**Figure 8D** and **Table S4**).

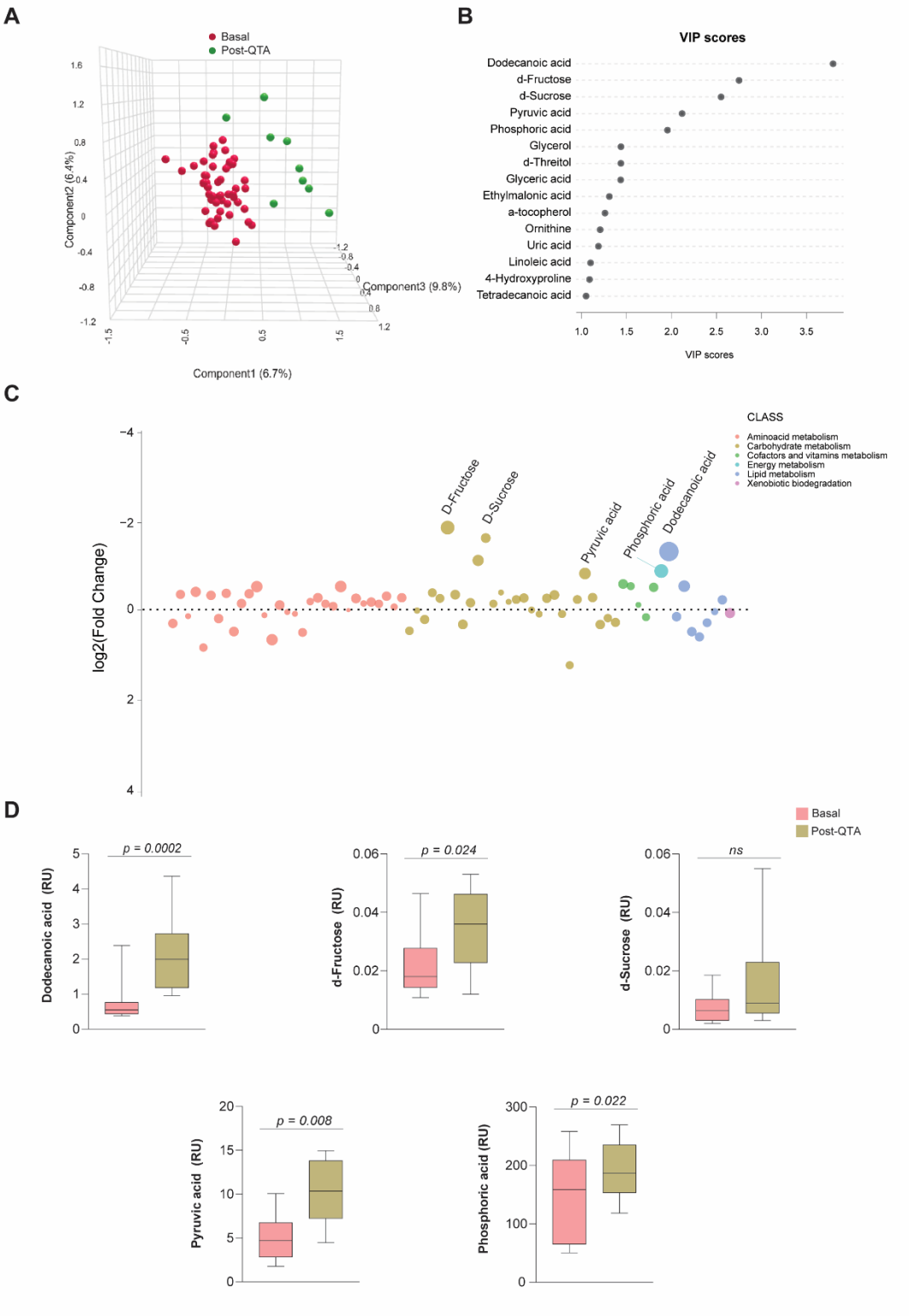

Figure 8. **Identification of overall variations and discriminant biomarkers in basal and post-QTA state based on metabolomic profiles**. (A) Partial Least Squares Discriminant Analysis (PLS-DA), (B) Variable Importance in Projection (VIP) score of statically significant variables, (C) bubble plot with all analyzed metabolites categorized according to their principal pathways and most significant ones highlighted and annotated, (D) boxplot for most significant metabolomic variables. The significance was determined by the Mann-Whitney U-test, $p < 0.05$. QTA: adjuvant chemotherapy.

3.5. *Circulatory alterations in BC patients after undergoing radiotherapy*

Firstly, a PLS-DA was generated with basal and post-radiotherapy samples based on their metabolomic profiles, and it revealed remarkable differences between the groups

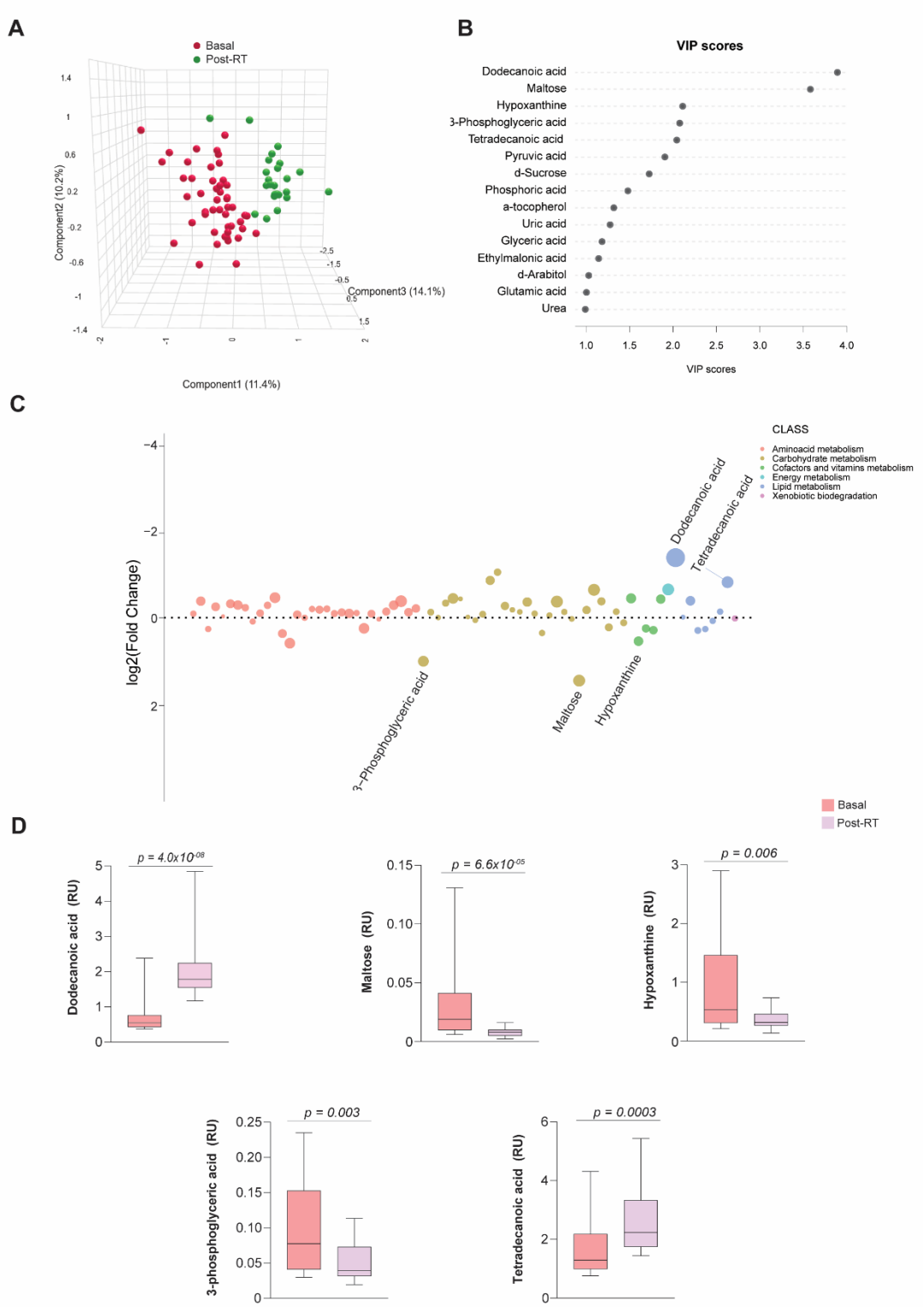

Figure 9. **Identification of overall variations and discriminant biomarkers in basal and post-RT state based on metabolomic profiles**. (A) Partial Least Squares Discriminant Analysis (PLS-DA), (B) Variable Importance in Projection (VIP) score of statically significant variables, (C) bubble plot with all analyzed metabolites categorized according to their principal pathways and most significant ones highlighted and annotated, (D) boxplot for most significant metabolomic variables. The significance was determined by the Mann-Whitney U-test, $p < 0.05$. RT: radiotherapy.

(**Figure 9A**). VIP score analysis was undertaken to display the most predictable and statistically significant metabolites (**Figure 9B**). Furthermore, a bubble plot was generated, representing the expression of all analyzed metabolites categorized according to their principal pathways. Alterations in carbohydrate, lipid, and cofactors and vitamins metabolism were seen (**Figure 9C**).

From this analysis, it was observed that dodecanoic acid and tetradecanoic acid showed increased levels in post-RT samples, while maltose, hypoxanthine, and 3-phosphoglyceric acid were decreased in this group (**Figure 9D** and **Table S5**).

3.6. Circulatory alterations in BC patients between surgery and radiotherapy

In addition to the comparisons between basal and post-treatment samples, which display the cumulative metabolic response of patients to the complete treatment strategy, individual comparisons were performed to evaluate the specific effect of each therapy. For this purpose, post-surgery and post-QTA were compared, but no significant differences were found. Then, post-surgery and post-RT samples were evaluated. In this case, evident disparities could be seen among both groups (**Figure 10A**).

Following that, VIP score analysis was conducted to show the metabolites that exhibit the highest predictability and statistical significance (**Figure 10B**). Moreover, alterations in amino acid, carbohydrate, and cofactors and vitamins metabolism were observed (**Figure 10C**).

Based on these findings, it was noted that 3-hydroxybutyric acid exhibited elevated levels in post-RT samples, whereas maltose, d-fructose, 3-phosphoglyceric acid, and hypoxanthine demonstrated decreased levels in this group (**Figure 10D** and **Table S6**). Finally, a ROC curve was made to show radiotherapy's individual discriminatory power and its impact on BC patients (AUC = 0.855) (**Figure S2**).

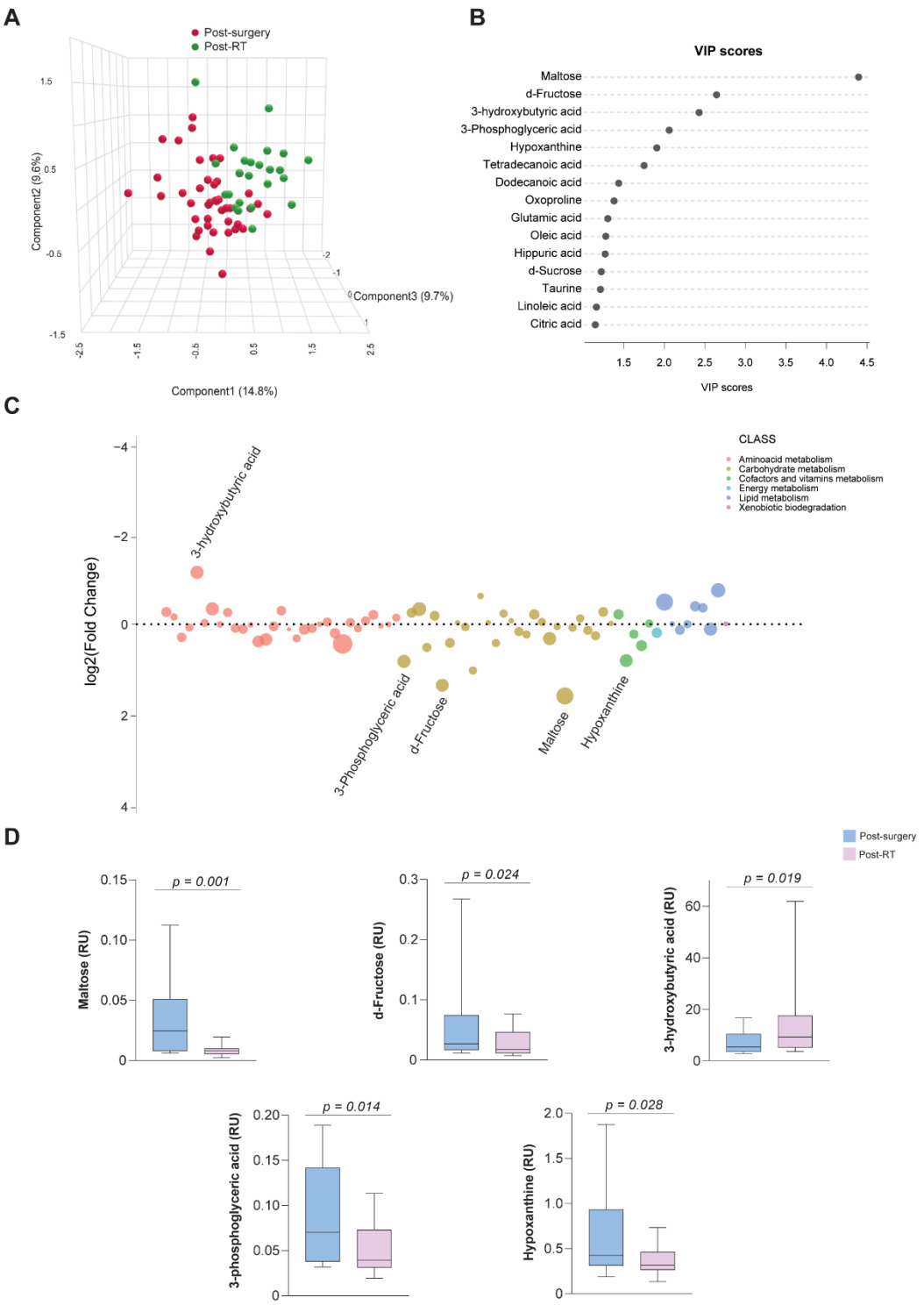

Figure 10. **Identification of overall variations and discriminant biomarkers in post-surgery and post-RT state based on metabolomic profiles**. (A) Partial Least Squares Discriminant Analysis (PLS-DA), (B) Variable Importance in Projection (VIP) score of statically significant variables, (C) bubble plot with all analyzed metabolites categorized according to their principal pathways and most significant ones highlighted and annotated, (D) boxplot for most significant metabolomic variables. The significance was determined by the Mann-Whitney U-test, $p < 0.05$. RT: radiotherapy.

