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TITLE: Tumor Metabolism as the Achilles Heel in Prostate Cancer

PRINCIPAL INVESTIGATOR: Nagalakshmi Nadiminty, PhD

CONTRACTING ORGANIZATION: The University of Toledo

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Toledo
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3000 Arlington Avenue
Toledo, OH 43614

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The hypothesis of this proposal was that the inhibition of monocarboxylate transporters (MCTs) can overcome resistance to enzalutamide in therapy-resistant prostate cancer cells. In advanced prostate cancer, glycolysis produces high levels of the toxic by-product lactate. Hence, prostate cancer cells upregulate the expression of MCTs to aid in lactate export. High levels of expression of MCTs have been associated with poor prognosis and biochemical failure in prostate cancer. Our findings so far show that the inhibition of MCT activity can suppress survival and proliferation of enzalutamide-resistant cells preferentially and can inhibit the growth of enzalutamide-resistant xenografts. In addition, we found that the combination of MCT inhibitors with enzalutamide reduced basal and compensatory glycolysis as well as extracellular acidification rates in enzalutamide-resistant prostate cancer cells. Once completed, our project may have an enormous impact on the future of prostate cancer research.

15. SUBJECT TERMS NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

PCa energetic metabolism is unique. Normal prostate cells use glucose oxidation to synthesize and secrete citrate, resulting in incomplete Krebs cycle and minimal oxidative phosphorylation for energy production. In contrast, PCa cells do not secrete citrate, but reactivate the Krebs cycle as the energy source. The accumulation of the metabolic end-product lactate in either case is toxic. In response, cancer cells upregulate the expression of monocarboxylate transporters (MCTs) to increase lactate efflux, thereby reducing extracellular acidification. Our preliminary data showed that: 1) the expression of MCTs is higher in PCa tissues and PCa cells resistant to enzalutamide; 2) MCT antagonists resensitize resistant cells to enzalutamide; and 3) the inhibition of MCTs has no significant effects on normal prostate cells. Hence, we hypothesized that the **inhibition of MCTs in therapy-resistant PCa cells may augment the efficacy of targeted therapeutics such as enzalutamide**.

Specific aims proposed:

1) Test the combination of MCT inhibitors with enzalutamide in PCa cells in vitro
2) Test the combination of MCT inhibitors with enzalutamide in PCa cells and xenografts in vivo
3) Analyze the mechanisms governing the synergism between MCT inhibitors and enzalutamide

We believe MCT inhibition would be a highly innovative strategy to overcome enzalutamide resistance in PCa.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Prostate cancer, enzalutamide, androgen receptor, monocarboxylate transporter, metabolism, glycolysis, resistance

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Research-Specific Tasks:

Specific Aim 1: To test the combination of MCT inhibitors with enzalutamide in PCa cells *in vitro*				
Major Task 1: To characterize the effects of MCT inhibitors in				

enzalutamide-resistant cells in vitro				
Subtask 1: Treat cell lines with AR-C155858, AZD3965, or syrosingopine either singly or in combination with enzalutamide and analyze Cell lines used: C4-2B parental, C4-2B-MDVR; 22Rv1 parental, 22Rv1-MDVR; VCaP parental, VCaP-MDVR	1-6	Sayani Bhattacharjee	Completed; see Annual Report 2021	
Subtask 2: Confirm above effects with MCT shRNAs Cell lines used: C4-2B parental, C4-2B-MDVR; 22Rv1 parental, 22Rv1-MDVR; VCaP parental, VCaP-MDVR	7-12	Sayani Bhattacharjee	Generation of C4-2B-MDVR cells shRNAs completed; Viability assays completed; other assays in progress	
Subtask 3: Explore structure-activity relationships to optimize effects of MCT inhibitors in collaboration with College of Pharmacy, UT	9-12	TBD	In progress; no results to report yet	
Subtask 4: Generate resistant cell lines and analyze relative activities of MCT inhibitors	10-14	Dr. Nadiminty, Sayani Bhattacharjee	Cell lines resistant to AR-C, AZD, or syrosingopine generated; viability assays completed; other assays in progress	
Milestone(s) Achieved: Characterization of the effects of MCT inhibitors in vitro				
Specific Aim 2: To test the combination of MCT inhibitors with enzalutamide in PCa cell xenografts and patient-derived xenografts *in vivo*				
Major Task 2: To characterize the effects of MCT inhibitors in enzalutamide-resistant cells and PDX models in vivo				
Subtask 1: Submit documents for ACURO approvals	8-12	Dr. Nadiminty	Completed; approval granted 04/13/2021	
Subtask 2: Generate xenografts of enzalutamide-resistant cells in SCID mice, treat with MCT inhibitors either singly or in combination with	12-16	Dr. Nadiminty, Sayani Bhattacharjee	Completed work with C4-2B and C4-2B-MDVR cells treated with AR-C155858 and AZD3965 and reported in	

enzalutamide Cell lines used: C4-2B parental, C4-2B-MDVR; VCaP parental, VCaP-MDVR Animals requested: 352; Supplier: Charles River			Annual Report 2021; In Annual report 2022, we report work with C4-2B/C4-2B-MDVR cell xenografts treated with syrosingopine; and VCaP/VCaP-MDVR cell xenografts treated with AR-C155858, AZD3965, or syrosingopine singly or in combination with enzalutamide	
Subtask 3: Pharmacokinetic analyses of MCT inhibitors in SCID mice	15-18	Dr. Nadiminty, Sayani Bhattacharjee	Planned in the next reporting period due to Dr. Sarver's retirement	
Subtask 4: Analyze the effects of MCT inhibitors in PDX models Animals requested: 120; Supplier: Living Tumor lab	15-21	Dr. Nadiminty, Dr. Petros, Sayani Bhattacharjee	In progress since Summer 2022; no results to report yet	
Milestone(s) Achieved: Characterization of the effects of MCT inhibitors and their pharmacokinetic/toxicity profiles in vivo				
Specific Aim 3: Analyze the mechanisms governing the synergistic activity of MCT inhibitor and enzalutamide combination				
Major Task 3: Metabolomic analyses to analyze the pathways involved in enzalutamide resistance and the effects of MCT inhibitors				
Subtask 1: Analyze extracellular flux, glucose metabolism, intracellular lactate levels, cellular acidification, and cell death in cells treated with MCT inhibitors	21-24	Dr. Nadiminty, Sayani Bhattacharjee	Completed experiments with extracellular acidification, glycolysis, and cell death; see Annual Report 2021	
Subtask 2: Perform LC-MS metabolomic analyses of cellular metabolite levels	24-30	Dr. Nadiminty, Dr. Matam Vijay Kumar, Sayani Bhattacharjee	Targeted Metabolomics analyses of central carbon metabolites completed; data being analyzed; see accomplishments in Annual report 2022	

Subtask 3: Analyze results and repeat/optimize experiments, if necessary	30-34	Dr. Nadiminty, Sayani Bhattacharjee	We have submitted MCT inhibitor and combination-treated samples for gene expression analyses using the Nanostring nCounter technology	
Major Task 4: Prepare data for publication and submit 1-2 manuscripts	30-36	Dr. Nadiminty, Sayani Bhattacharjee and/or Graduate student (TBD)	We are preparing a manuscript for publication with results obtained so far	
Milestones achieved: 1-2 peer-reviewed journal articles				

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities:

a) To generate C4-2B-MDVR or VCaP-MDVR cells expressing specific shRNAs against MCTs 1,2, or 4 and confirm effects are due to MCT expression
b) To generate C4-2B-MDVR cells resistant to AR-C155858, AZD3965, or syrosngopine and assess response to MCT inhibitors
c) To generate C4-2B/C4-2B-MDVR cell xenografts in SCID mice and treat with syrosingopine singly or in combination with enzalutamide
d) To generate VCaP/VCaP-MDVR cell xenografts in SCID mice and treat with AR-C155858, AZD3965, or syrosingopine singly or in combination with enzalutamide
e) Perform targeted metabolomics analyses of C4-2B/C4-2B-MDVR cells treated with MCT inhibitors singly or in combination with enzalutamide.