3.7. *Significant treatment-induced changes in BC patients*

Hypoxanthine, maltose, and 3-phosphoglyceric acid were identified as exhibiting not only a high discriminatory power between the control basal groups, but also statistically significant differences between some of the treatments to which patients had been subjected (**Figure 11**). In general, an increase in the concentration of these metabolites was observed at the moment of the diagnosis. As they progressed with treatments, the concentration tended to normalize, resembling the individuals in the control group. In addition, a reduction in the dispersion of the levels of these metabolites was observed as treatment progressed, indicating less variability between patients.

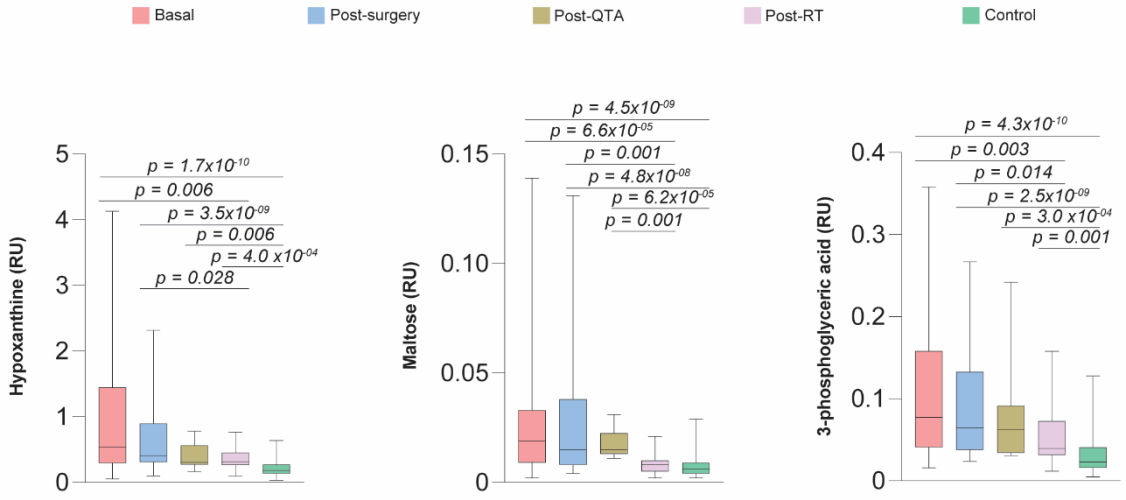

Figure 11. **Progression of specific metabolite levels with treatment strategy**. (A) boxplot for metabolomic variables. The significance was determined by the Mann-Whitney U-test, p< 0.05. QTA: adjuvant chemotherapy; RT: radiotherapy.

3.8. *Circulatory alterations associated with clinicopathological characteristics of BC patients*

We also aimed to investigate differences in the overall treatment response among BC patients by grouping them based on their clinicopathological characteristics. Firstly, molecular subtype was used, and this analysis showed that while phosphoric acid, ethylamonic acid, sedoheptulose, α-tocopherol, pyruvic acid, and tetradecanoic acid were decreased in the luminal A group after treatment, glyceric acid and 3-phosphoglyceric acid could be seen increased in luminal B group (**Figure 12**). Moreover, carbohydrate and lipid metabolism were displayed to be decreased in luminal A patients compared to luminal B patients (fumaric acid, glycerol-1-phosphate, and malic acid). On

the other side, both groups had increased maltose levels and decreased dodecanoic acid levels at the end of the treatment.

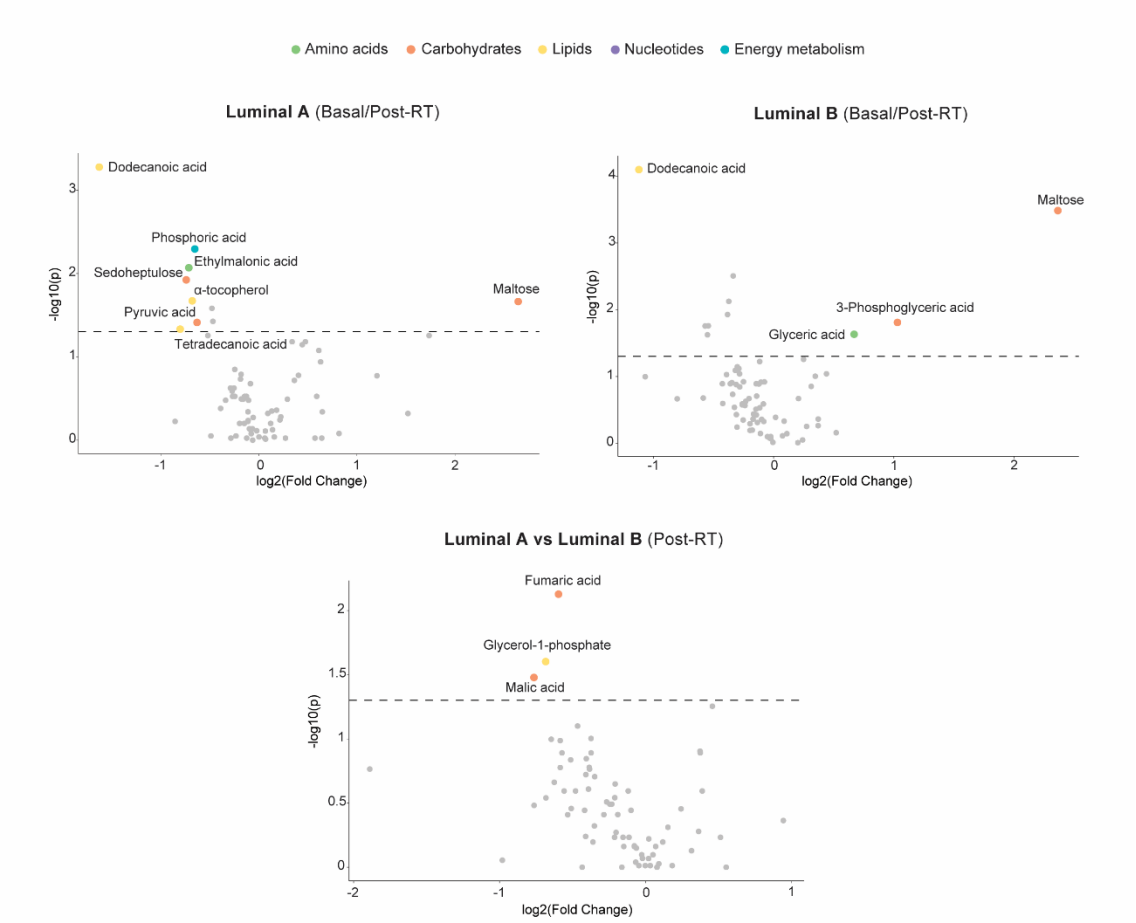

Figure 12. **Molecular subtype of tumor was associated with metabolic alterations**. Mean log2 for energy metabolism was represented. Significative variables were colored depending on the class. Significance was determined by the Mann-Whitney U-test, $p < 0.05$. RT: radiotherapy.

HER2+ and TNBC patients had to be excluded from this comparison due to the lack of samples, which did not allow a proper evaluation.

In addition, with regard to histological grade, similarities were seen between grade I and luminal A patients as well as grade II and luminal B patients when comparing metabolomic profiles obtained. Ethylamonic acid, α-tocopherol, sedoheptulose, tetradecanoic acid, and pyruvic acid were shown to be decreased in grade I group, whereas glyceric acid and phosphoglyceric acid were increased in grade II group, were d-arbitol showed decreased concentration (**Figure 13**). Again, both groups had increased maltose levels and decreased dodecanoic acid levels at the end of the treatment strategy.

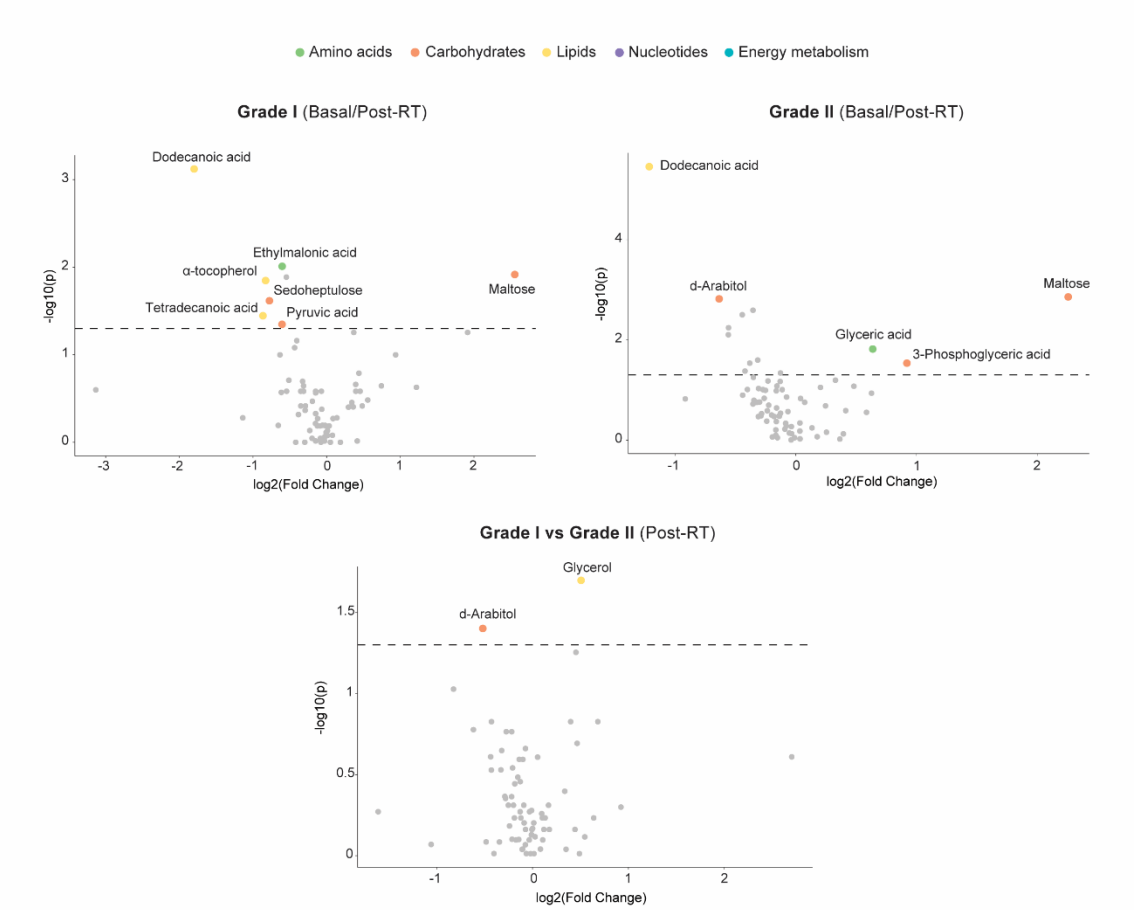

Figure 13. **Histological grade of tumor was associated with metabolic alterations**. Mean log2 for energy metabolism was represented. Significative variables were colored depending on the class. Significance was determined by the Mann-Whitney U-test, $p < 0.05$. RT: radiotherapy.

Grade III patients were excluded from this analysis due to the insufficient availability of samples, which precluded a comprehensive evaluation.

Finally, the menopausal state was used to look for differences between pre- and post-menopausal patients. Nucleotide metabolism was increased in premenopausal state after treatments, as hypoxanthine and uracil showed increased levels. Furthermore, sedoheptulose was decreased in premenopausal patients, whereas d-sucrose and tetradecanoic acid displayed diminished levels in postmenopausal women. Additionally, maltose showed an increased presence in this group. Both groups showed reduced levels of dodecanoic acid, pyruvic acid, and phosphoric acid post-RT (**Figure 14**). Moreover, carbohydrate metabolism decreased in premenopausal state (threonic acid and α-ketoglutaric acid).

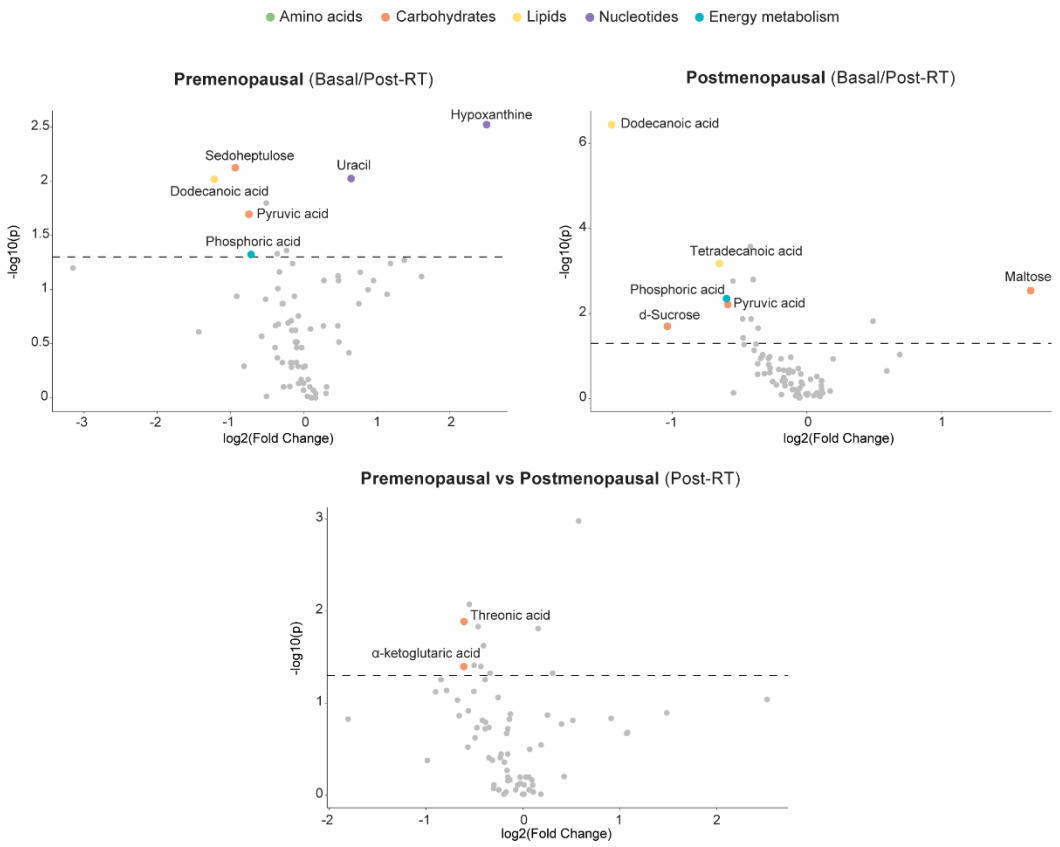

Figure 14. **Menopausal state of patients was associated with metabolic alterations**. Mean log2 for energy metabolism was represented. Significative variables were colored depending on the class. Significance was determined by the Mann-Whitney U-test, $p < 0.05$. RT: radiotherapy.

Other studies with clinicopathological characteristics of patients wanted to be done such as pathological anatomy of the tumor (ductal or lobular) but more samples are needed to perform them in order to allow a proper analysis.

4. Discussion

Metabolomics has emerged as a significant scientific breakthrough exploring novel biomarkers across various diseases. This technique has simplified the discovery of biomarkers that enable early diagnosis of disease, aid in personalized patient treatment, and provide valuable insights into patients' treatment responses.

Within the current context, where the understanding of the metabolic response of BC patients to treatments remains incomplete, this study aims to investigate potential alterations in the metabolic profiles of patients undergoing different therapies (surgery, chemotherapy, and radiotherapy). By comprehensively characterizing the metabolic changes associated with various therapeutic interventions, this research contributes to the understanding of treatment efficacy. It enables the development of personalized approaches for BC management.

In this study, we compared BC patients in a basal state (at the time of the diagnosis) with our control group. This comparison aimed to provide insights into our patient's metabolomic profiles and uncover differences in contrast to a healthy reference group, establishing the baseline metabolic status of BC patients for a better understanding of the impact of various treatments on their metabolic profiles.

As previously explained, metabolic reprogramming is one of the main characteristics of cancer. Among the metabolic pathways involved, alterations in carbohydrate metabolism have been identified. It needs to be considered that patients in this study had overweight (BMI = 27.4 kg/m^2), and other studies showed a direct correlation between overweight, hyperglycemia, increased risk of cancer [42, 43], and poorer prognosis when talking about BC [44]. Our study revealed elevated glycolytic intermediate 3-phosphoglyceric acid concentrations, which can be attributed to increased glycolysis in cancer cells. Notably, this metabolite is crucial in serine synthesis [45]. It is also necessary to produce sphingolipids and headgroups of phospholipids [46]. Moreover, our analysis revealed that maltose was increased while xylonic acid was decreased compared to the control group, which has yet to be described by the literature.

Lipidic metabolism is also reprogrammed in cancer to meet increased energy and macromolecule requirements. Among the most significant metabolites, glycerol was decreased, which can be explained by its use by cancerous cells to enter the glycolytic

pathway to obtain energy [47]. It is also used for the synthesis of TAGs, also recurrent in cancer cells for membrane synthesis [48].

Moving to ethylmalonic acid, it is formed during the breakdown of valine and is not usually associated with cancer. However, as shown in our results, other studies found low concentrations of ethylmalonic acid in BC patients [49], but the reason has not been described yet. This acid has been reported to affect the activity of short-chain acyl-CoA dehydrogenase (SCAD) in the β-oxidation of fatty acids, leading to a decrease in this pathway [50]. A low concentration of this acid may support increased fatty acid oxidation in cancer cells, which is crucial for their growth. However, further investigation is needed to understand the mechanisms underlying these low acid levels.

Finally, the most crucial discriminant metabolite showed by VIP score analysis was hypoxanthine, which is related to purine metabolism deregulation. Purines are catabolized by tumoral cells to obtain energy, and hypoxanthine is one of the subproducts of purine nucleotide degradation [51]. It supports abnormal tumoral cell proliferation and was previously found to increase in BC patients in other studies [52, 53]. Cancer cells can also use it to produce new purine nucleotides [54].

With these six metabolites: hypoxanthine, maltose, 3-phosphoglyceric acid, xylonic acid, ethylmalonic acid, and glycerol, we constructed a ROC curve with an AUC of 1, resulting in a novel biomarker signature with absolute discriminant capacity to diagnose BC using plasma samples.

Once observed our patients' metabolic alterations, we aimed to analyze their response to the different treatments. Firstly, we started with surgery: although specific literature on these metabolites is limited, the elevation of sugar concentration, such as sucrose and fructose, could be attributed to inflammatory response, surgical stress, and the medications administered during the operative period. Regarding lipidic metabolism, we observed increased dodecanoic acid levels in our patients. It was found decreased in BC patients by other studies as it is a medium-chain fatty acid absorbed directly into the blood vessels, which can be used to obtain energy via β-oxidation [55, 56]. Additionally, dodecanoic acid has been shown to induce antiproliferative and pro-apoptotic effects in breast cancerous cells, importantly increasing reactive oxygen species levels [57]. Hence, the observed increase in dodecanoic acid levels in the blood after tumor removal can be explained by the tumor cells' reduced consumption of this acid.

Concerning energy metabolism, there is an increase in phosphoric acid concentration in BC patients after surgery. Other studies previously saw increased phosphoric acid in the post-operative state of patients with other cancers, and it was related to unfavorable prognosis. It was proposed to be produced by treatment-related complications like infections, organ failures, or the development of cardiovascular complications [58].

Three-hydroxybutyric acid is a ketone body (KB) that is an important alternative energy source for glucose during nutrient deprivation [59]. Dysregulation of KB metabolism also plays a role in tumorigenesis in cancer. Cancer cells favor increased glycolysis to support their proliferation, which can lead to glucose starvation by non-cancerous cells, where KBs, among other alternative energy sources, will be consumed as metabolic fuel [60]. That can explain the increased concentration seen by other studies in cancer patients [61]. In the absence of tumor cells or when their presence does not significantly impact blood glucose and sugar concentrations, there may be a reduced demand for the overproduction of ketone bodies. This reduced metabolic demand can explain the decreased concentrations that our study reported.

The significant differences observed in the metabolome of cancer patients before and after surgery, as indicated by the ROC curve with an AUC of 0.966, provide statistical evidence of the real impact of surgery on the metabolic profile, indicating that the surgical intervention induces significant changes in the metabolome which can be indeed attributed to the eradication of cancerous cells.

By comparing basal and post-QTA samples, we found significant differences in the metabolic profiles of patients, very similar to that found in the previous comparison (basal vs. post-surgery). However, when comparing post-surgery samples with post-QTA samples to isolate the specific effects of chemotherapy, no significant differences were found. We conclude that these basal/post-QTA differences are caused by the cumulative impact of surgery and chemotherapy rather than chemotherapy alone. On the other side, other studies showed alterations caused by chemotherapy in lipid profiles [62], weight gain [63], and increased sugar levels [64].

The final treatment that our patients received was radiotherapy. We wanted to investigate the metabolic impact of radiotherapy in cancer patients who had previously undergone the whole treatment strategy (surgery, chemotherapy, and radiotherapy). Still, we also wanted to isolate the specific effect of radiotherapy, so we compared the

metabolomics data between post-surgery and post-radiotherapy samples (as chemotherapy did not show any impact).

The cumulative effect of treatments showed altered lipid metabolism; increased dodecanoic acid as in the post-surgery state, and increased tetradecanoic acid. Tetradecanoic acid can be used to obtain energy, as a cell membrane component, and for protein modification. We can find that literature points out that radiotherapy can cause cell death in both cancerous and healthy cells [65]. This can lead to tissue damage and increased levels of lipids in our body, which may indicate an early response to radiotherapy [62]. We have also found contradictions in this case as this acid has been found increased in BC patients compared to a control group [66]. In contrast, we found it increased in BC patients after being submitted to all treatments.

Significant changes in metabolite levels provide valuable insights into the normalization of metabolic pathways after radiotherapy in BC patients. Notably, 3-phosphoglyceric acid and hypoxanthine exhibited decreased levels post-RT, in contrast to the increased levels reported in BC patients compared to control groups. This suggests a restoration of carbohydrate and nucleotide metabolism thanks to the eradication of cancer cells. Furthermore, sugars such as maltose and fructose also presented decreased levels post-RT, returning to normal levels compared to the basal or post-surgery state. However, the increased levels of 3-hydroxybutyric acid in this state contradict the observed pattern after surgery, showing the need for further investigation into the underlying reasons.

The observed substantial differences in the metabolome of cancer patients pre- and post-radiotherapy, supported by an AUC of 0.855 in the ROC curve constructed with maltose, fructose, 3-hydroxybutyric acid, 3-phosphoglyceric acid, and hypoxanthine, offer compelling evidence of the actual impact of radiotherapy on the metabolic profile.

Now that all treatments have been described, we also realized three metabolites showed statistically significant differences in the basal vs. control comparison. Still, they also showed significance across different treatments, indicating their potential relevance to the disease. Notably, these metabolites also emerged as some of the most discriminant metabolites among other groups, as evident from their high VIP scores. These metabolites were hypoxanthine, maltose, and 3-phosphoglyceric acid. Subsequently, we created boxplots for the concentrations of these metabolites in each treatment, comparing them to the basal state. This helped us understand their dynamic changes

throughout the treatments. The boxplots showed a progressive decrease in their levels with each treatment, eventually reaching levels comparable to those of the control group. Noticeably, among the treatments, radiotherapy demonstrated the highest significance and exhibited the most pronounced decrease in these metabolite levels. This could describe the potential of RT in BC therapy strategy. However, these metabolite levels may be naturally normalizing post-surgery and post-QTA, independent of the effects of radiotherapy, due to the temporal aspect. However, ethical constraints prevent studying a group of BC patients who do not receive radiotherapy to confirm this hypothesis. Notably, the deviation in metabolite levels exhibited a significant reduction with each successive treatment. This observation may imply that the treatments are effectively modulating and normalizing the metabolic profiles of BC patients, promoting a more consistent and regulated metabolic state. Despite all that has been explained, further studies are needed to clarify the specific mechanisms by which these treatments impact the metabolic pathways associated with the described metabolites.

Given the extensive heterogeneity of BC, it is crucial to carefully stratify and assess the clinicopathological characteristics of patients for an accurate pathology interpretation. In this case, we decided to look for the differences in the whole treatment response, as doing it with each treatment was impossible despite the lack of samples. This analysis exposed significant alterations between the molecular subtype of tumors and changes in the metabolome of patients. In this case, we saw increased fumaric acid, glycerol-1-phosphate, and malic acid in luminal B patients compared to luminal A. Luminal B BC is associated with more aggressive tumor characteristics and a higher proliferation rate compared to luminal A BC. Our results showed alterations in TCA cycle as well as in lipid metabolism and their increased levels in luminal B patients might reflect dysregulation in these pathways.

Moving on to histological grade, our study revealed a significant association between histological grade and circulatory alterations in BC patients. Specifically, we observed a notable decrease in glycerol levels in grade II patients, whereas a substantial decrease in d-arabitol was seen in grade I patients. Grade II BC may involve distinct metabolic reprogramming of lipid metabolism leading to lower glycerol production. On the other hand, grade I BC is typically associated with a less aggressive phenotype, and alterations in metabolite concentration, such as d-arabitol, may reflect distinct metabolic adaptations in these tumors.

Finally, when classifying patients by their menopausal state, we observed a notable increase in threonic acid and α-ketoglutaric acid in postmenopausal individuals without further associated information.

The possible limitation of this study is the reduced sample size, which may limit the generalizability of the findings. Conducting a future study with a larger cohort of BC patients would be necessary to explore the possible variations in the results and drive more definitive conclusions.

5. Conclusion

Significant alterations in the metabolome of BC patients were seen when compared to a control group. Our group found a novel biomarker signature (composed of hypoxanthine, maltose, 3-phosphoglyceric acid, xylonic acid, ethylmalonic acid, and glycerol), which showed absolute predictive power to discriminate BC patients from healthy individuals.

Concerning treatments, both surgery and radiotherapy exhibited a significant influence on the metabolome of BC patients. These distinctive alterations could be potentially related to the eradication or absence of cancerous cells when compared to BC patients in the basal state. Consequently, a list of metabolites emerged, which holds promise as potential biomarkers for studying and assessing patients' responses to treatments. Moreover, hypoxanthine, maltose, and 3-phosphoglyceric acid allowed for a deeper exploration of the dynamic metabolic changes occurring in BC patients, showing consecutive decreasing levels with each treatment arriving to resemble the control group.

Finally, altered metabolic profiles have been seen concerning molecular subtype, histological grade, and menopausal state, which should be studied in the future to investigate these metabolic profiles and their potential clinical implications comprehensively.

6. Future perspectives

As future perspectives, replication of this study with a larger cohort would provide a robust validation of the observed results and ensure the generalizability of the findings. Additionally, the biomarkers of BC detection identified in this study should undergo rigorous validation to ensure their reliability and diagnostic accuracy.

In terms of alterations in the metabolome of BC patients after the different treatment strategies, conducting in-depth analyses would provide insights into their potential in guiding treatment decisions and evaluating treatment effectiveness.

Finally, future studies in pre-QTNA patients would be interesting, as they would provide a comprehensive understanding of their metabolic landscape, which may differ from that of the pre-surgery patients we analyzed.