2) Specific objectives:

a) We generated C4-2B-MDVR cells expressing shRNAs against MCTs 1, 2, or 4 by stable transfection. The cells were treated with vehicle-DMSO or 20 μM enzalutamide for 72 h. Cell viability was measured using the Cell Viability Fluor kit (Promega).

b) We generated VCaP-MDVR cells expressing shRNAs against MCTs 1, 2, or 4 by stable transfection. The cells were treated with vehicle-DMSO or 20 μM enzalutamide for 72 h. Cell viability was measured using the Cell Viability Fluor kit (Promega).
c) We generated C4-2B-MDVR cells resistant to AR-C155858 or AZD3965 by chronic culture in increasing concentrations from 20 μM-2 mM. C4-2B-MDVR cells resistant to syrosingopine were generated by chronic culture in increasing concentrations of syrosingopine from 10 μM-100 μM. Resistance to the respective MCT inhibitors was confirmed by passaging in the highest concentration attained for at least 5 passages. Cells were subsequently maintained in media containing MCT inhibitors. These cells grow slowly and are very sensitive to even slight changes in growth conditions. C4-2B-MDVR cells resistant to AR-C155858 were treated with 5 or 10 μM AZD3965 or syrosingopine; cells resistant to AZD3965 were treated with 5 or 10 μM AR-C155858 or syrosingopine; and cells resistant to syrosingopine were treated with 5 or 10 μM AR-C155858 or AZD3965 for 72 h. Cell viability was measured using the Cell Viability Flour kit (Promega). Other assays are in progress.
d) We generated sub-cutaneous xenografts of C4-2B and C4-2B-MDVR cells in SCID mice (n=10/group). After the tumors were palpable, we divided them randomly into 4 groups and treated via oral gavage daily with 1) vehicle, 2) 25 mg/kg enzalutamide, 3) 10 mg/kg Syrosingopine, or 4) Enza+Syrosingopine. We measured tumor growth twice a week and mouse weights twice a week. When the control tumors reached a size of 2 cm^3, the mice were euthanized, and tumor tissues were collected.
e) We generated sub-cutaneous xenografts of VCaP and VCaP-MDVR cells in SCID mice (n=10/group). After the tumors were palpable, we divided them randomly into 8 groups and treated via oral gavage daily with 1) vehicle, 2) 25 mg/kg enzalutamide, 3) 10 mg/kg AR-C155858, 4) 50 mg/kg AZD3965, 5) 10 mg/kg syrosingopine, 6) Enza+AR-C155858, 7) Enza+AZD3965, or 8) Enza+Syrosingopine. We measured tumor growth twice a week and mouse weights twice a week. When the control tumors reached a size of 2 cm^3, the mice were euthanized, and tumor tissues were collected.
f) We performed Targeted metabolomics of central carbon metabolites using the C-Scope assay of Human Metabolome Technologies Inc. We followed sample preparation instructions provided by the service provider (please see appended files). The data were analyzed to confirm that MCT inhibitors trapped lactate in the treated cells. Further analyses are in progress.
g) We are analyzing the significant results and preparing a manuscript. The graduate student working on this project, Sayani Bhattacharjee, is set to graduate by the end of the Fall 2022 semester.

3) Key outcomes: (please see pages at the end of this document for figures)

a) We confirmed using shRNAs against MCTs 1, 2, and 4 that the observed effects of MCT inhibitors on cell survival and proliferation are due to MCT expression (Fig. 1A and B).
b) C4-2B-MDVR cells resistant to each of the MCT inhibitors showed that even with metabolic adaptation due to resistance, the cells retain sensitivity to other MCT inhibitors (Fig. 2A, B, and C). Confirmatory experiments are under way. These findings may have implications in translating the use of MCT inhibitors to the clinic.

c) Growth of C4-2B-MDVR xenografts was suppressed significantly when treated with a combination of syrosingopine and enzalutamide (Fig. 3A and B), confirming our previous results with AR-C155858 and AZD3965.
d) Growth of VCaP-MDVR xenografts was significantly inhibited when treated with combinations of AR-C155858 (Fig. 4A and B), AZD3965 (Fig. 4C and D), or syrosingopine (Fig. 4E and F) with enzalutamide. These results confirmed that enzalutamide-resistant PCa cells are more sensitive to MCT inhibition.
e) Targeted metabolomics revealed that MCT inhibitors either singly or in combination with enzalutamide succeeded in trapping lactate inside the PCa cells. In line with the higher expression of MCTs in MDVR cells, very low lactate concentrations were found in MDVR cells treated with vehicle. These results indicated that MCTs are actively pumping lactate produced from glycolysis out of the cells. These results also confirmed our previous results from Glycolytic rate assays that extracellular acidification rates were higher in C4-2B-MDVR cells compared with C4-2B parental cells. Similarly, we also confirmed that lactate retention is higher in MDVR cells treated with the combination of enzalutamide with MCT inhibitors. Collectively, these results demonstrate that: a) enzalutamide-resistant PCa cells have higher rates of glycolysis; b) MCT expression is higher in enzalutamide-resistant PCa cells; c) MCT inhibitors trap lactate inside the cells; d) more lactate is produced and trapped in MDVR cells; e) more lactate is trapped in MDVR cells treated with Enza+MCT inhibitors.
f) **Conclusions**: Taken together, our results so far confirm our hypothesis that enzalutamide-resistance may be overcome using MCT inhibition. Work under way will shed more light on the mechanisms and pathways potentially involved.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project was not intended to provide training opportunities. However, the graduate student working on the project, Sayani Bhattacharjee, was able to attend the Annual Meeting of the American Association for Cancer Research (AACR) 2022 in New Orleans, LA and present the following poster in person.

Bhattacharjee S, Doan JP, Wynn RM, Nadiminty N. Targeting MCT inhibition to overcome enzalutamide resistance in prostate cancer. AACR Annual Meeting 2022 Proceedings.

Work from this project also enabled the PI, Nagalakshmi Nadiminty, to apply for and be selected to participate in the 2021-22 FDA-AACR Oncology Educational Fellowship held virtually due to COVID restrictions.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Our original plan for the 2021-2022 reporting period included pharmacokinetic analyses in collaboration with Dr. Sarver in the College of Pharmacy. However, these plans have received a set back due to Dr. Sarver's retirement in late 2021. We hope to be able to find a replacement soon and plan to complete these analyses in the next reporting period. We will also complete the PDX model assays in the next reporting period.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We will find a replacement for Dr. Sarver to perform the pharmacokinetic analyses.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No changes to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Bhattacharjee, Doan JP, Wynn RM, Sindhwani P, Petros FG, Nadiminty N. (in preparation) Monocarboxylate transporter inhibition overcomes enzalutamide resistance in prostate cancer cells. (To be submitted after including shRNA results)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Bhattacharjee S, Doan JP, Wynn RM, Nadiminty N. Targeting MCT inhibition to overcome enzalutamide resistance in prostate cancer. AACR Annual Meeting 2022 Proceedings.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1) Name:	Nagalakshmi Nadiminty
Project Role:	PI
ORCID:	0000-0003-3408-3206
Nearest person months worked:	3
Contribution to project:	Dr. Nadiminty has performed work with vertebrate animals and generated resistant cells.
Funding support:	College of Medicine and Life Sciences, University of Toledo
2) Name:	Sayani Bhattacharjee
Project Role:	Graduate Student
ORCID:	0000-0001-8781-5867
Nearest person months worked:	12
Contribution to project:	Ms. Bhattacharjee performed most of the in vitro work and assisted in the in vivo work.
Funding support:	None
3) Name:	Rebecca Wynn
Project Role:	Research Associate
ORCID:	0000-0002-2801-6798
Nearest person months worked:	3
Contribution to project:	Ms. Wynn assisted in in vivo work and acted as lab manager.
Funding support:	Department of Urology, COMLS, University of Toledo

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

All the following pages contain data and images that are unpublished. Please protect.

1) We transfected C4-2B-MDVR (Fig. 1A); or VCaP-MDVR (Fig. 1B) with specific shRNAs against MCT1, MCT2, or MCT4. The cells were then treated with vehicle-DMSO or 20 μM enzalutamide. Cell viability was assessed using the Cell viability assay kit (Promega).
2) We generated C4-2B-MDVR cells resistant to 2 mM AR-C155858, 2 mM AZD3965, or 100 μM syrosingopine by chronic culture in increasing concentrations of the respective MCT inhibitors. C-42B-MDVR cells resistant to AR-C were treated with 5 or 10 μM AZD3965 or syrosingopine; C4-2B-MDVR cells resistant to AZD were treated with 5 or 10 μM AR-C155858 or syrosingopine; C4-2B-MDVR cells resistant to syrosingopine were treated with 5 or 10 μM AR-C155858 or AZD3965; either singly or in combination with enzalutamide. Cell viability was assessed using the Cell viability assay kit (Promega). The results showed that even though metabolic adaptation blunted the efficacy of MCT inhibitors, PCa cells resistant to one of the MCT inhibitors still showed responses to the others. For example, C4-2B-MDVR cells resistant to AR-C155858 (Fig. 2A) were not responsive to treatment with AZD3965, but were still sensitive to syrosingopine. C4-2B-MDVR cells resistant to AZD3965 (Fig. 2B) were still responsive to AR-C155858 and syrosingopine, though at different levels. Similarly, C4-2B-MDVR cells resistant to syrosingopine (Fig. 2C) were still responsive to treatment with AR-C155858, but did not show appreciable response to AZD3965. These cells grow slowly and are very sensitive to even slight changes in growth conditions. We are continuing to analyze these cells using other measures of response such as clonogenic assays and glycolytic rate assays.
3) In the annual report for the 2020-2021 reporting period, we reported in vivo xenograft assays with C4-2B or C4-2B-MDVR cells treated with AR-C155858 or AZD3965 either singly or in combination with enzalutamide. Here we report xenograft assays with C4-2B and C4-2B-MDVR cell xenografts treated with syrosingopine either singly or in combination with enzalutamide. C4-2B-MDVR cell xenografts treated with a combination of syrosingopine and enzalutamide showed significant tumor growth inhibition compared with C4-2B parental cell xenografts (Fig. 3A and 3B).
4) We also performed in vivo mouse xenograft assays with VCaP and VCaP-MDVR cells treated with AR-C155858 (Fig. 4A and B), AZD3965 (Fig. 4C and D), or syrosingopine (Fig. 4E and F) either singly or in combination with enzalutamide. These assays indicated that MCT inhibitors synergize with enzalutamide to reduce tumor growth kinetics.
5) We performed targeted metabolomics analyses of 4-2B and C4-2B-MDVR cells treated with MCT inhibitors either singly or in combination with enzalutamide using the C-Scope assay from Human Metabolome Technologies Inc. This uses CE-TOFMS and CE-QqQMS technology to quantitatively measure 116 metabolites involved in glycolysis, pentose phosphate pathway, TCA cycle, urea cycle, and polyamine, creatine, purine, glutathione, nicotinamide, choline, and amino acid metabolism pathways. The results revealed that MCT inhibitors successfully trapped lactate inside the prostate cancer cells. Trapped lactate levels were much higher in cells treated with a combination of enzalutamide and MCT inhibitors. Higher amounts of lactate were retained in C4-2B-MDVR cells (Fig. 5; please also see appended files), indicating that enzalutamide-resistant cells produce higher levels of lactate due to higher levels of glycolysis. We are doing more in-depth analyses of the data with the help of tech support at Human Metabolome Technologies Inc. to reveal inter-relationships between metabolic pathways that may yield more insights into the mechanisms involved.