7. Acknowledgements

En primer lugar, me gustaría dar las gracias al Prof. Jorge Joven por permitirme realizar las prácticas en la *Unitat de Recerca Biomédica* (URB) y proporcionarme orientación y consejos los cuales estoy seguro que me serán de utilidad en mi futuro como investigador. A la Dra. Meritxell Arenas por dejarme formar parte de su estudio oncológico e introducirme en el complejo mundo del cáncer.

También agradecer a la Dra. Anna Hernández por ayudarme en todo momento. Tu amabilidad, paciencia y disposición para responder a todas mis preguntas han sido realmente reconfortantes.

Gracias a Andrea, gracias por tu sólida guía, respaldo y por confiar en mí a lo largo de esta etapa. Gracias por compartir conmigo todos tus conocimientos y por ser paciente durante mi aprendizaje. Helena, gracias por tu firme apoyo y por infundir entusiasmo en nuestro trabajo de investigación. Ambas me acogisteis en el grupo desde el primer día y no puedo estar más agradecido. Vuestra presencia y vuestra generosidad han hecho que mi estada de prácticas fuera increíble, sois modelos ejemplares a seguir.

Agradecer a mi familia, a mis padres y a mi hermana, por darme apoyo incondicional a lo largo de toda mi etapa académica. Siempre han sabido entenderme, ayudarme y tenderme una mano en aquellos momentos en los que lo necesitaba.

Finalmente agradecer a mis amigos: Vicente, Carla, Gabriela y Nagore. Gracias por hacer que estos cuatro años hayan superado toda expectativa posible, espero que esto sea solo el comienzo de una larga amistad.

8. Bibliography

1. Wilkinson, L., & Gathani, T. (2022). Understanding breast cancer as a global health concern. *The British journal of radiology*, 95(1130), 20211033. https://doi.org/10.1259/bjr.20211033
2. Rodríguez-Tomàs, E., Arenas, M., Baiges-Gaya, G., Acosta, J., Araguas, P., Malave, B., Castañé, H., Jiménez-Franco, A., Benavides-Villarreal, R., Sabater, S., Solà-Alberich, R., Camps, J., & Joven, J. (2022). Gradient Boosting Machine Identified Predictive Variables for Breast Cancer Patients Pre- and Post-Radiotherapy: Preliminary Results of an 8-Year Follow-Up Study. *Antioxidants* (Basel, Switzerland), 11(12), 2394. https://doi.org/10.3390/antiox11122394
3. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: *a cancer journal for clinicians*, 71(3), 209–249. https://doi.org/10.3322/caac.21660
4. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, accessed [13 May 2023].
5. ECIS - European Cancer Information System. European Union. Available from https://ecis.jrc.ec.europa.eu, accessed on [13 May 2023].
6. Kashyap, D., Pal, D., Sharma, R., Garg, V. K., Goel, N., Koundal, D., Zaguia, A., Koundal, S., & Belay, A. (2022). Global Increase in Breast Cancer Incidence: Risk Factors and Preventive Measures. *BioMed research international*, 2022, 9605439. https://doi.org/10.1155/2022/9605439
7. Fragomeni, S. M., Sciallis, A., & Jeruss, J. S. (2018). Molecular Subtypes and Local-Regional Control of Breast Cancer. *Surgical oncology clinics of North America*, 27(1), 95–120. https://doi.org/10.1016/j.soc.2017.08.005
8. Orrantia-Borunda E, Anchondo-Nuñez P, Acuña-Aguilar LE, et al. Subtypes of Breast Cancer. In: Mayrovitz HN, editor. Breast Cancer [Internet]. Brisbane (AU): Exon Publications; 2022 Aug 6. Chapter 3. Available from: https://www.ncbi.nlm.nih.gov/books/NBK583808/ doi: 10.36255/exon-publications-breast-cancer-subtypes
9. Waks, A. G., & Winer, E. P. (2019). Breast Cancer Treatment: A Review. *JAMA*, 321(3), 288–300. https://doi.org/10.1001/jama.2018.19323

10. Patel, A., Unni, N., & Peng, Y. (2020). The Changing Paradigm for the Treatment of HER2-Positive Breast Cancer. Cancers, 12(8), 2081. https://doi.org/10.3390/cancers12082081
11. Kazama, T., Takahara, T., & Hashimoto, J. (2022). Breast Cancer Subtypes and Quantitative Magnetic Resonance Imaging: A Systemic Review. *Life* (Basel, Switzerland), 12(4), 490. https://doi.org/10.3390/life12040490
12. Surveillance, Epidemiology, and End Results (SEER) Program Populations (1969-2020). National Cancer Institute, DCCPS, Surveillance Research Program, released February 2022. Available from: https://www.seer.cancer.gov/statfacts, accessed on [14 May 2023].
13. Moccia, C., & Haase, K. (2021). Engineering Breast Cancer On-chip-Moving Toward Subtype Specific Models. *Frontiers in bioengineering and biotechnology,* 9, 694218. https://doi.org/10.3389/fbioe.2021.694218
14. Missiroli, S., Perrone, M., Genovese, I., Pinton, P., & Giorgi, C. (2020). Cancer metabolism and mitochondria: Finding novel mechanisms to fight tumours. *EBioMedicine*, 59, 102943. https://doi.org/10.1016/j.ebiom.2020.102943
15. Schmidt, D. R., Patel, R., Kirsch, D. G., Lewis, C. A., Vander Heiden, M. G., & Locasale, J. W. (2021). Metabolomics in cancer research and emerging applications in clinical oncology. CA: *a cancer journal for clinicians*, 71(4), 333–358. https://doi.org/10.3322/caac.21670
16. Camps, J., Castañé, H., Rodríguez-Tomàs, E., Baiges-Gaya, G., Hernández-Aguilera, A., Arenas, M., Iftimie, S., & Joven, J. (2021). On the Role of Paraoxonase-1 and Chemokine Ligand 2 (C-C motif) in Metabolic Alterations Linked to Inflammation and Disease. A 2021 Update. *Biomolecules*, 11(7), 971. https://doi.org/10.3390/biom11070971
17. Mishra, A., Srivastava, A., Pateriya, A., Tomar, M. S., Mishra, A. K., & Shrivastava, A. (2021). Metabolic reprograming confers tamoxifen resistance in breast cancer. *Chemico-biological interactions*, 347, 109602. https://doi.org/10.1016/j.cbi.2021.109602
18. Martínez-Reyes, I., & Chandel, N. S. (2021). Cancer metabolism: looking forward. *Nature reviews. Cancer*, 21(10), 669–680. https://doi.org/10.1038/s41568-021-00378-6

19. Sciacovelli, M., Gaude, E., Hilvo, M., & Frezza, C. (2014). The Metabolic Alterations of Cancer Cells. *Methods in Enzymology* (pp. 1-23). Academic Press. https://doi.org/10.1016/b978-0-12-416618-9.00001-7
20. Jeong, DW., Lee, S. & Chun, YS. How cancer cells remodel lipid metabolism: strategies targeting transcription factors. *Lipids Health Dis 20*, 163 (2021). https://doi.org/10.1186/s12944-021-01593-8
21. Hsu, C. C., Tseng, L. M., & Lee, H. C. (2016). Role of mitochondrial dysfunction in cancer progression. *Experimental biology and medicine* (Maywood, N.J.), 241(12), 1281–1295. https://doi.org/10.1177/1535370216641787
22. Luo, Y., Ma, J., & Lu, W. (2020). The Significance of Mitochondrial Dysfunction in Cancer. *International journal of molecular sciences*, 21(16), 5598. https://doi.org/10.3390/ijms21165598
23. Chiu, H., Tay, E. X. Y., Ong, D. S. T., & Taneja, R. (2020). Mitochondrial Dysfunction at the Center of Cancer Therapy. *Antioxidants & Redox Signaling*, 32(5), 309-330. https://doi.org/10.1089/ars.2019.7898
24. Srinivasan, S., Guha, M., Kashina, A., & Avadhani, N. G. (2017). Mitochondrial dysfunction and mitochondrial dynamics-The cancer connection. *Biochimica et biophysica acta. Bioenergetics*, 1858(8), 602–614. https://doi.org/10.1016/j.bbabio.2017.01.004
25. Anderson, N. M., & Simon, M. C. (2020). The tumor microenvironment. *Current biology*: CB, 30(16), R921–R925. https://doi.org/10.1016/j.cub.2020.06.081
26. Dias, A. S., Almeida, C. R., Helguero, L. A., & Duarte, I. F. (2019). Metabolic crosstalk in the breast cancer microenvironment. *European journal of cancer* (Oxford, England : 1990), 121, 154–171. https://doi.org/10.1016/j.ejca.2019.09.002
27. Tse, T., Sehdev, S., Seely, J., Gravel, D. H., Clemons, M., Cordeiro, E., & Arnaout, A. (2021). Neoadjuvant Chemotherapy in Breast Cancer: Review of the Evidence and Conditions That Facilitated Its Use during the Global Pandemic. *Current oncology* (Toronto, Ont.), 28(2), 1338–1347. https://doi.org/10.3390/curroncol28020127
28. Wang, H., & Mao, X. (2020). Evaluation of the Efficacy of Neoadjuvant Chemotherapy for Breast Cancer. *Drug design, development and therapy*, 14, 2423–2433. https://doi.org/10.2147/DDDT.S253961

29. Masood S. (2016). Neoadjuvant chemotherapy in breast cancers. *Women's health* (London, England), 12(5), 480–491. https://doi.org/10.1177/1745505716677139
30. Lovelace, D. L., McDaniel, L. R., & Golden, D. (2019). Long-Term Effects of Breast Cancer Surgery, Treatment, and Survivor Care. *Journal of midwifery & women's health*, 64(6), 713–724. https://doi.org/10.1111/jmwh.13012
31. Admoun, C., & Mayrovitz, H. (2021). Choosing Mastectomy vs. Lumpectomy-With-Radiation: Experiences of Breast Cancer Survivors. *Cureus*, 13(10), e18433. https://doi.org/10.7759/cureus.18433
32. Abass, M. O., Gismalla, M. D. A., Alsheikh, A. A., & Elhassan, M. M. A. (2018). Axillary Lymph Node Dissection for Breast Cancer: Efficacy and Complication in Developing Countries. *Journal of global oncology*, 4, 1–8. https://doi.org/10.1200/JGO.18.00080
33. Pondé, N. F., Zardavas, D., & Piccart, M. (2019). Progress in adjuvant systemic therapy for breast cancer. *Nature reviews. Clinical oncology*, 16(1), 27–44. https://doi.org/10.1038/s41571-018-0089-9
34. Hortobagyi G. (2000). Adjuvant therapy for breast cancer. *Annual review of medicine*, 51, 377–392. https://doi.org/10.1146/annurev.med.51.1.377
35. Haussmann, J., Corradini, S., Nestle-Kraemling, C., Bölke, E., Njanang, F. J. D., Tamaskovics, B., Orth, K., Ruckhaeberle, E., Fehm, T., Mohrmann, S., Simiantonakis, I., Budach, W., & Matuschek, C. (2020). Recent advances in radiotherapy of breast cancer. *Radiation oncology* (London, England), 15(1), 71. https://doi.org/10.1186/s13014-020-01501-x
36. Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. *Cancers*, 13(17), 4287. https://doi.org/10.3390/cancers13174287
37. Kaidar-Person, O., & Offersen, B. V. (2021b). Radiation therapy in breast cancer: a narrative review on current standards and future perspectives. *Annals of breast surgery*, 6, 28. https://doi.org/10.21037/abs-21-16
38. Koenig, J. L., Kozak, M. M., Sabolch, A., Horst, K., Tsai, J., Wapnir, I. L., & Pollom, E. (2019). Use of Preoperative Radiation Therapy in Early-stage and Locally Advanced Breast Cancer. *Cureus*, 11(9), e5748. https://doi.org/10.7759/cureus.5748

39. Tremont, A., Lu, J., & Cole, J. T. (2017). Endocrine Therapy for Early Breast Cancer: Updated Review. *The Ochsner journal*, 17(4), 405–411.
40. Greenblatt K, Khaddour K. Trastuzumab. [Updated 2022 Nov 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532246/
41. Han, J., Li, Q., Chen, Y., & Yang, Y. (2021). Recent Metabolomics Analysis in Tumor Metabolism Reprogramming. *Frontiers in molecular biosciences*, 8, 763902. https://doi.org/10.3389/fmolb.2021.763902
42. Ramteke, P., Deb, A., Shepal, V., & Bhat, M. K. (2019). Hyperglycemia Associated Metabolic and Molecular Alterations in Cancer Risk, Progression, Treatment, and Mortality. *Cancers*, 11(9), 1402. https://doi.org/10.3390/cancers11091402
43. Schwartsburd P. (2019). Cancer-Induced Reprogramming of Host Glucose Metabolism: "Vicious Cycle" Supporting Cancer Progression. *Frontiers in oncology*, 9, 218. https://doi.org/10.3389/fonc.2019.00218
44. Monzavi-Karbassi, B., Gentry, R., Kaur, V., Siegel, E. R., Jousheghany, F., Medarametla, S., Fuhrman, B. J., Safar, A. M., Hutchins, L. F., & Kieber-Emmons, T. (2016). Pre-diagnosis blood glucose and prognosis in women with breast cancer. *Cancer & metabolism*, 4, 7. https://doi.org/10.1186/s40170-016-0147-7
45. Sadiqa, A., Rasul, A., Hassan, M., Sultana, S., & Jabeen, F. (2022). Identification of Novel Natural Inhibitors to Human 3-Phosphoglycerate Dehydrogenase (PHGDH) for Cancer Treatment. *Molecules* (Basel, Switzerland), 27(18), 6108. https://doi.org/10.3390/molecules27186108
46. Mattaini, K. R., Sullivan, M. R., & Vander Heiden, M. G. (2016). The importance of serine metabolism in cancer. *The Journal of cell biology*, 214(3), 249–257. https://doi.org/10.1083/jcb.201604085
47. Karagiota, A., Chachami, G., & Paraskeva, E. (2022). Lipid Metabolism in Cancer: The Role of Acylglycerolphosphate Acyltransferases (AGPATs). *Cancers*, 14(1), 228. https://doi.org/10.3390/cancers14010228
48. Broadfield, L. A., Pane, A. A., Talebi, A., Swinnen, J. V., & Fendt, S. M. (2021). Lipid metabolism in cancer: New perspectives and emerging mechanisms. *Developmental cell*, 56(10), 1363–1393. https://doi.org/10.1016/j.devcel.2021.04.013
49. Wang, X., Zhao, X., Chou, J., Yu, J., Yang, T., Liu, L., & Zhang, F. (2018). Taurine, glutamic acid and ethylmalonic acid as important metabolites for detecting

human breast cancer based on the targeted metabolomics. *Cancer biomarkers: section A of Disease markers*, 23(2), 255–268. https://doi.org/10.3233/CBM-181500

50. Ruiz-Sala, P., & Peña-Quintana, L. (2021). Biochemical Markers for the Diagnosis of Mitochondrial Fatty Acid Oxidation Diseases. *Journal of clinical medicine*, 10(21), 4855. https://doi.org/10.3390/jcm10214855
51. Oh, S. H., Choi, S. Y., Choi, H. J., Ryu, H. M., Kim, Y. J., Jung, H. Y., Cho, J. H., Kim, C. D., Park, S. H., Kwon, T. H., & Kim, Y. L. (2019). The emerging role of xanthine oxidase inhibition for suppression of breast cancer cell migration and metastasis associated with hypercholesterolemia. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 33(6), 7301–7314. https://doi.org/10.1096/fj.201802415RR
52. Wei, Y., Jasbi, P., Shi, X., Turner, C., Hrovat, J., Liu, L., Rabena, Y., Porter, P., & Gu, H. (2021). Early Breast Cancer Detection Using Untargeted and Targeted Metabolomics. *Journal of proteome research*, 20(6), 3124–3133. https://doi.org/10.1021/acs.jproteome.1c00019
53. Park, J., Shin, Y., Kim, T. H., Kim, D. H., & Lee, A. (2019). Plasma metabolites as possible biomarkers for diagnosis of breast cancer. *PloS one*, 14(12), e0225129. https://doi.org/10.1371/journal.pone.0225129
54. Yin, J., Ren, W., Huang, X., Deng, J., Li, T., & Yin, Y. (2018). Potential Mechanisms Connecting Purine Metabolism and Cancer Therapy. *Frontiers in immunology*, 9, 1697. https://doi.org/10.3389/fimmu.2018.01697
55. Roopashree, P. G., Shetty, S. S., Shetty, V. V., & Nalilu, S. K. (2022). Medium-Chain Fatty Acids and Breast Cancer Risk by Receptor and Pathological Subtypes. *Nutrients*, 14(24), 5351. https://doi.org/10.3390/nu14245351
56. Nukaga, S., Mori, T., Miyagawa, Y., Fujiwara-Tani, R., Sasaki, T., Fujii, K., Mori, S., Goto, K., Kishi, S., Nakashima, C., Ohmori, H., Kawahara, I., Luo, Y., & Kuniyasu, H. (2020). Combined administration of lauric acid and glucose improved cancer-derived cardiac atrophy in a mouse cachexia model. *Cancer science*, 111(12), 4605–4615. https://doi.org/10.1111/cas.14656
57. Lappano, R., Sebastiani, A., Cirillo, F., Rigiracciolo, D. C., Galli, G. R., Curcio, R., Malaguarnera, R., Belfiore, A., Cappello, A. R., & Maggiolini, M. (2017). The lauric acid-activated signaling prompts apoptosis in cancer cells. *Cell death discovery*, 3, 17063. https://doi.org/10.1038/cddiscovery.2017.63

58. Ye, Z., Palazzo, J. P., Lin, L., Lai, Y., Guiles, F., Myers, R. E., Han, J., Xing, J., & Yang, H. (2013). Postoperative hyperphosphatemia significantly associates with adverse survival in colorectal cancer patients. *Journal of gastroenterology and hepatology*, 28(9), 1469–1475. https://doi.org/10.1111/jgh.12237
59. Bartmann, C., Janaki Raman, S. R., Flöter, J., Schulze, A., Bahlke, K., Willingstorfer, J., Strunz, M., Wöckel, A., Klement, R. J., Kapp, M., Djuzenova, C. S., Otto, C., & Kämmerer, U. (2018). Beta-hydroxybutyrate (3-OHB) can influence the energetic phenotype of breast cancer cells, but does not impact their proliferation and the response to chemotherapy or radiation. *Cancer & metabolism*, 6, 8. https://doi.org/10.1186/s40170-018-0180-9
60. Hwang, C. Y., Choe, W., Yoon, K. S., Ha, J., Kim, S. S., Yeo, E. J., & Kang, I. (2022). Molecular Mechanisms for Ketone Body Metabolism, Signaling Functions, and Therapeutic Potential in Cancer. *Nutrients*, 14(22), 4932. https://doi.org/10.3390/nu14224932
61. Hilvo, M., de Santiago, I., Gopalacharyulu, P., Schmitt, W. D., Budczies, J., Kuhberg, M., Dietel, M., Aittokallio, T., Markowetz, F., Denkert, C., Sehouli, J., Frezza, C., Darb-Esfahani, S., & Braicu, E. I. (2016). Accumulated Metabolites of Hydroxybutyric Acid Serve as Diagnostic and Prognostic Biomarkers of Ovarian High-Grade Serous Carcinomas. *Cancer research*, 76(4), 796–804. https://doi.org/10.1158/0008-5472.CAN-15-2298
62. Arlauckas, S. P., Browning, E. A., Poptani, H., & Delikatny, E. J. (2019). Imaging of cancer lipid metabolism in response to therapy. *NMR in biomedicine*, 32(10), e4070. https://doi.org/10.1002/nbm.4070
63. Qi, A., Li, Y., Yan, S., Sun, H., Zhao, M., & Chen, Y. (2021). Effect of postoperative chemotherapy on blood glucose and lipid metabolism in patients with invasive breast cancer. *Gland surgery*, 10(4), 1470–1477. https://doi.org/10.21037/gs-21-141
64. Qi, A., Li, Y., Yan, S., Sun, H., & Chen, Y. (2021). Effect of anthracycline-based postoperative chemotherapy on blood glucose and lipid profiles in patients with invasive breast cancer. *Annals of palliative medicine*, 10(5), 5502–5508. https://doi.org/10.21037/apm-21-533
65. Little JB. Principal Cellular and Tissue Effects of Radiation. In: Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. *Holland-Frei Cancer Medicine*. 6th edition.

Hamilton (ON): BC Decker; 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK12344/

66. Matta, M., Deubler, E., Chajes, V., Vozar, B., Gunter, M. J., Murphy, N., & Gaudet, M. M. (2022). Circulating plasma phospholipid fatty acid levels and breast cancer risk in the Cancer Prevention Study-II Nutrition Cohort. *International journal of cancer*, 151(12), 2082–2094. https://doi.org/10.1002/ijc.34216

9. Supplementary material

Table S1. Energy metabolism characteristics of pre-surgery and pre-QTNA patients.

	Pre-surgery (n=48)	**Pre-QTNA** (n=29)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.847
d-Mannonic acid	0.03 (0.02-0.03)	0.03 (0.02-0.04)	0.966
Galactose metabolism			
d-Galactitol	0.02 (0.01-0.02)	0.02 (0.01-0.02)	0.225
Glycolysis			
3-Phosphoglyceric acid	0.08 (0.04-0.15)	0.07 (0.05-0.14)	0.856
Glucose 6-phosphate	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.773
Lactic acid	61.3 (55.7-73.8)	62.9 (52.9-81.6)	0.949
Nucleotide sugar			
d-Arabinose	0.02 (0.02-0.03)	0.02 (0.01-0.03)	0.461
d-Threitol	0.03 (0.02-0.03)	0.02 (0.02-0.03)	0.696
d-Xylose	0.01 (0.01-0.02)	0.01 (0.01-0.01)	0.708
Erythronic acid	0.03 (0.02-0.04)	0.03 (0.02-0.04)	0.521
Threonic acid	0.4 (0.3-0.5)	0.3 (0.3-0.4)	0.455
Xylonic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.320
Pentose glucoronate interconversion			
d-Arabitol	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.893
d-Xylitol	0.001 (0.001-0.002)	0.002 (0.001-0.002)	0.942
Galacturonic acid	0.02 (0.02-0.02)	0.02 (0.01-0.02)	0.325
Myo-Inositol	0.6 (0.5-0.7)	0.6 (0.4-0.7)	0.505
Sucrose metabolism			
d-Sucrose	0.008 (0.003-0.01)	0.009 (0.00-0.01)	0.818
Maltose	0.02 (0.003-0.01)	0.02 (0.003-0.002)	0.372
TCA			
α-ketoglutaric acid	0.2 (0.1-0.2)	0.1 (0.1-0.2)	0.642
Citric acid	20.8 (15.8-27.3)	18.8 (14.9-23.7)	0.488
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.03-0.04)	0.160
Fumaric acid	0.2 (0.1-0.2)	0.1 (0.09-0.1)	0.242
Glutamine	33.8 (26.5-40.3)	31.5 (25.3-40.6)	0.572
Malic acid	0.07 (0.06-0.09)	0.07 (0.05-0.1)	0.339
Pyruvic acid	4.6 (2.8-6.6)	5.4 (2.8-6.7)	0.860
Succinic acid	0.07 (0.06-0.1)	0.06 (0.05-0.09)	0.202
Glyoxylate and dicarboxylate			
Glycolic acid	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.898
Pentose phosphate pathway			
Sedoheptulose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.596
Amino acid metabolism			
Alanine and aspartate			
Alanine	24.3 (21.3-28.6)	24.5 (21.2-28.7)	0.898
Glutamic acid	5.6 (3.5-7.8)	5.3 (4.1-9.03)	0.623
Arginine and proline			
2-Hydroxyisobutyric acid	1.4 (1.1-1.7)	1.5 (1.1-1.8)	0.773
Urea	33.8 (26.9-40.9)	30.8 (25.2-41.4)	0.586
Proline	30.2 (24.2-41.4)	30.1 (22.1-36.4)	0.468
4-Hydroxyproline	1.4 (1.1-1.9)	1.2 (0.8-1.5)	0.054
Ornithine	12.8 (9.7-14.8)	12.3 (9.8-15.1)	0.915
Oxoproline	25.3 (21.03-28.8)	23.3 (21.01-28.1)	0.448
Glycine and Serine			
Glyceric acid	0.5 (0.4-0.6)	0.6 (0.4-0.7)	0.753
Glycine	6.7 (6.1-7.2)	6.9 (6.4-7.1)	0.852
Serine	8.2 (6.5-9.5)	7.4 (6.7-9.6)	0.818
Threonine	7.9 (6.8-8.9)	7.9 (6.3-9.8)	0.860
Tyrosine metabolism			

Vanillylmandelic acid	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.527
Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.9 (2.8-5.6)	3.6 (2.7-5.6)	0.881
3-methyl-2-oxobutyric acid	0.6 (0.4-0.7)	0.4 (0.4-0.8)	0.402
3-hydroxybutyric acid	8.6 (5.1-24.7)	8.1 (5.1-17.9)	0.658
2-Hydroxyisovaleric acid	5.2 (3.7-7.5)	6.8 (4.5-11.5)	0.066
2-keto-3-methylvaleric acid	1.1 (0.9-1.3)	1.04 (0.9-1.5)	0.814
3-Hydroxyisovaleric acid	0.8 (0.7-1.2)	1.0 (0.7-1.2)	0.785
Ethylmalonic acid	2.2 (1.7-2.9)	2.1 (1.7-3.3)	0.881
Isoleucine	4.6 (3.8-5.3)	4.6 (3.9-6.5)	0.488
Leucine	8.9 (7.8-10.7)	9.2 (7.7-11.7)	0.631
Valine	16.9 (14.8-20.3)	18.0 (14.3-21.7)	0.568
Cysteine and methionine metabolism			
Methionine	3.6 (3.4-3.9)	3.5 (3.2-3.9)	0.745
Tryptophan metabolism			
Indole-3-propanoic acid	0.2 (0.1-0.2)	0.1 (0.07-0.2)	0.536
Indolelactic acid	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.571
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.13-0.23)	0.2 (0.11-0.24)	0.729
Benzoic acid	0.5 (0.5-0.7)	0.6 (0.5-0.7)	0.308
Hippuric acid	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.272
Hydrocinnamic acid	0.05 (0.03-0.09)	0.06 (0.04-0.1)	0.860
Phenylalanine	6.4 (5.7-7.2)	6.5 (5.5-7.4)	0.852
Lipid metabolism			
Lipids			
Dodecanoic acid	0.6 (0.4-0.8)	0.7 (0.5-1.1)	0.198
Linoleic acid	2.8 (1.6-4.7)	2.4 (1.2-3.5)	0.283
Oleic acid	17.9 (11.4-24.8)	21.9 (8.8-24.9)	0.881
Tetradecanoic acid	1.3 (1.0-2.1)	1.6 (1.1-2.6)	0.405
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.873
Glycerol	0.8 (0.5-1.0)	0.7 (0.5-0.9)	0.843
Glycerol-1-phosphate	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.928
Primary bile acid biosynthesis			
Taurine	0.9 (0.6-1.2)	0.8 (0.5-1.4)	0.461
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.2 (0.1-0.3)	0.3 (0.2-0.3)	0.627
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.5 (0.3-1.4)	0.5 (0.3-0.9)	0.586
Ribonic acid	0.004 (0.003-0.005)	0.005 (0.003-0.07)	0.966
Uracil	0.02 (0.02-0.03)	0.02 (0.01-0.03)	0.615
Uric acid	10.3 (6.7-19.8)	11.6 (7.1-20.4)	0.685
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.873
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	186.3 (154.5-233.9)	204.7 (172.2-260.3)	0.172

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**). QTNA: neoadjuvant chemotherapy.

Table S2. Energy metabolism characteristics of basal patients and control group.