Fig. 1A

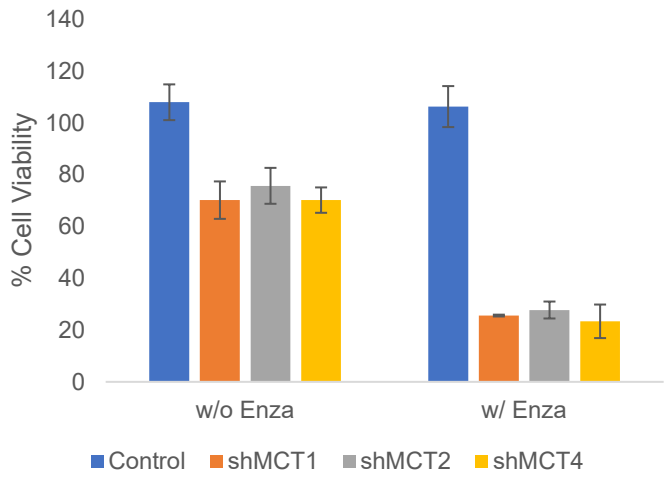


Fig. 1B

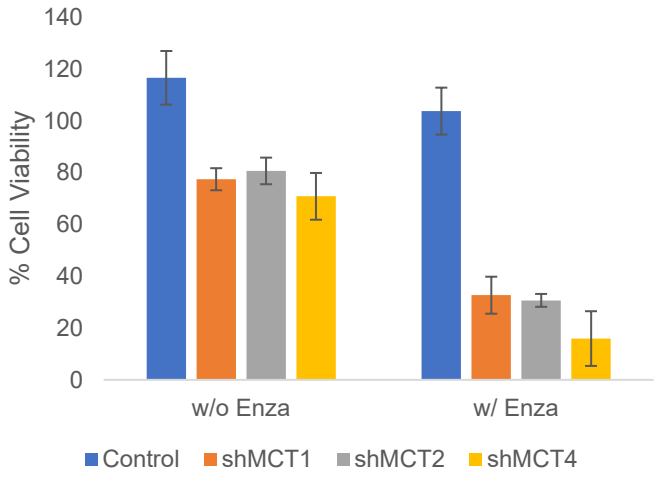


Fig. 2A

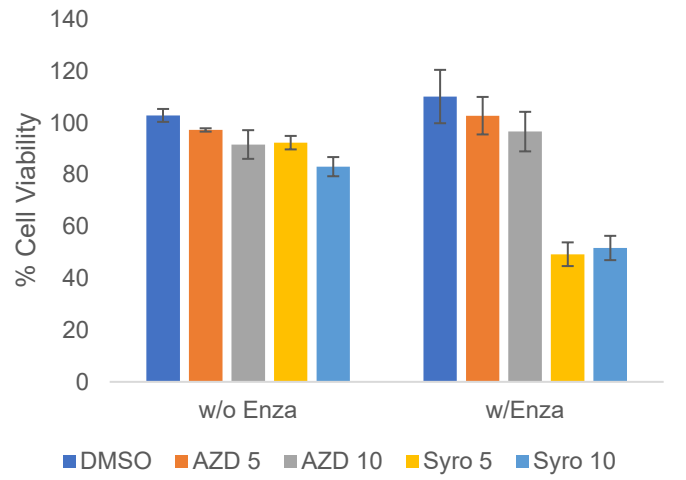


Fig. 2B

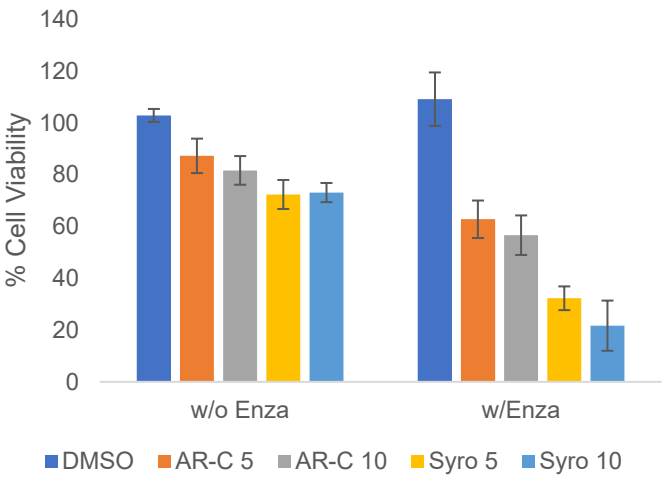


Fig. 2C

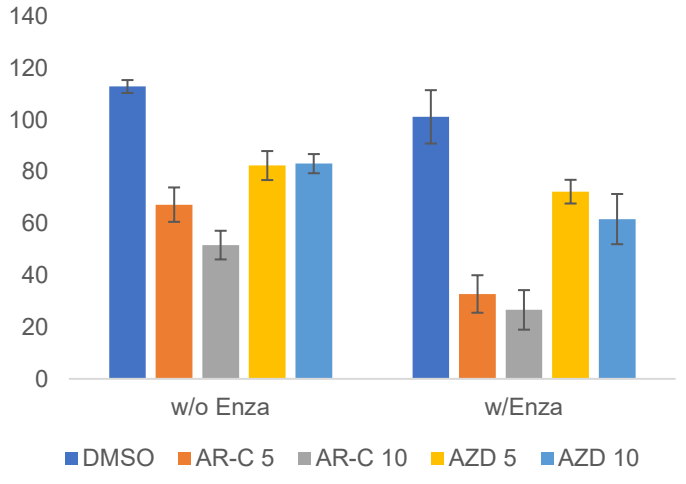


Fig. 3A

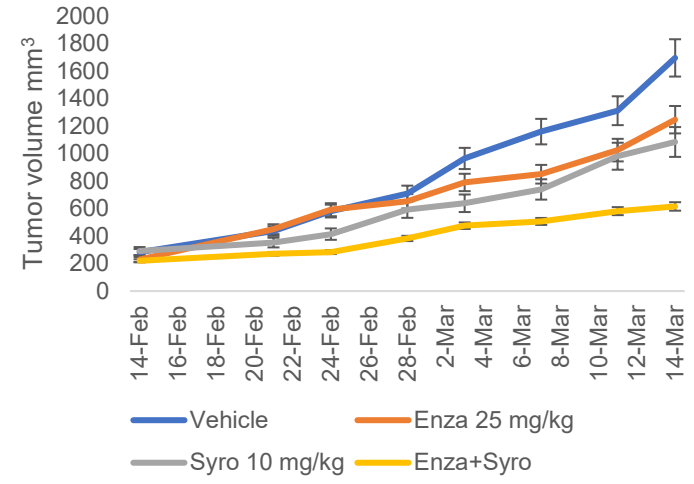


Fig. 3B

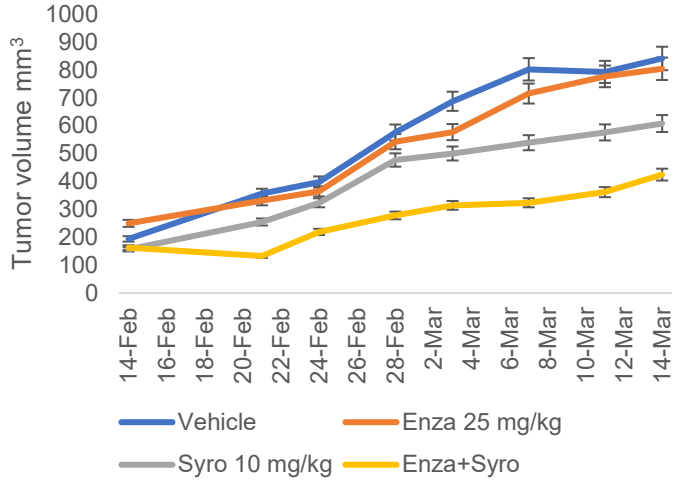


Fig. 4A

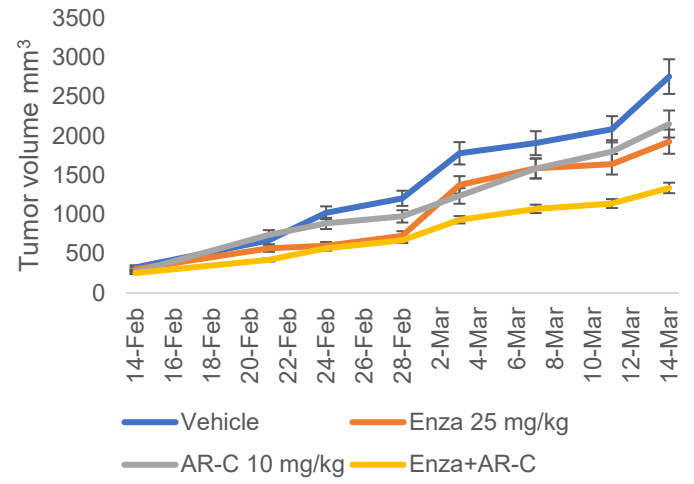


Fig. 4B

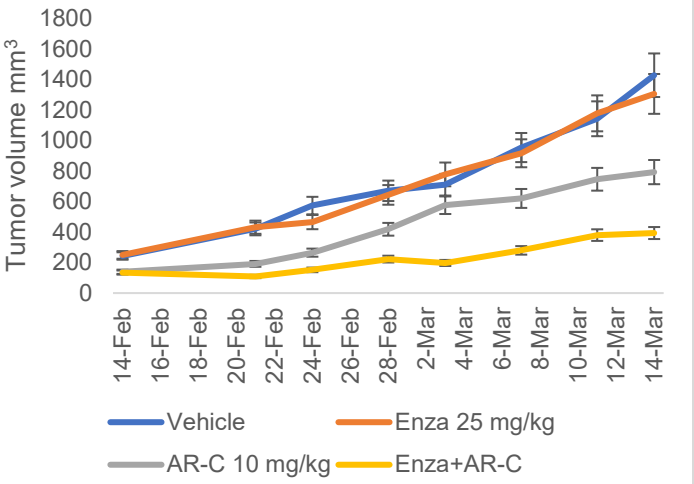


Fig. 4C