	Basal (n=48)	**Control** (n=50)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.02 (0.01-0.03)	0.01 (0.01-0.02)	0.052
d-Mannonic acid	0.03 (0.02-0.03)	0.02 (0.02-0.03)	**0.013**
Galactose metabolism			
d-Galactitol	0.02 (0.01-0.02)	0.02 (0.02-0.03)	0.053
Glycolysis			
3-Phosphoglyceric acid	0.08 (0.04-0.2)	0.02 (0.02-0.04)	**4.3×10^{-10}**
Glucose 6-phosphate	0.01 (0.01-0.02)	0.02 (0.01-0.02)	**5.8×10^{-08}**
Lactic acid	61.3 (55.7-73.8)	56.0 (43.0-62.5)	**0.002**
Nucleotide sugar			
d-Arabinose	0.02 (0.02-0.03)	0.02 (0.01-0.02)	0.064
d-Threitol	0.03 (0.02-0.03)	0.02 (0.02-0.02)	**1.1×10^{-06}**
d-Xylose	0.008 (0.01-0.02)	0.01 (0.01-0.01)	0.050
Erythronic acid	0.03 (0.02-0.04)	0.02 (0.01-0.03)	**4.7×10^{-06}**
Threonic acid	0.3 (0.3-0.5)	0.2 (0.2-0.23)	**7.9×10^{-05}**
Xylonic acid	0.01 (0.01-0.02)	0.05 (0.04-0.05)	**2.1×10^{-16}**
Pentose glucoronate interconversion			
d-Arabitol	0.009 (0.01-0.01)	0.01 (0.01-0.02)	**1.8×10^{-04}**
d-Xylitol	0.001 (0.001-0.002)	0.001 (0.001-0.001)	0.160
Galacturonic acid	0.02 (0.02-0.02)	0.02 (0.01-0.02)	0.496
Myo-Inositol	0.6 (0.5-0.7)	0.4 (0.4-0.5)	**4.5×10^{-05}**
Sucrose metabolism			
d-Sucrose	0.008 (0.003-0.01)	0.005 (0.002-0.005)	**0.002**
Maltose	0.02 (0.01-0.04)	0.01 (0.01-0.02)	**4.5×10^{-09}**
TCA			
α-ketoglutaric acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.730
Citric acid	20.8 (15.8-27.3)	16.9 (14.0-19.6)	**0.016**
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.03-0.05)	0.555
Fumaric acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.924
Glutamine	33.8 (26.5-40.3)	35.5 (28.2-45.1)	0.128
Malic acid	0.07 (0.06-0.09)	0.06 (0.05-0.07)	**0.004**
Pyruvic acid	4.6 (2.8-6.6)	4.8 (3.3-6.2)	0.798
Succinic acid	0.07 (0.06-0.1)	0.06 (0.05-0.06)	**1.5×10^{-04}**
Glyoxylate and dicarboxylate			
Glycolic acid	0.3 (0.1-0.3)	0.2 (0.1-0.2)	**3.1×10^{-06}**
Pentose phosphate pathway			
Sedoheptulose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.393
Amino acid metabolism			
Alanine and aspartate			
Alanine	24.3 (21.3-28.6)	20.6 (17.8-24.2)	**4.4×10^{-04}**
Glutamic acid	5.6 (3.4-7.8)	3.7 (3.1-4.4)	**4.7×10^{-05}**
Arginine and proline			
2-Hydroxyisobutyric acid	1.4 (1.1-1.7)	1.1 (0.9-1.3)	**0.005**
Urea	33.8 (26.9-40.9)	31.5 (22.9-36.9)	**0.048**
Proline	30.2 (24.2-41.4)	26.8 (21.6-33.7)	0.113
4-Hydroxyproline	1.4 (1.1-1.9)	1.4 (1.1-1.9)	0.834
Ornithine	12.8 (9.7-14.8)	13.1 (10.0-16.7)	0.426
Oxoproline	25.3 (21.0-28.8)	25.7 (21.7-29.7)	0.551
Glycine and Serine			
Glyceric acid	0.5 (0.4-0.6)	0.6 (0.5-0.8)	**0.008**
Glycine	6.7 (6.1-7.2)	6.5 (6.0-7.0)	0.422
Serine	8.2 (6.5-9.5)	8.8 (7.4-9.5)	0.142
Threonine	7.9 (6.8-8.9)	8.7 (7.1-10.1)	**0.032**
Tyrosine metabolism			
Vanillylmandelic acid	0.007 (0.01-0.02)	0.01 (0.01-0.01)	**0.002**
Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.9 (2.8-5.6)	3.1 (2.0-4.2)	**0.011**

3-methyl-2-oxobutyric acid	0.6 (0.4-0.7)	0.6 (0.5-0.7)	0.649
3-hydroxybutyric acid	8.6 (5.1-24.7)	8.6 (5.3-20.7)	0.691
2-Hydroxyisovaleric acid	5.2 (3.7-7.5)	4.3 (3.2-5.3)	**0.031**
2-keto-3-methylvaleric acid	1.1 (0.9-1.3)	1.3 1.0-1.5)	0.056
3-Hydroxyisovaleric acid	0.9 (0.7-1.2)	0.9 (0.6-1.1)	0.621
Ethylmalonic acid	2.2 (1.7-2.9)	5.3 (4.4-6.3)	**3.7×10^{-15}**
Isoleucine	4.6 (3.8-5.3)	4.5 (3.9-5.1)	0.823
Leucine	8.9 (7.8-10.7)	8.9 (8.1-9.8)	0.870
Valine	16.9 (14.8-20.3)	16.4 (15.4-18.3)	0.495
Cysteine and methionine metabolism			
Methionine	3.6 (3.4-3.9)	3.6 (3.5-3.8)	0.212
Tryptophan metabolism			
Indole-3-propanoic acid	0.2 (0.1-0.2)	0.1 (0.1-0.2)	0.115
Indolelactic acid	0.1 (1-0.2)	0.2 (0.1-0.2)	**0.034**
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.069
Benzoic acid	0.6 (0.5-0.7)	0.5 (0.3-0.6)	0.101
Hippuric acid	0.2 (0.1-0.4)	0.3 (0.2-0.4)	0.466
Hydrocinnamic acid	0.05 (0.03-0.09)	0.07 (0.04-0.1)	0.412
Phenylalanine	6.4 (5.7-7.2)	6.2 (5.9-6.7)	0.462
Lipid metabolism			
Lipids			
Dodecanoic acid	0.6 (0.4-0.8)	0.5 (0.4-0.7)	0.203
Linoleic acid	2.8 (1.6-4.7)	2.9 (1.6-4.2)	0.744
Oleic acid	17.9 (11.4-24.8)	14.6 (7.9-25.6)	0.172
Tetradecanoic acid	1.3 (1.0-2.1)	1.6 (1.2-2.1)	0.406
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.893
Glycerol	0.8 (0.5-1.0)	1.6 (1.3-2.1)	**2.3×10^{-13}**
Glycerol-1-phosphate	0.2 (0.1-0.2)	0.3 (0.1-0.2)	**0.020**
Primary bile acid biosynthesis			
Taurine	0.9 (0.6-1.2)	0.5 (0.4-0.7)	**1.0×10^{-08}**
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.2 (0.1-0.3)	0.2 (0.1-0.4)	0.352
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.5 (0.3-1.4)	0.2 (0.1-0.3)	**1.7×10^{-10}**
Ribonic acid	0.004 (0.003-0.005)	0.003 (0.002-0.004)	**0.012**
Uracil	0.02 (0.02-0.03)	0.02 (0.01-0.02)	0.076
Uric acid	10.3 (6.7-19.8)	9.7 (6.3-15.2)	0.176
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0.453
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	186.3 (154.5-233.9)	158.7 (67.0-209.0)	**0.020**

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**).

Table S3. Energy metabolism characteristics of basal and post-surgery patients.

	Basal (n=48)	**Post-surgery** (n=42)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.02 (0.01-0.03)	0.03 (0.02-0.07)	**0.012**
d-Mannonic acid	0.03 (0.02-0.03)	0.03 (0.03-0.04)	**0.006**
Galactose metabolism			
d-Galactitol	0.02 (0.01-0.02)	0.02 (0.02-0.03)	0.061
Glycolysis			
3-Phosphoglyceric acid	0.08 (0.04-0.2)	0.07 (0.04-0.1)	0.431
Glucose 6-phosphate	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.928
Lactic acid	61.3 (55.7-73.8)	79.2 (66.7-108.8)	**1.5×10^{-4}**
Nucleotide sugar			
d-Arabinose	0.02 (0.02-0.03)	0.03 (0.02-0.04)	**0.001**
d-Threitol	0.03 (0.02-0.03)	0.03 (0.02-0.03)	0.275
d-Xylose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.086
Erythronic acid	0.03 (0.02-0.04)	0.03 (0.02-0.03)	0.195
Threonic acid	0.3 (0.3-0.5)	0.3 (0.3-0.4)	0.306
Xylonic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.287
Pentose glucoronate interconversion			
d-Arabitol	0.01 (0.01-0.01)	0.01 (0.01-0.02)	0.056
d-Xylitol	0.001 (0.001-0.002)	0.002 (0.001-0.002)	**0.036**
Galacturonic acid	0.02 (0.02-0.02)	0.02 (0.02-0.03)	**0.005**
Myo-Inositol	0.6 (0.5-0.7)	0.7 (0.6-0.9)	**0.021**
Sucrose metabolism			
d-Sucrose	0.008 (0.003-0.01)	0.01 (0.01-0.02)	**0.004**
Maltose	0.02 (0.01-0.04)	0.02 (0.01-0.05)	0.925
TCA			
α-ketoglutaric acid	0.2 (0.1-0.2)	0.1 (0.1-0.2)	0.608
Citric acid	20.8 (15.8-27.3)	14.6 (12.8-22.6)	**0.004**
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.03-0.05)	0.507
Fumaric acid	0.1 (0.1-0.12)	0.2 (0.1-0.2)	0.078
Glutamine	33.8 (26.5-40.3)	29.8 (25.3-33.3)	**0.044**
Malic acid	0.07 (0.06-0.09)	0.07 (0.05-0.08)	0.739
Pyruvic acid	4.6 (2.8-6.6)	7.67 (4.8-10.3)	**0.003**
Succinic acid	0.07 (0.06-0.1)	0.07 (0.05-0.1)	0.655
Glyoxylate and dicarboxylate			
Glycolic acid	0.1 (0.1-0.2)	0.2 (0.2-0.3)	**0.002**
Pentose phosphate pathway			
Sedoheptulose	0.01 (0.01-0.02)	0.02 (0.02-0.02)	**1.0×10^{-4}**
Amino acid metabolism			
Alanine and aspartate			
Alanine	24.3 (21.3-28.6)	28.6 (25.5-31.8)	**0.004**
Glutamic acid	5.6 (3.5-7.8)	5.7 (4.3-8.4)	0.718
Arginine and proline			
2-Hydroxyisobutyric acid	1.4 (1.1-1.7)	1.7 (1.3-2.1)	**0.032**
Urea	33.8 (26.9-41.0)	44.1 (35.8-55.2)	**2.8×10^{-5}**
Proline	30.2 (24.2-41.3)	34.7 (27.5-41.4)	0.481
4-Hydroxyproline	1.4 (1.1-1.9)	1.2 (0.9-1.7)	0.079
Ornithine	12.8 (9.7-14.8)	14.7 (11.4-18.7)	**0.009**
Oxoproline	25.3 (21.1-28.8)	27.8 (24.1-32.8)	**0.014**
Glycine and Serine			
Glyceric acid	0.5 (0.4-0.6)	0.4 (0.3-0.6)	0.183
Glycine	6.7 (6.1-7.2)	7.4 (6.6-7.8)	**0.001**

Serine	8.2 (6.5-9.5)	7.8 (7.3-9.6)	0.549
Threonine	7.9 (6.8-8.9)	8.4 (6.9-9.6)	0.329
Tyrosine metabolism			
Vanillylmandelic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.257
Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.9 (2.8-5.6)	3.8 (2.12-5.6)	0.347
3-methyl-2-oxobutyric acid	0.5 (0.4-0.7)	0.6 (0.5-0.7)	0.894
3-hydroxybutyric acid	8.6 (5.1-24.7)	5.4 (3.7-10.2)	**0.006**
2-Hydroxyisovaleric acid	5.2 (3.7-7.5)	5.8 (4.2-7.8)	0.347
2-keto-3-methylvaleric acid	1.1 (0.9-1.3)	1.4 (1.2-1.7)	**2.0×10^{-4}**
3-Hydroxyisovaleric acid	0.9 (0.7-1.2)	1.1 (0.8-1.3)	0.119
Ethylmalonic acid	2.2 (1.7-2.9)	3.1 (2.5-4.2)	**1.1×10^{-4}**
Isoleucine	4.6 (3.8-5.3)	5.3 (4.5-6.0)	**0.014**
Leucine	8.9 (7.8-10.7)	10.0 (8.5-11.6)	0.062
Valine	16.9 (14.8-20.3)	19.5 (16.7-21.7)	**0.013**
Cysteine and methionine metabolism			
Methionine	3.6 (3.4-3.9)	3.8 (3.4-4.1)	0.248
Tryptophan metabolism			
Indole-3-propanoic acid	0.1 (0.1-0.2)	0.2 (0.2-0.3)	**0.018**
Indolelactic acid	0.1 (0.1-0.2)	0.2 (0.2-0.3)	**0.004**
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.1-0.2)	0.2 (0.2-0.2)	0.155
Benzoic acid	0.6 (0.5-0.7)	0.7 (0.5-0.9)	**0.006**
Hippuric acid	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.359
Hydrocinnamic acid	0.05 (0.03-0.1)	0.07 (0.03-0.1)	0.138
Phenylalanine	6.4 (5.7-7.2)	6.3 (5.8-7.5)	0.565
Lipid metabolism			
Lipids			
Dodecanoic acid	0.6 (0.4-0.8)	1.3 (1.0-1.7)	**2.5×10^{-9}**
Linoleic acid	2.8 (1.6-4.7)	1.9 (1.1-2.9)	**0.008**
Oleic acid	17.9 (11.4-24.8)	12.2 (7.1-21.7)	**0.020**
Tetradecanoic acid	1.3 (1.0-2.1)	1.8 (1.3-2.2)	**0.049**
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.845
Glycerol	0.78 (0.5-1.0)	1.1 (0.9-1.3)	**3.5×10^{-5}**
Glycerol-1-phosphate	0.2 (0.1-0.3)	0.2 (0.1-0.2)	0.080
Primary bile acid biosynthesis			
Taurine	0.9 (0.6-1.2)	1.1 (0.7-1.4)	0.112
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.2 (0.1-0.3)	0.3 (0.2-0.3)	0.252
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.5 (0.3-1.4)	0.4 (0.3-0.9)	0.353
Ribonic acid	0.004 (0.003-0.005)	0.004 (0.003-0.005)	0.721
Uracil	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.906
Uric acid	10.3 (6.7-19.8)	12.9 (10.6-22.4)	**0.023**
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.304
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	186.3 (154.5-233.9)	320.0 (267.5-374.3)	**1.9×10^{-12}**

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**).