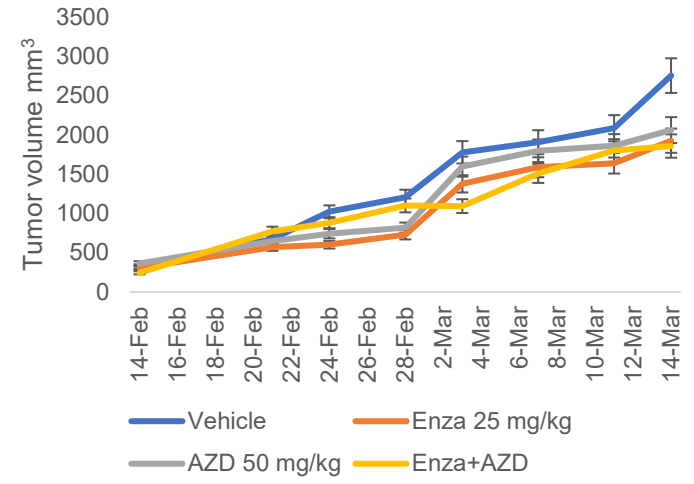


Fig. 4D

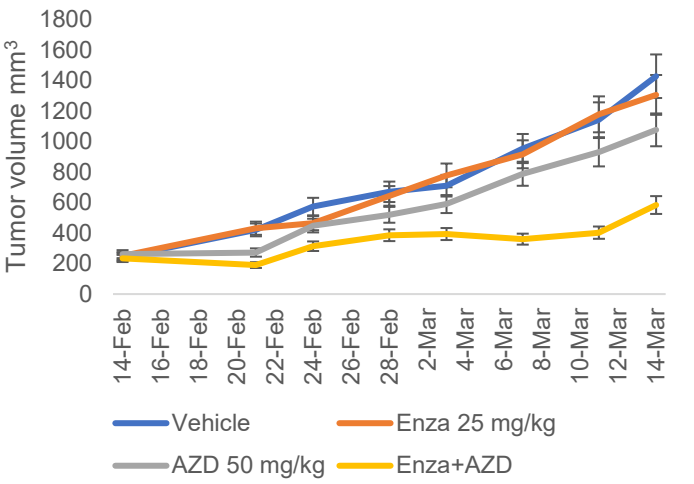


Fig. 4E

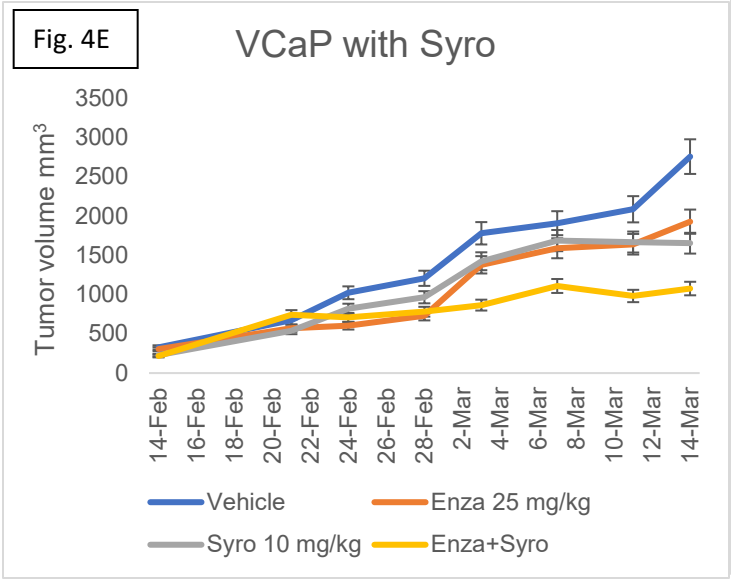


Fig. 4F

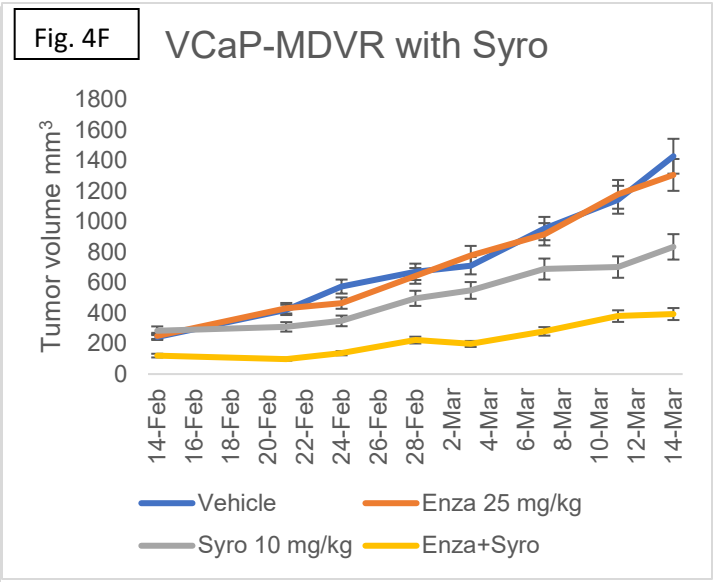
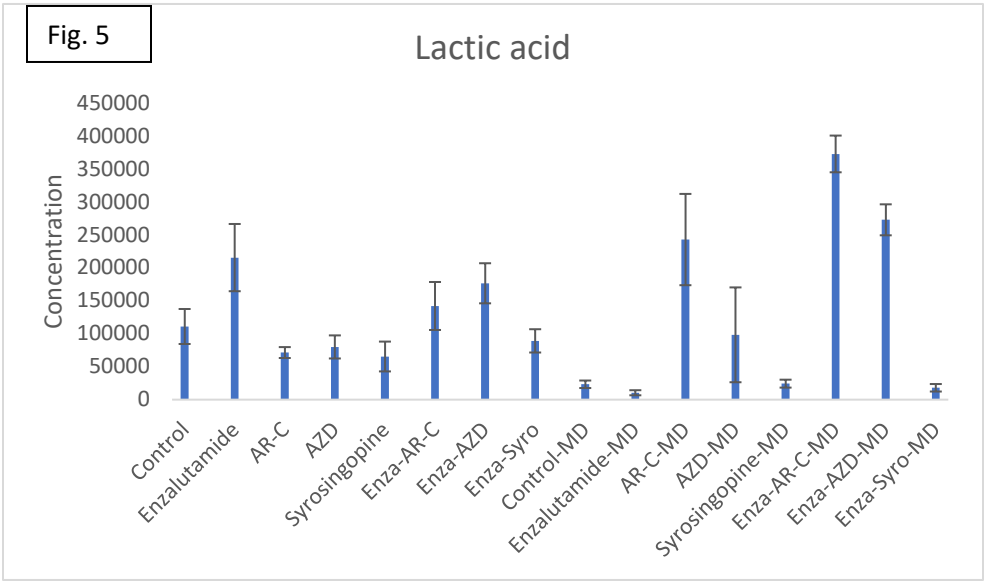


Fig. 5



Research Report

(C-SCOPE：Selected component analysis)

Metabolome Profiles of　Prostate Cancer Cells by CE-TOFMS and CE-QqQMS Analysis

Client: University of Toledo

Report Number：TLDUV-HMT-001

Report Date: August 18, 2022

Human Metabolome Technologies, Inc.