Table S4. Energy metabolism characteristics of basal and post-QTA patients.

	Basal (n=48)	**Post-QTA** (n=9)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.02 (0.01-0.03)	0.04 (0.03-0.05)	**0.024**
d-Mannonic acid	0.03 (0.02-0.03)	0.04 (0.03-0.04)	**0.046**
Galactose metabolism			
d-Galactitol	0.02 (0.01-0.02)	0.02 (0.02-0.03)	0.134
Glycolysis			
3-Phosphoglyceric acid	0.08 (0.04-0.2)	0.06 (0.04-0.08)	0.365
Glucose 6-phosphate	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.704
Lactic acid	61.3 (55.7-73.8)	65.9 (57.7-95.5)	0.376
Nucleotide sugar			
d-Arabinose	0.02 (0.02-0.03)	0.03 (0.01-0.04)	0.447
d-Threitol	0.03 (0.02-0.03)	0.03 (0.03-0.03)	0.254
d-Xylose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.751
Erythronic acid	0.03 (0.02-0.04)	0.03 (0.03-0.04)	0.792
Threonic acid	0.3 (0.3-0.5)	0.3 (0.2-0.4)	0.442
Xylonic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.179
Pentose glucoronate interconversion			
d-Arabitol	0.01 (0.01-0.01)	0.01 (0.01-0.02)	0.280
d-Xylitol	0.001 (0.001-0.002)	0.002 (0.001-0.002)	0.462
Galacturonic acid	0.02 (0.02-0.02)	0.02 (0.02-0.03)	0.220
Myo-Inositol	0.6 (0.5-0.7)	0.7 (0.5-0.9)	0.287
Sucrose metabolism			
d-Sucrose	0.008 (0.003-0.01)	0.01 (0.01-0.02)	0.056
Maltose	0.02 (0.01-0.04)	0.01 (0.01-0.02)	0.776
TCA			
α-ketoglutaric acid	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.598
Citric acid	20.8 (15.8-27.3)	18.3 (12.7-28.7)	0.376
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.03-0.04)	0.198
Fumaric acid	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.278
Glutamine	33.8 (26.5-40.3)	32.6 (32.5-34.7)	0.658
Malic acid	0.07 (0.06-0.09)	0.08 (0.05-0.09)	0.681
Pyruvic acid	4.6 (2.8-6.6)	10.3 (7.17-10.6)	**0.008**
Succinic acid	0.07 (0.06-0.1)	0.06 (0.05-0.07)	0.167
Glyoxylate and dicarboxylate			
Glycolic acid	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.555
Pentose phosphate pathway			
Sedoheptulose	0.01 (0.01-0.02)	0.02 (0.01-0.02)	0.197
Amino acid metabolism			
Alanine and aspartate			
Alanine	24.3 (21.3-28.6)	27.4 (23.1-30.4)	0.158
Glutamic acid	5.6 (3.4-7.8)	5.1 (4.7-6.4)	0.899
Arginine and proline			
2-Hydroxyisobutyric acid	1.4 (1.1-1.7)	1.5 (1.2-2.0)	0.487
Urea	33.8 (26.9-40.9)	32.6 (28.7-60.5)	0.448
Proline	30.2 (24.2-41.4)	39.1 (21.1-51.8)	0.658
4-Hydroxyproline	1.4 (1.1-1.9)	1.1 (0.8-1.4)	0.073
Ornithine	12.8 (9.7-14.8)	18.8 (14.9-20.7)	**0.011**
Oxoproline	25.3 (21.0-28.8)	23.9 (23.5-27.1)	0.866
Glycine and Serine			
Glyceric acid	0.5 (0.4-0.6)	0.3 (0.3-0.4)	**0.015**
Glycine	6.7 (6.1-7.2)	6.9 (6.7-7.9)	0.195
Serine	8.2 (6.5-9.5)	8.6 (8.2-10.3)	0.238
Threonine	7.9 (6.8-8.9)	8.4 (6.3-10.9)	0.513
Tyrosine metabolism			
Vanillylmandelic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.244

Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.9 (2.8-5.6)	2.6 (2.0-4.7)	0.273
3-methyl-2-oxobutyric acid	0.6 (0.4-0.7)	0.4 (0.3-0.7)	0.354
3-hydroxybutyric acid	8.6 (5.1-24.7)	9.4 (5.2-10.1)	0.555
2-Hydroxyisovaleric acid	5.2 (3.7-7.5)	5.5 (4.1-7.1)	0.950
2-keto-3-methylvaleric acid	1.1 (0.9-1.3)	1.3 (1.1-1.7)	0.114
3-Hydroxyisovaleric acid	0.9 (0.7-1.2)	1.0 (0.9-1.4)	0.080
Ethylmalonic acid	2.2 (1.7-2.9)	3.8 (2.2-4.2)	**0.023**
Isoleucine	4.6 (3.8-5.3)	6.5 (4.5-7.0)	0.088
Leucine	8.9 (7.8-10.7)	12.0 (8.7-12.8)	0.214
Valine	16.9 (14.8-20.3)	20.0 (17.7-20.5)	0.332
Cysteine and methionine metabolism			
Methionine	3.6 (3.4-3.9)	3.7 (3.4-4.1)	0.411
Tryptophan metabolism			
Indole-3-propanoic acid	0.2 (0.1-0.2)	0.1 (0.07-0.1)	0.129
Indolelactic acid	0.1 (1-0.2)	0.2 (0.1-0.3)	0.500
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.1-0.2)	0.3 (0.1-0.3)	0.184
Benzoic acid	0.6 (0.5-0.7)	0.7 (0.5-0.8)	0.246
Hippuric acid	0.2 (0.1-0.4)	0.3 (0.2-0.5)	0.697
Hydrocinnamic acid	0.05 (0.03-0.09)	0.07 (0.05-0.08)	0.712
Phenylalanine	6.4 (5.7-7.2)	8.3 (7.1-8.7)	**0.011**
Lipid metabolism			
Lipids			
Dodecanoic acid	0.6 (0.4-0.8)	2.0 (1.4-2.4)	**2.0×10^{-04}**
Linoleic acid	2.8 (1.6-4.7)	2.2 (1.3-3.1)	0.246
Oleic acid	17.9 (11.4-24.8)	18.9 (7.0-29.2)	0.613
Tetradecanoic acid	1.3 (1.0-2.1)	2.1 (1.5-2.5)	0.077
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.080
Glycerol	0.8 (0.5-1.0)	1.0 (0.9-1.4)	**0.017**
Glycerol-1-phosphate	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.188
Primary bile acid biosynthesis			
Taurine	0.9 (0.6-1.2)	1.1 (0.9-1.2)	0.292
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.2 (0.1-0.3)	0.2 (0.2-0.5)	0.229
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.5 (0.3-1.4)	0.3 (0.3-0.6)	0.234
Ribonic acid	0.004 (0.003-0.005)	0.005 (0.002-0.007)	0.958
Uracil	0.02 (0.02-0.03)	0.02 (0.01-0.03)	0.665
Uric acid	10.3 (6.7-19.8)	17.3 (11.9-26.3)	0.152
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.056
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	186.3 (154.5-233.9)	358.2 (223.2-483.6)	**0.022**

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**). QTA: adjuvant chemotherapy.

Table S5. Energy metabolism characteristics of basal and post-RT patients.

	Basal (n=48)	**Post-RT** (n=24)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.02 (0.01-0.03)	0.02 (0.01-0.04)	0.703
d-Mannonic acid	0.03 (0.02-0.03)	0.03 (0.02-0.04)	0.171
Galactose metabolism			
d-Galactitol	0.02 (0.01-0.02)	0.02 (0.01-0.02)	0.777
Glycolysis			
3-Phosphoglyceric acid	0.08 (0.04-0.2)	0.04 (0.03-0.07)	**0.003**
Glucose 6-phosphate	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.459
Lactic acid	61.3 (55.7-73.8)	62.2 (52.4-92.4)	0.853
Nucleotide sugar			
d-Arabinose	0.02 (0.02-0.03)	0.02 (0.02-0.04)	0.320
d-Threitol	0.03 (0.02-0.03)	0.03 (0.02-0.04)	0.205
d-Xylose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.477
Erythronic acid	0.03 (0.02-0.04)	0.03 (0.02-0.04)	0.926
Threonic acid	0.3 (0.3-0.5)	0.4 (0.3-0.5)	0.481
Xylonic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.271
Pentose glucoronate interconversion			
d-Arabitol	0.01 (0.01-0.02)	0.02 (0.01-0.02)	**0.005**
d-Xylitol	0.001 (0.001-0.002)	0.002 (0.001-0.002)	0.069
Galacturonic acid	0.02 (0.01-0.02)	0.02 (0.02-0.03)	0.091
Myo-Inositol	0.6 (0.5-0.7)	0.7 (0.5-0.8)	0.095
Sucrose metabolism			
d-Sucrose	0.008 (0.003-0.01)	0.01 (0.01-0.02)	0.055
Maltose	0.02 (0.01-0.04)	0.01 (0.01-0.02)	**6.6×10^{-5}**
TCA			
α-ketoglutaric acid	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.350
Citric acid	20.8 (15.8-27.3)	21.2 (17.6-25.0)	0.835
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.04-0.05)	0.669
Fumaric acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.086
Glutamine	33.8 (26.5-40.3)	36.1 (25.3-38.9)	0.853
Malic acid	0.07 (0.06-0.09)	0.06 (0.06-0.08)	0.449
Pyruvic acid	4.6 (2.8-6.6)	7.9 (5.5-10.3)	**5.9×10^{-4}**
Succinic acid	0.07 (0.06-0.1)	0.07 (0.05-0.07)	0.116
Glyoxylate and dicarboxylate			
Glycolic acid	0.1 (0.1-0.2)	0.1 (0.2-0.3)	**1.6×10^{-4}**
Pentose phosphate pathway			
Sedoheptulose	0.01 (0.01-0.02)	0.02 (0.01-0.02)	**0.032**
Amino acid metabolism			
Alanine and aspartate			
Alanine	24.3 (21.3-28.6)	28.4 (20.9-30.0)	0.192
Glutamic acid	5.6 (3.5-7.8)	4.07 (3.0-7.0)	0.127
Arginine and proline			
2-Hydroxyisobutyric acid	1.4 (1.1-1.8)	1.8 (1.3-2.4)	**0.032**
Urea	33.8 (26.9-41.0)	45.3 (35.7-53.4)	**0.001**
Proline	30.2 (24.2-41.4)	32.8 (26.1-38.6)	0.945
4-Hydroxyproline	1.4 (1.1-1.9)	1.4 (1.0-2.0)	0.712
Ornithine	12.8 (9.7-14.8)	12.4 (9.9-16.7)	0.862
Oxoproline	25.3 (21.0-28.8)	21.6 (17.1-25.6)	**0.009**
Glycine and Serine			
Glyceric acid	0.5 (0.4-0.6)	0.3 (0.3-0.5)	**0.002**
Glycine	6.7 (6.1-7.2)	7.0 (6.4-7.4)	0.103
Serine	8.2 (6.5-9.5)	8.8 (7.3-10.2)	0.182
Threonine	7.9 (6.8-9.0)	9.2 (7.4-10.3)	**0.030**
Tyrosine metabolism			
Vanillylmandelic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.067

Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.9 (2.8-5.6)	4.0 (3.2-6.4)	0.474
3-methyl-2-oxobutyric acid	0.6 (0.4-0.7)	0.7 (0.6-0.8)	**0.014**
3-hydroxybutyric acid	8.6 (5.1-24.7)	9.3 (5.3-15.2)	0.945
2-Hydroxyisovaleric acid	5.2 (3.7-7.5)	5.0 (4.0-6.4)	0.699
2-keto-3-methylvaleric acid	1.1 (0.9-1.3)	1.3 (1.0-1.8)	0.079
3-Hydroxyisovaleric acid	0.9 (0.7-1.2)	1.1 (0.9-1.4)	**0.038**
Ethylmalonic acid	2.2 (1.7-2.9)	3.2 (2.3-4.2)	**0.003**
Isoleucine	4.6 (3.9-5.3)	4.6 (4.4-6.0)	0.350
Leucine	8.9 (7.8-10.7)	9.8 (8.8-10.9)	0.092
Valine	16.9 (14.8-20.3)	18.8 (17.4-21.1)	**0.035**
Cysteine and methionine metabolism			
Methionine	3.6 (3.4-3.9)	3.7 (3.6-4.1)	**0.019**
Tryptophan metabolism			
Indole-3-propanoic acid	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.100
Indolelactic acid	0.2 (0.1-0.2)	0.2 (0.1-0.20)	0.243
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.1-0.2)	0.2 (0.2-0.3)	0.332
Benzoic acid	0.6 (0.5-0.7)	0.6 (0.5-0.9)	0.470
Hippuric acid	0.2 (0.1-0.4)	0.3 (0.2-0.4)	0.583
Hydrocinnamic acid	0.1 (0.03-0.09)	0.07 (0.04-0.1)	0.202
Phenylalanine	6.4 (5.7-7.2)	6.7 (6.4-7.3)	0.133
Lipid metabolism			
Lipids			
Dodecanoic acid	0.6 (0.4-0.8)	1.8 (1.6-2.2)	**4.0×10^{-8}**
Linoleic acid	2.8 (1.6-4.7)	2.5 (1.4-3.5)	0.406
Oleic acid	17.9 (11.34-24.8)	17.7 (8.4-22.4)	0.533
Tetradecanoic acid	1.3 (1.0-2.1)	2.4 (1.8-3.3)	**3.0×10^{-4}**
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.972
Glycerol	0.8 (0.5-1.0)	1.0 (0.7-1.3)	**0.028**
Glycerol-1-phosphate	0.2 (0.1-0.3)	0.2 (0.1-0.2)	0.310
Primary bile acid biosynthesis			
Taurine	0.9 (0.6-1.2)	0.8 (0.5-1.0)	0.341
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.2 (0.1-0.3)	0.3 (0.3-0.4)	**0.008**
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.5 (0.3-1.4)	0.3 (0.3-0.5)	**0.006**
Ribonic acid	0.004 (0.003-0.005)	0.004 (0.002-0.005)	0.193
Uracil	0.02 (0.02-0.03)	0.02 (0.01-0.02)	**0.029**
Uric acid	10.3 (6.7-19.8)	15.1 (12.4-20.0)	0.043
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0.303
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	186.3 (154.5-233.9)	277.07 (212.2-357.7)	**9.6×10^{-5}**

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**). RT: radiotherapy.

Table S6. Energy metabolism characteristics of post-surgery and post-RT patients.

	Post-surgery (n=42)	**Post-RT** (n=24)	**p-value**
Carbohydrate metabolism			
Fructose and mannose			
d-Fructose	0.03 (0.02-0.07)	0.02 (0.01-0.04)	**0.024**
d-Mannonic acid	0.03 (0.03-0.04)	0.03 (0.02-0.04)	0.393
Galactose metabolism			
d-Galactitol	0.02 (0.02-0.03)	0.02 (0.02-0.02)	0.171
Glycolysis			
3-Phosphoglyceric acid	0.07 (0.04-0.14)	0.04 (0.03-0.07)	**0.014**
Glucose 6-phosphate	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.556
Lactic acid	79.2 (66.7-108.8)	62.2 (52.4-92.4)	**0.021**
Nucleotide sugar			
d-Arabinose	0.03 (0.02-0.04)	0.02 (0.02-0.04)	0.280
d-Threitol	0.03 (0.02-0.03)	0.03 (0.02-0.04)	0.743
d-Xylose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.451
Erythronic acid	0.03 (0.02-0.03)	0.03 (0.02-0.04)	0.423
Threonic acid	0.3 (0.3-0.4)	0.4 (0.30-0.5)	0.185
Xylonic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.841
Pentose glucoronate interconversion			
d-Arabitol	0.01 (0.01-0.02)	0.02 (0.01-0.02)	0.159
d-Xylitol	0.002 (0.001-0.002)	0.002 (0.001-0.002)	0.930
Galacturonic acid	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.313
Myo-Inositol	0.7 (0.6-0.9)	0.7 (0.5-0.8)	0.501
Sucrose metabolism			
d-Sucrose	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.483
Maltose	0.02 (0.01-0.05)	0.01 (0.01-0.02)	**0.001**
TCA			
α-ketoglutaric acid	0.1 (0.1-0.2)	0.2 (0.1-0.2)	0.156
Citric acid	14.6 (12.8-22.6)	21.2 (17.6-25.0)	**0.008**
DL-2-Hydroxyglutaric acid	0.04 (0.03-0.05)	0.04 (0.04-0.05)	0.894
Fumaric acid	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.904
Glutamine	29.8 (25.3-33.3)	36.1 (25.3-38.9)	0.098
Malic acid	0.07 (0.05-0.08)	0.06 (0.06-0.08)	0.631
Pyruvic acid	7.7 (4.8-10.3)	7.9 (5.5-10.3)	0.472
Succinic acid	0.07 (0.05-0.1)	0.07 (0.05-0.07)	0.334
Glyoxylate and dicarboxylate			
Glycolic acid	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.540
Pentose phosphate pathway			
Sedoheptulose	0.02 (0.02-0.03)	0.02 (0.01-0.02)	0.301
Amino acid metabolism			
Alanine and aspartate			
Alanine	28.6 (25.5-31.8)	28.4 (21.0-30.0)	0.298
Glutamic acid	5.8 (4.3-8.4)	4.1 (3.0-7.0)	**0.047**
Arginine and proline			
2-Hydroxyisobutyric acid	1.7 (1.3-2.1)	1.8 (1.3-2.4)	0.650
Urea	44.1 (35.8-55.2)	45.3 (35.7-53.4)	0.926
Proline	34.7 (27.5-41.4)	32.8 (26.1-38.6)	0.505
4-Hydroxyproline	1.2 (0.9-1.7)	1.4 (1.0-2.0)	0.292
Ornithine	14.8 (11.4-18.7)	12.4 (9.9-16.7)	0.110
Oxoproline	27.8 (24.-32.8)	21.6 (17.1-25.6)	**5.7×10^{-5}**
Glycine and Serine			
Glyceric acid	0.4 (0.3-0.6)	0.3 (0.3-0.5)	**0.033**
Glycine	7.4 (6.6-7.8)	7.0 (6.4-7.4)	0.158
Serine	7.8 (7.3-9.6)	8.8 (7.3-10.2)	0.317
Threonine	8.4 (6.9-9.6)	9.2 (7.4-10.3)	0.166
Tyrosine metabolism			
Vanillylmandelic acid	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.533

Valine, leucine and isoleucine			
2-Hydroxybutyric acid	3.8 (2.1-5.6)	4.0 (3.2-6.4)	0.129
3-methyl-2-oxobutyric acid	0.6 (0.5-0.7)	0.7 (0.6-0.8)	**0.018**
3-hydroxybutyric acid	5.4 (3.7-10.2)	9.3 (5.3-15.2)	**0.019**
2-Hydroxyisovaleric acid	5.8 (4.2-7.8)	5.0 (4.0-6.4)	0.220
2-keto-3-methylvaleric acid	1.4 (1.2-1.7)	1.3 (1.0-1.8)	0.435
3-Hydroxyisovaleric acid	1.1 (0.8-1.3)	1.1 (0.9-1.4)	0.472
Ethylmalonic acid	3.1 (2.5-4.2)	3.2 (2.3-4.2)	0.862
Isoleucine	5.3 (4.5-6.0)	4.6 (4.4-6.0)	0.252
Leucine	10.0 (8.5-11.6)	9.8 (8.8-10.9)	0.905
Valine	19.5 (16.7-21.7)	18.8 (17.4-21.1)	0.810
Cysteine and methionine metabolism			
Methionine	3.8 (3.4-4.1)	3.7 (3.6-4.1)	0.311
Tryptophan metabolism			
Indole-3-propanoic acid	0.2 (0.2-0.3)	0.2 (0.1-0.2)	0.553
Indolelactic acid	0.2 (0.2-0.3)	0.2 (0.1-0.2)	0.081
Phenylalanine metabolism			
4-hydroxyPhenyllactic acid	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.739
Benzoic acid	0.7 (0.5-0.9)	0.6 (0.5-0.9)	0.218
Hippuric acid	0.2 (0.1-0.4)	0.3 (0.2-0.4)	0.198
Hydrocinnamic acid	0.07 (0.03-0.1)	0.07 (0.04-0.1)	0.995
Phenylalanine	6.3 (5.8-7.5)	6.7 (6.4-7.3)	0.246
Lipid metabolism			
Lipids			
Dodecanoic acid	1.3 (1.0-1.7)	1.8 (1.6-2.2)	**0.001**
Linoleic acid	1.9 (1.1-2.9)	2.5 (1.4-3.5)	0.114
Oleic acid	12.2 (7.1-21.7)	17.7 (8.4-22.4)	0.225
Tetradecanoic acid	1.8 (1.3-2.2)	2.4 (1.8-3.3)	**0.005**
Glycolipid metabolism			
Ethanolamine	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.883
Glycerol	1.1 (0.9-1.3)	1.0 (0.7-1.3)	0.185
Glycerol-1-phosphate	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.497
Primary bile acid biosynthesis			
Taurine	1.1 (0.7-1.4)	0.8 (0.5-1.0)	**0.018**
Metabolism of cofactors and vitamins			
Cofactor biosynthesis			
α-tocopherol	0.3 (0.2-0.3)	0.3 (0.3-0.4)	0.116
Nucleotide metabolism			
Purine and pyrimidine			
Hypoxanthine	0.4 (0.3-0.9)	0.3 (0.3-0.5)	**0.028**
Ribonic acid	0.004 (0.003-0.005)	0.004 (0.002-0.005)	0.322
Uracil	0.02 (0.02-0.03)	0.02 (0.01-0.02)	0.068
Uric acid	12.9 (10.6-22.4)	15.1 (12.4-20.0)	0.497
Xenobiotic biodegradation			
Benzoate degradation			
4-Hydroxybenzoic acid	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.963
Energy metabolism			
Oxidative phosphorylation			
Phosphoric acid	320.0 (267.5-374.3)	277.1 (212.2-357.7)	0.096

Values are provided as median (interquartile range) in RU (relative units). Significance was decided using Mann-Whitney U test. Values in bold indicate statistical significance (**p<0,05**). RT: radiotherapy.

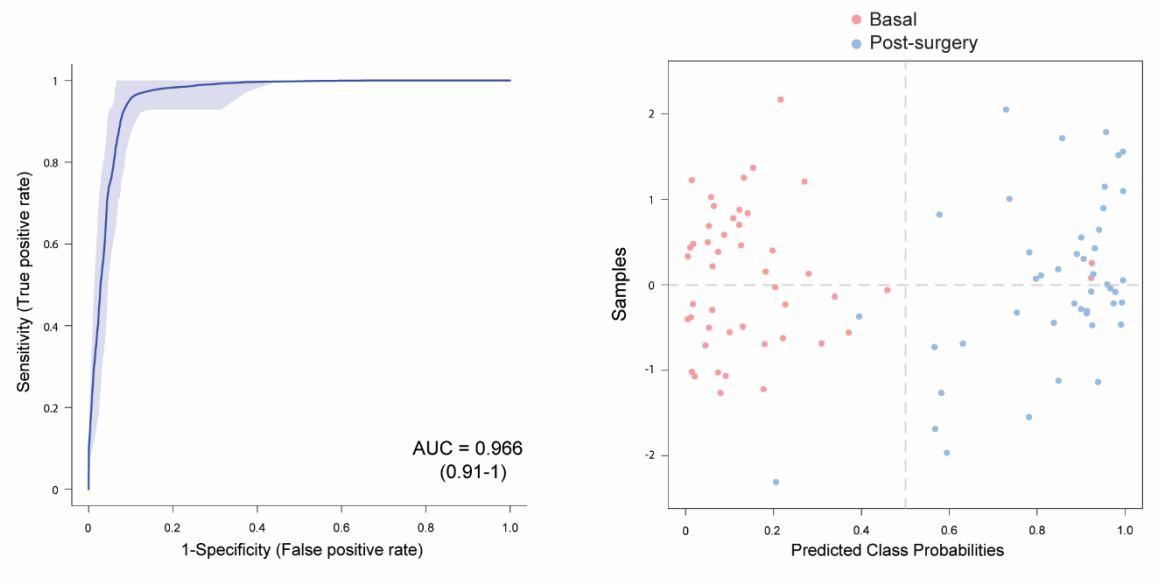

Figure S1. Receiver Operating Characteristic (ROC) curve and complexity matrix of d-sucrose, dodecanoic acid, d-fructose, phosphoric acid and 3-hydroxybutyric acid, based on comparison of basal/post-surgery samples.

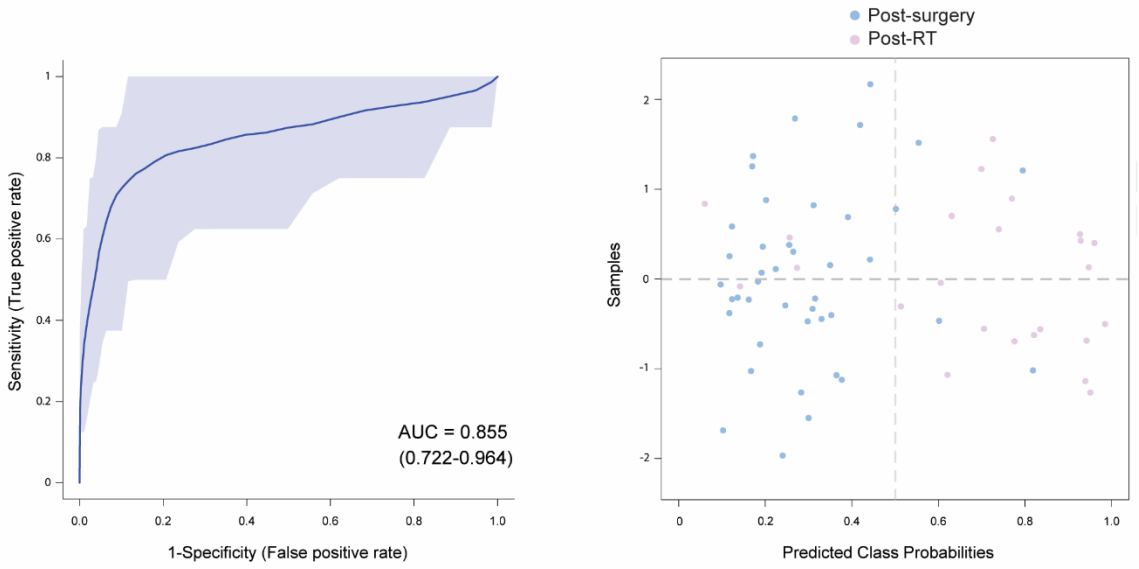

Figure S2. Receiver Operating Characteristic (ROC) curve and complexity matrix of maltose, d-fructose, 3-hydroxybutyric acid, 3-phosphoglyceric acid and hypoxanthine, based on comparison of post-surgery/post-RT samples. RT: radiotherapy.