This report consists of 57 pages including the title page.

TABLE OF CONTENTS

1. Study Summary

1-1. Purpose of Study

Analyzing the ionic metabolites in the prostate cancer cells by CE-TOFMS and CE-QqQMS.

1-2. Analysis Summary

Targeted quantitative analysis was performed on 50 samples of prostate cancer cells using capillary electrophoresis mass spectrometry (CE-TOFMS and CE-QqQMS) in the cation and anion analysis modes for analyzing cationic and anionic metabolites, respectively. A total of 116 metabolites (54 and 62 metabolites in the cation and anion mode, respectively) involved in glycolysis, pentose phosphate pathway, tricarboxylic acid (TCA) cycle, urea cycle, and polyamine, creatine, purine, glutathione, nicotinamide, choline, and amino acid metabolisms were annotated based on the HMT metabolite database.

2. Materials and Methods

2-1. Materials

The samples were sent from University of Toledo (In this report, it is written as "University of Toledo".) to HMT. The sample information is listed in Table 1.

Table 1: Sample Information

Name	Amount ($\times 10^6$ cells)	Group	Dilution (Cation) §	Dilution (Anion) §
C4-2B-1-1	3.6996		1	5
C4-2B-1-2	3.6996	Control	1	5
C4-2B-1-3	3.6996		1	5
C4-2B-2-1	3.5370		1	5
C4-2B-2-2	3.5370	Enzalutamide	1	5
C4-2B-2-3	3.5370		1	5
C4-2B-3-1	4.1309		1	5
C4-2B-3-2	4.1309	AR-C	1	5
C4-2B-3-3	4.1309		1	5
C4-2B-4-1	6.1772		1	5
C4-2B-4-2	6.1772	AZD	1	5
C4-2B-4-3	6.1772		1	5
C4-2B-5-1	4.5254		1	5
C4-2B-5-2	4.5254	Syrosingopine	1	5
C4-2B-5-3	4.5254		1	5
C4-2B-6-1	4.3612		1	5
C4-2B-6-2	4.3612	Enza-AR-C	1	5
C4-2B-6-3	4.3612		1	5
C4-2B-7-1	4.0663		1	5
C4-2B-7-2	4.0663	Enza-AZD	1	5
C4-2B-7-3	4.0663		1	5
C4-2B-8-1	3.1532		1	5
C4-2B-8-2	3.1532	Enza-Syro	1	5
C4-2B-8-3	3.1532		1	5
C4-2B-MD-1-1	6.0100		1	5
C4-2B-MD-1-2	6.0100	Control-MD	1	5
C4-2B-MD-1-3	6.0100		1	5
C4-2B-MD-2-1	4.5208		1	5
C4-2B-MD-2-2	4.5208	Enzalutamide-MD	1	5
C4-2B-MD-2-3	4.5208		1	5
C4-2B-MD-3-1	3.7309		1	5
C4-2B-MD-3-2	3.7309	AR-C-MD	1	5
C4-2B-MD-3-3	3.7309		1	5
C4-2B-MD-4-1	3.8100		1	5
C4-2B-MD-4-2	3.8100	AZD-MD	1	5
C4-2B-MD-4-3	3.8100		1	5
C4-2B-MD-5-1	5.2320		1	5
C4-2B-MD-5-2	5.2320	Syrosingopine-MD	1	5
C4-2B-MD-5-3	5.2320		1	5
C4-2B-MD-6-1	3.0866		1	5
C4-2B-MD-6-2	3.0866	Enza-AR-C-MD	1	5
C4-2B-MD-6-3	3.0866		1	5
C4-2B-MD-7-1	4.3390		1	5
C4-2B-MD-7-2	4.3390	Enza-AZD-MD	1	5
C4-2B-MD-7-3	4.3390		1	5
C4-2B-MD-8-1	4.5250		1	5
C4-2B-MD-8-2	4.5250	Enza-Syro-MD	1	5
C4-2B-MD-8-3	4.5250		1	5
22Rv1-1	3.1734	Control-Rv1	1	5
22Rv1-MDVR-1	2.7512	Control-Rv1-MD	1	5

§ Dilution factors for Measurement

2-2. Sample Preparation

Culture medium was removed and the cells were washed twice with mannitol solution. The cells were then treated with methanol and homogenized for 30 sec. Next, Milli-Q water containing internal standards (10 μM) was added to the cell extract, followed by further homogenization for 30 sec. The cell extract was then centrifuged at 2,300 × *g*, 4°C for 5 min, after which the supernatant was centrifugally filtered at 4°C through a 5-kDa cut-off filter (ULTRAFREE-MC-PLHCC, Human Metabolome Technologies, Yamagata, Japan) to remove macromolecules. The filtrate was evaporated to dryness under vacuum and was reconstituted in Milli-Q water for metabolome analysis.

2-3. Cation Analysis

Cationic compounds were measured in the cation mode of metabolome analysis as shown in Table 2 based on the methods described in the references (1–3). The samples were diluted as shown in Table 1 to improve analysis qualities of the CE-TOFMS analysis.

Table 2: Device and Analytical Condition in Cation Measurement

Device	
CE-TOFMS	Agilent CE-TOFMS system (Agilent Technologies) Machine No. 13
Capillary	Fused silica capillary, i.d. 50 μm × 80 cm
Analytical Condition	
Run buffer	Cation buffer solution (p/n: H3301-1001)
Rinse buffer	Cation buffer solution (p/n: H3301-1001)
Sample injection	Pressure injection at 50 mbar, 5 s
CE voltage	Positive, 30 kV
MS ionization	ESI Positive
MS capillary voltage	4,000 V
MS scan range	*m/z* 50–1,000
Sheath liquid	HMT sheath liquid (p/n: H3301-1020)

2-4. Anion Analysis

Anionic compounds were measured in the positive or negative mode of metabolome analysis using CE-MS/MS as shown in Table 3 based on the methods described in the references (1–3). The samples were diluted as shown in Table 1 to improve analysis qualities of the CE-QqQMS analysis.

Table 3: Device and Analytical Condition in Anion Measurement

Device	
CE	Agilent CE system
MS	Agilent 6460 TripleQuad LC/MS Machine No. QqQ1
Capillary	Fused silica capillary, i.d. 50 μm × 80 cm
Analytical Condition	
Run buffer	Anion buffer solution (p/n: H3302-1023)
Rinse buffer	Anion buffer solution (p/n: H3302-1023)
Sample injection	Pressure injection at 50 mbar for 25 s
CE voltage	30 kV
MS ionization	ESI Positive and negative
MS capillary voltage	4500 V for positive and negative mode
Sheath liquid	HMT sheath liquid (p/n: I3301-1030)

2-5. Data Processing

Peaks detected in CE-TOFMS analysis were extracted using automatic integration software (MasterHands ver.2.19.0.2 developed at Keio University) (4) and those in CE-QqQMS analysis were extracted using automatic integration software (MassHunter Quantitative Analysis B.06.00 Agilent Technologies, Santa Clara, CA, USA) in order to obtain peak information, which includes *m/z*, migration time (MT), and peak area. The peak area was then converted to relative peak area by the following equation[1]. The peaks were annotated based on the migration times in CE and *m/z* values determined by TOFMS.

$$\text{†1 Relative peak Area} = \frac{\text{Metabolite Peak Area}}{\text{Internal Standard Peak Area} \times \text{Normalization Factor}^{\dagger 2}}$$

†2 Normalization factor is calculated if the volume or amount of samples used in the analysis is varied depending on the samples.

Putative metabolites were then assigned from HMT metabolite database on the basis of *m/z* and MT. The tolerance was ±0.5 min in MT and ±10 ppm†3 in *m/z*.

$$\text{†3 Mass error (ppm)} = \frac{\text{Measured Value} - \text{Theoretical Value}}{\text{Measured Value}} \times 10^6$$

In addition, absolute quantification was performed on 116 metabolites including glycolytic and TCA cycle intermediates, amino acids, and nucleic acids. All the metabolite concentrations were calculated by normalizing the peak area of each metabolite with respect to the area of the internal standard and by using standard curves, which were obtained by three-point calibrations.

2-6. Statistical Analysis

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) (5) were performed using statistical analysis software (developed at HMT). The analysis results are shown in the attached Excel file in detail.

2-7. Plotting on Pathway Maps

Detected metabolites were plotted on metabolic pathway maps using VANTED (Visualization and Analysis of Networks containing Experimental Data) software (6). The abbreviations of some metabolites used in VANTED are different from those in the HMT metabolite database (Table 3). The pathway map in VANTED was prepared based on the metabolic pathways that are known to exist in human cells.

3. Results

3-1. Absolute Concentration

Absolute concentration of 116 compounds in the prostate cancer cells is listed in Table 4 in ID order.

Table 4: Absolute Concentration of 116 Compounds

ID	HMT DB Compound name	Concentration (pmol/10^6 cells)																	
		Control		Enzalutamide		AR-C		AZD		Syrosingopine		Enza-AR-C		Enza-AZD		Enza-Syro		Control-MD		Enzalutamide-MD	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
A_0001	NAD+	8,146	287	6,384	1,009	8,538	2,040	4,981	164	5,775	655	4,530	631	4,296	407	6,314	578	7,038	457	6,664	818
A_0002	cAMP	7.9	0.6	4.7	1.5	8.6	2.4	4.8	0.5	6.2	0.14	4.3	0.2	3.6	0.2	6.1	0.08	5.2	0.2	4.3	0.7
A_0003	cGMP	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0004	NADH	565	74	762	312	633	106	332	33	461	28	494	19	490	55	604	102	325	48	438	47
A_0005	Xanthine	73	9.6	79	31	57	15	52	14	84	17	71	11	78	14	99	24	217	62	376	52
A_0006	ADP-ribose	15	2.3	23	11	14	4.0	13	2.8	6.5	2.3	11	1.4	12	1.8	11	1.4	11	3.3	10	2.5
A_0007	Mevalonic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0008	UDP-glucose	1,540	45	857	105	1,684	89	1,245	28	1,562	149	818	212	688	86	1,117	106	1,261	165	1,046	205
A_0009	Uric acid	16	6.5	N.A.	N.A.	N.A.	N.A.	9.2	6.9	23	24	8.7	N.A.	3.8	N.A.	3.8	N.A.	29	18	83	18
A_0010	NADP+	824	43	392	130	617	89	358	49	497	46	335	57	306	45	456	23	414	138	345	104
A_0011	IMP	58	3.0	52	17	40	2.5	26	2.2	37	6.5	43	3.3	44	4.4	51	6.7	50	37	34	3.8
A_0012	Sedoheptulose 7-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0013	Glucose 6-phosphate	950	20	933	174	1,276	251	944	88	554	139	516	51	622	55	445	46	659	16	292	48
A_0014	Fructose 6-phosphate	294	2.7	268	67	382	83	263	8.1	144	41	149	18	168	12	118	21	201	15	81	21
A_0015	Fructose 1-phosphate	32	41	N.A.	N.A.	31	N.A.	48	39	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0016	Galactose 1-phosphate	52	4.2	36	19	68	15	35	9.6	61	6.3	37	5.7	36	4.2	50	8.5	39	11	22	7.4
A_0017	Glucose 1-phosphate	204	19	184	75	201	7.0	128	46	116	17	104	15	119	4.6	121	22	107	16	77	5.5
A_0018	Acetoacetyl CoA	N.A.	N.A.	18	N.A.	22	4.2	12	N.A.	14	1.2	N.A.	N.A.	16	0.7	22	1.5	N.A.	N.A.	N.A.	N.A.
A_0019	Acetyl CoA	4.6	3.6	7.8	5.4	8.1	9.1	2.2	1.3	6.3	2.3	2.1	0.4	4.6	4.8	3.3	1.7	2.5	3.7	1.8	1.7
A_0020	Folic acid	13	4.5	10	2.8	7.6	2.0	8.4	1.3	7.8	3.6	10	3.2	10	1.7	10	1.6	4.4	0.9	4.9	1.1
A_0021	Ribose 5-phosphate	5.8	N.A.	9.4	7.5	0.2	N.A.	18	5.4	N.A.	N.A.	17	1.3	9.1	4.3	5.8	N.A.	18	9.3	17	N.A.
A_0022	CoA	277	39	124	40	275	18	130	64	183	11	138	20	116	27	230	6.9	91	69	160	10
A_0023	Ribose 1-phosphate	26	2.7	41	N.A.	29	14	22	2.6	27	9.5	25	8.3	33	16	32	3.0	24	8.7	25	0.3
A_0024	Ribulose 5-phosphate	10	8.6	10	2.6	9.5	6.3	14	8.1	8.8	4.6	14	6.8	17	14	0.14	N.A.	N.A.	N.A.	N.A.	N.A.
A_0025	Xylulose 5-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1,789	410	3,779	907
A_0026	Erythrose 4-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0027	HMG CoA	8.7	0.5	8.4	2.1	7.1	0.8	4.3	0.4	7.4	1.1	6.2	1.1	6.3	0.5	9.2	1.3	4.9	1.7	5.3	1.5
A_0028	Glyceraldehyde 3-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0029	NADPH	410	37	526	247	495	52	301	33	440	35	403	93	381	61	630	122	376	80	581	101
A_0030	Malonyl CoA	9.6	0.9	9.4	0.4	10	2.4	6.1	0.3	6.5	0.4	7.0	0.8	7.2	0.2	N.A.	N.A.	6.3	0.9	6.8	0.5
A_0031	Phosphocreatine	25,200	1,310	23,989	7,565	23,172	1,738	14,567	1,732	15,990	1,236	17,144	1,331	17,221	1,486	20,860	2,617	14,962	2,214	20,119	1,178
A_0032	XMP	3.8	0.8	3.4	4.4	3.2	1.8	2.8	0.9	9.0	0.8	9.9	0.9	10	3.1	14	3.1	3.4	3.4	3.4	0.7
A_0033	Dihydroxyacetone phosphate	N.A.	N.A.	62	N.A.	N.A.	N.A.	104	10	N.A.	N.A.	N.A.	N.A.	14	N.A.	N.A.	N.A.	95	N.A.	N.A.	N.A.
A_0034	Adenylosuccinic acid	11	1.1	14	6.7	11	1.4	7.1	0.9	11	0.3	21	6.5	11	0.9	15	2.4	12	8.7	8.9	0.8
A_0035	Fructose 1,6-diphosphate	278	40	160	70	320	37	228	27	350	229	165	30	154	41	204	24	200	29	98	27
A_0036	6-Phosphogluconic acid	99	14	107	49	94	18	62	7.5	58	5.6	66	8.2	69	3.7	80	6.1	58	5.8	58	4.0
A_0037	*N*-Carbamoylaspartic acid	18	2.7	27	3.0	11	1.7	8.9	3.1	6.0	2.4	11	2.6	14	3.0	14	0.9	3.6	1.6	4.4	0.5
A_0038	PRPP	230	26	231	22	233	12	142	14	146	20	194	15	247	40	242	37	107	14	182	29
A_0039	2-Phosphoglyceric acid	51	7.5	36	13	55	11	27	0.9	39	5.8	43	10	41	3.5	66	3.5	30	4.7	37	5.3
A_0040	2,3-Diphosphoglyceric acid	159	19	182	86	240	22	141	8.4	163	20	246	67	230	34	377	37	47	7.0	117	44
A_0041	3-Phosphoglyceric acid	357	2.8	241	76	366	93	185	6.5	282	67	292	41	288	41	455	20	199	30	258	62
A_0042	Phosphoenolpyruvic acid	105	15	N.A.	N.A.	76	43	19	7.3	59	34	66	34	44	10	134	3.2	31	19	70	35
A_0043	GMP	424	38	536	132	429	78	319	27	564	27	442	69	492	87	724	13	408	115	382	45
A_0044	AMP	493	350	1,035	258	658	376	671	332	1,376	179	1,213	351	1,169	258	2,216	145	931	355	769	357
A_0045	2-Oxoisovaleric acid	130	25	315	156	134	26	83	11	107	11	210	31	247	43	267	29	75	29	111	4.0
A_0046	GDP	361	181	542	161	509	71	189	149	485	112	510	105	479	126	781	107	300	102	352	103
A_0047	Lactic acid	110,923	26,525	215,383	50,982	71,398	8,269	79,927	17,499	65,417	22,616	142,016	36,375	176,436	30,388	89,124	17,648	23,290	5,691	10,437	3,851
A_0048	ADP	1,407	575	1,840	965	1,707	104	689	593	2,716	472	2,951	1,095	2,624	637	5,314	1,211	290	132	742	456
A_0049	GTP	12,755	842	9,379	1,177	10,998	164	6,393	341	8,070	1,094	7,669	1,338	7,939	760	9,449	538	9,444	443	10,201	1,606
A_0050	Glyoxylic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0051	ATP	48,385	1,981	31,166	2,371	48,123	5,329	27,782	2,289	36,952	4,368	27,428	6,616	24,578	2,356	39,311	3,427	43,179	717	52,024	8,111
A_0052	Glycerol 3-phosphate	1,001	41	3,536	240	1,103	202	803	132	538	107	1,184	190	1,879	107	701	64	466	162	348	52
A_0053	Glycolic acid	1,778	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	191	N.A.	N.A.	N.A.	N.A.	N.A.
A_0054	Pyruvic acid	1,157	148	1,328	333	1,056	372	828	195	1,094	240	1,069	196	1,036	195	817	62	1,516	N.A.	460	16
A_0055	*N*-Acetylglutamic acid	301	16	148	30	269	58	250	49	304	52	241	128	162	16	530	46	581	112	1,452	200
A_0056	2-Hydroxyglutaric acid	545	63	173	30	480	94	386	41	493	64	208	61	208	32	636	89	411	148	87	14
A_0057	Carbamoylphosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0058	Succinic acid	1,070	308	510	282	588	283	717	263	798	202	563	197	540	100	724	103	477	112	364	14
A_0059	Malic acid	6,188	910	N.A.	N.A.	3,373	2,102	3,318	636	2,553	334	1,831	632	1,329	98	346	440	1,579	1,975	240	203
A_0060	2-Oxoglutaric acid	955	162	598	244	734	198	874	312	502	188	655	137	855	158	312	366	1,023	201	237	327

ID consists of analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficience of the data.
† The ratio is of computed by using averaged detection values. The latter was used as denominator.
‡ The p-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

Table 4: Absolute Concentration of 116 Compounds

ID	HMT DB Compound name	Concentration (pmol/10^6 cells)												Comparative Analysis					
		AR-C-MD		AZD-MD		Syrosingopine-MD		Enza-AR-C-MD		Enza-AZD-MD		Enza-Syro-MD		Control-Rv1	Control-Rv1-MD	Control-MD vs Control		Enzalutamide vs Control		AR-C vs Control	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	Mean	Ratio ¶	*p*-value ‖	Ratio ¶	*p*-value ‖	Ratio ¶	*p*-value ‖
A_0001	NAD+	10,343	2,854	9,566	1,992	6,405	476	5,956	650	4,845	617	7,926	2,095	9,818	7,268	0.9	0.031 *	0.8	0.084	1.0	0.772
A_0002	cAMP	7.8	2.4	6.4	0.8	5.1	0.3	4.7	0.7	3.7	0.5	6.1	1.6	45	15	0.6	0.008 **	0.6	0.051	1.1	0.703
A_0003	cGMP	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0004	NADH	810	217	535	40	397	55	679	68	529	51	453	82	753	781	0.6	0.013 *	1.3	0.388	1.1	0.417
A_0005	Xanthine	489	110	376	63	331	43	661	85	535	110	516	102	69	43	3.0	0.054	1.1	0.775	0.8	0.206
A_0006	ADP-ribose	19	1.9	20	5.6	9.8	3.4	20	5.5	21	0.9	12	4.1	20	30	0.7	0.158	1.6	0.299	1.0	0.844
A_0007	Mevalonic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0008	UDP-glucose	1,799	524	1,888	345	1,054	117	1,005	187	853	84	1,478	325	12,526	2,124	0.8	0.091	0.6	0.003 **	1.1	0.090
A_0009	Uric acid	5.2	N.A.	54	23	92	31	32	N.A.	23	16	73	88	16	126	1.8	0.354	<1	N.A.	<1	N.A.
A_0010	NADP+	547	193	400	49	385	63	250	49	181	4.1	441	91	429	225	0.5	0.027 *	0.5	0.020 *	0.7	0.038 *
A_0011	IMP	241	72	59	11	32	4.1	91	3.0	64	8.6	35	5.4	96	165	0.9	0.756	0.9	0.620	0.7	0.002 **
A_0012	Sedoheptulose 7-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0013	Glucose 6-phosphate	1,146	512	952	169	493	76	587	120	407	67	746	45	869	493	0.7	5.8E-05 ***	1.0	0.883	1.3	0.153
A_0014	Fructose 6-phosphate	334	137	264	30	136	26	141	26	120	6.3	211	34	217	128	0.7	0.007 **	0.9	0.573	1.3	0.205
A_0015	Fructose 1-phosphate	99	36	70	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	334	N.A.	<1	N.A.	<1	N.A.	1.0	N.A.
A_0016	Galactose 1-phosphate	72	23	74	30	34	12	21	5.2	24	5.6	45	18	172	30	0.7	0.155	0.7	0.267	1.3	0.211
A_0017	Glucose 1-phosphate	170	63	139	14	97	15	133	21	84	15	122	22	213	142	0.5	0.003 **	0.9	0.685	1.0	0.777
A_0018	Acetoacetyl CoA	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	1<	N.A.	1<	N.A.
A_0019	Acetyl CoA	3.4	1.1	1.3	1.4	0.7	0.3	6.5	5.4	2.0	0.6	2.6	3.7	37	2.6	0.5	0.511	1.7	0.451	1.8	0.584
A_0020	Folic acid	8.1	4.4	6.2	1.7	5.6	1.5	5.2	0.8	5.5	0.7	4.1	0.4	11	12	0.3	0.078	0.8	0.469	0.6	0.171
A_0021	Ribose 5-phosphate	25	20	41	13	8.6	N.A.	19	24	36	7.3	12	11	52	36	3.1	N.A.	1.6	N.A.	0.03	N.A.
A_0022	CoA	259	47	234	54	93	21	181	30	137	23	167	79	351	419	0.3	0.025 *	0.4	0.009 **	1.0	0.931
A_0023	Ribose 1-phosphate	38	15	24	0.5	20	4.6	42	3.2	33	11	20	N.A.	52	116	0.9	0.785	1.6	N.A.	1.1	0.742
A_0024	Ribulose 5-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	39	10	<1	N.A.	1.0	0.951	0.9	0.921
A_0025	Xylulose 5-phosphate	2,705	549	3,416	1,514	2,998	403	1,938	477	1,763	511	3,085	1,957	N.A.	N.A.	1<	N.A.	N.A.	N.A.	N.A.	N.A.
A_0026	Erythrose 4-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0027	HMG CoA	13	2.3	11	0.4	6.4	0.9	11	2.0	8.9	1.1	6.3	1.3	23	12	0.6	0.048 *	1.0	0.824	0.8	0.048 *
A_0028	Glyceraldehyde 3-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	215	98	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0029	NADPH	701	149	657	152	387	11	565	91	482	100	605	184	450	372	0.9	0.551	1.3	0.501	1.2	0.091
A_0030	Malonyl CoA	13	3.1	9.2	0.7	N.A.	N.A.	12	1.5	8.9	0.9	8.5	1.1	41	13	0.7	0.009 **	1.0	0.664	1.1	0.824
A_0031	Phosphocreatine	27,396	10,062	18,064	906	16,108	1,941	15,753	2,166	13,064	1,330	20,599	3,738	26,899	18,981	0.6	0.005 **	1.0	0.809	0.9	0.187
A_0032	XMP	7.2	2.3	7.2	3.8	2.9	2.3	11	4.3	7.1	2.1	3.4	1.9	N.A.	13	0.9	0.899	0.9	0.914	0.8	0.613
A_0033	Dihydroxyacetone phosphate	223	N.A.	307	171	56	53	309	N.A.	305	80	230	97	661	204	1<	N.A.	1<	N.A.	N.A.	N.A.
A_0034	Adenylosuccinic acid	17	5.0	16	7.4	8.9	2.8	17	2.5	13	1.9	9.1	1.6	17	12	1.1	0.899	1.2	0.536	0.9	0.479
A_0035	Fructose 1,6-diphosphate	362	104	266	17	217	23	139	34	115	15	257	75	559	635	0.7	0.057	0.6	0.080	1.2	0.253
A_0036	6-Phosphogluconic acid	126	37	84	8.1	63	7.9	94	8.7	70	2.6	70	10	167	159	0.6	0.022 *	1.1	0.807	0.9	0.699
A_0037	*N*-Carbamoylaspartic acid	94	36	35	45	5.4	0.3	141	15	102	6.6	3.6	0.8	121	125	0.2	0.003 **	1.5	0.020 *	0.6	0.027 *
A_0038	PRPP	684	184	214	51	119	7.6	274	36	203	13	140	11	1,013	4,380	0.5	0.005 **	1.0	0.945	1.0	0.843
A_0039	2-Phosphoglyceric acid	60	21	42	10	25	3.3	57	3.9	48	4.3	26	4.7	59	54	0.6	0.020 *	0.7	0.167	1.1	0.673
A_0040	2,3-Diphosphoglyceric acid	258	59	113	38	64	15	202	16	159	23	56	3.8	283	142	0.3	0.005 **	1.1	0.691	1.5	0.009 **
A_0041	3-Phosphoglyceric acid	408	144	266	52	170	22	373	41	312	41	170	30	485	366	0.6	0.012 *	0.7	0.119	1.0	0.874
A_0042	Phosphoenolpyruvic acid	64	38	65	25	22	6.2	82	13	115	30	25	34	93	74	0.3	0.007 **	<1	N.A.	0.7	0.360
A_0043	GMP	829	253	626	92	365	52	754	139	563	100	470	66	293	215	1.0	0.838	1.3	0.275	1.0	0.932
A_0044	AMP	2,266	1,131	1,528	763	441	214	1,971	457	1,438	417	900	486	969	512	1.9	0.203	2.1	0.103	1.3	0.608
A_0045	2-Oxoisovaleric acid	198	65	131	31	102	0.9	227	20	198	47	143	45	N.A.	187	0.6	0.183	2.4	0.174	1.0	0.892
A_0046	GDP	847	542	404	188	111	38	556	58	407	51	127	47	659	511	0.8	0.647	1.5	0.264	1.4	0.290
A_0047	Lactic acid	242,738	69,219	98,279	71,903	24,250	6,018	372,590	27,834	272,671	23,500	17,984	5,688	179,079	371,343	0.2	0.025 *	1.9	0.051	0.6	0.112
A_0048	ADP	2,487	2,305	558	144	487	199	2,009	167	1,100	215	476	239	3,964	2,252	0.2	0.071	1.3	0.549	1.2	0.463
A_0049	GTP	16,770	4,380	12,839	788	8,924	626	10,925	1,921	9,383	877	11,127	2,368	16,078	8,091	0.7	0.009 **	0.7	0.019 *	0.9	0.064
A_0050	Glyoxylic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0051	ATP	65,290	18,948	62,137	8,142	42,781	2,497	45,513	9,625	37,489	4,162	54,979	10,112	77,726	24,407	0.9	0.033 *	0.6	7.5E-04 ***	1.0	0.942
A_0052	Glycerol 3-phosphate	2,203	574	1,377	322	402	59	3,035	412	2,855	331	465	37	1,807	2,100	0.5	0.024 *	3.5	0.002 **	1.1	0.476
A_0053	Glycolic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	715	N.A.	N.A.	N.A.	N.A.	N.A.	<1	N.A.	<1	N.A.	<1	N.A.
A_0054	Pyruvic acid	1,499	205	1,026	420	N.A.	N.A.	2,306	1,036	1,864	255	861	376	3,496	N.A.	1.3	N.A.	1.1	0.481	0.9	0.769
A_0055	*N*-Acetylglutamic acid	1,030	312	639	161	668	113	999	192	880	122	649	82	735	312	1.9	0.047 *	0.5	0.005 **	0.9	0.442
A_0056	2-Hydroxyglutaric acid	473	105	280	104	395	30	261	25	223	22	333	62	491	96	0.8	0.254	0.3	0.003 **	0.9	0.382
A_0057	Carbamoylphosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
A_0058	Succinic acid	843	303	627	338	367	45	567	105	490	52	394	44	480	354	0.4	0.065	0.5	0.081	0.5	0.117
A_0059	Malic acid	5,547	2,063	3,319	3,708	1,003	437	2,849	1,130	2,881	655	659	159	4,751	N.A.	0.3	0.039 *	<1	N.A.	0.5	0.132
A_0060	2-Oxoglutaric acid	1,229	853	1,630	314	4.5	N.A.	2,246	517	2,638	272	584	935	2,517	607	1.1	0.729	0.6	0.113	0.8	0.212

ID consists of analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficience of the data.
¶ The ratio is of computed by using averaged detection values. The latter was used as denominator.
‖ The *p*-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

Table 4: Absolute Concentration of 116 Compounds

ID	HMT DB Compound name	Concentration (pmol/10^6 cells)																	
		Control		Enzalutamide		AR-C		AZD		Syrosingopine		Enza-AR-C		Enza-AZD		Enza-Syro		Control-MD		Enzalutamide-MD	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
A_0061	Fumaric acid	1,051	335	29	N.A.	568	351	528	124	452	185	401	114	252	171	15	N.A.	468	464	84	111
A_0062	Citric acid	22,229	1,630	19,745	4,441	22,827	5,566	12,550	913	14,998	1,505	14,171	2,219	14,899	1,209	20,336	1,425	10,083	877	9,277	2,004
A_0063	cis-Aconitic acid	307	10	351	37	306	57	194	14	200	4.3	247	41	270	36	347	23	156	10	151	36
A_0064	Isocitric acid	1,354	161	1,162	322	1,092	418	631	99	784	49	1,032	196	1,060	260	1,153	150	689	39	563	227
C_0001	Urea	27,823	2,473	20,340	5,771	17,450	824	17,108	5,203	28,231	5,812	23,159	2,484	24,774	3,855	31,984	3,868	11,948	3,239	14,311	1,359
C_0002	Gly	163,285	1,179	92,462	9,061	230,034	73,544	121,123	1,059	208,326	35,361	109,692	31,321	86,062	7,876	186,121	12,088	143,032	6,941	115,798	7,382
C_0003	Putrescine	50	11	N.A.	N.A.	67	59	14	N.A.	39	28	1.1	N.A.	N.A.	N.A.	3.0	N.A.	6.1	3.3	N.A.	N.A.
C_0004	β-Ala	4,702	241	4,445	203	4,082	935	3,396	568	4,428	459	3,718	364	4,025	551	4,408	326	2,711	346	3,597	132
C_0005	Sarcosine	792	39	513	57	2,029	275	1,599	133	1,363	166	1,337	218	1,309	154	1,306	55	362	184	361	37
C_0006	Ala	84,309	3,112	31,122	4,027	82,359	20,283	53,438	954	114,724	15,359	38,076	9,499	30,313	4,001	62,605	851	56,485	4,560	27,995	4,285
C_0007	*N,N*-Dimethylglycine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0008	γ-Aminobutyric acid	4,568	253	1,573	80	3,177	960	2,507	441	2,696	580	1,345	366	1,470	160	1,620	61	2,150	515	414	57
C_0009	Choline	N.A.	N.A.	409	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	200	264	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0010	Ser	32,740	894	8,878	3,547	35,515	8,447	22,786	3,263	58,134	4,369	14,925	5,895	10,366	1,532	34,731	1,374	26,496	3,251	30,633	1,601
C_0011	Carnosine	431	36	172	16	472	51	224	11	266	39	155	14	147	27	172	8.0	1,804	196	809	151
C_0012	Creatinine	684	107	500	41	588	27	452	71	614	83	409	62	461	54	575	26	343	13	390	27
C_0013	Pro	86,482	2,354	56,480	4,812	84,699	4,855	65,058	2,511	86,556	5,221	58,073	10,962	51,955	5,981	73,225	4,074	44,640	8,347	67,109	1,306
C_0014	Val	1,898	916	3,508	703	894	865	1,456	757	4,013	741	6,026	971	5,532	933	8,536	242	1,566	320	5,994	586
C_0015	Betaine	1,523	264	1,721	1,495	523	118	774	281	202	82	1,434	814	4,007	766	19	N.A.	840	N.A.	297	144
C_0016	Thr	10,177	753	7,611	721	6,419	307	5,474	1,055	9,954	588	9,462	1,488	8,895	1,329	14,905	445	3,933	160	8,012	660
C_0017	Homoserine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	8.1	N.A.	16	N.A.
C_0018	Betaine aldehyde	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0019	Cys	146	73	55	1.2	235	133	229	207	242	62	151	135	177	214	339	151	949	1,479	1,994	757
C_0020	Hydroxyproline	39,749	1,444	11,438	125	37,490	3,366	28,226	2,485	39,866	1,663	11,479	3,240	9,706	1,415	20,788	594	22,115	3,013	18,730	1,175
C_0021	Creatine	53,943	99	40,784	4,728	44,895	3,685	29,011	1,152	41,449	3,179	34,487	4,694	30,457	3,082	43,986	1,698	35,288	3,294	42,495	1,569
C_0022	Leu	6,252	973	7,408	1,518	4,147	1,149	4,633	756	8,755	742	9,456	1,920	8,473	1,419	13,746	829	4,421	264	10,503	847
C_0023	Ile	6,187	806	7,038	1,580	3,876	958	4,880	818	7,327	678	7,385	1,383	6,845	1,083	10,862	945	3,893	524	8,307	698
C_0024	Asn	48,292	643	18,439	1,894	37,694	867	32,293	2,766	50,258	2,009	19,536	5,882	15,935	2,065	37,714	1,628	29,368	3,815	29,929	402
C_0025	Ornithine	13,401	1,178	9,968	1,921	10,582	441	11,186	940	11,055	3,143	4,396	1,145	4,628	748	10,129	1,647	5,837	433	4,087	1,018
C_0026	Asp	16,343	1,275	1,913	497	14,669	1,132	10,373	1,717	23,962	1,013	4,327	2,313	2,593	520	12,799	1,564	25,728	3,686	42,022	1,449
C_0027	Homocysteine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
C_0028	Adenine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	2.3	N.A.	0.5	N.A.	9.2	N.A.	N.A.	N.A.	1.9	N.A.	17	8.4	4.3	1.9
C_0029	Hypoxanthine	614	52	607	149	835	192	582	48	2,355	584	1,471	289	1,361	354	2,024	46	890	424	837	5.0
C_0030	Spermidine	136	8.5	78	19	123	19	70	6.0	110	9.8	64	13	61	3.4	107	5.3	58	3.0	61	2.2
C_0031	Gln	2,399	223	1,059	69	2,579	1,290	2,010	151	3,045	924	1,389	344	1,555	367	1,594	333	6,317	2,083	3,800	360
C_0032	Lys	3,673	1,032	5,014	280	2,376	717	2,274	773	5,500	856	7,119	1,135	6,979	919	9,648	649	2,745	797	7,617	415
C_0033	Glu	84,276	2,853	31,295	1,633	84,546	12,896	57,654	3,223	83,094	4,456	42,329	12,157	35,110	4,659	65,456	4,287	100,546	2,844	91,748	10,589
C_0034	Met	1,079	218	1,742	223	906	90	1,015	260	1,735	421	2,456	354	2,624	527	3,108	610	895	175	2,162	475
C_0035	Guanine	169	7.1	173	39	228	62	162	13	560	137	387	107	284	105	527	36	152	58	113	14
C_0036	His	2,898	52	2,654	495	2,569	87	2,657	547	5,381	560	3,650	869	3,438	471	5,847	240	2,021	126	3,383	204
C_0037	Carnitine	300	101	N.A.	N.A.	295	189	451	55	217	115	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	374	89	1.9	N.A.
C_0038	Phe	1,569	418	2,439	299	983	323	1,011	329	2,586	287	3,638	488	3,419	534	5,321	151	968	189	3,180	270
C_0039	Arg	3,208	1,737	2,331	330	1,557	554	2,691	123	2,692	1,429	2,630	508	3,352	663	2,888	518	1,243	170	1,700	711
C_0040	Citrulline	135	34	200	33	105	8.5	118	18	94	29	108	25	154	24	61	23	23	11	11	N.A.
C_0041	Tyr	2,645	307	2,951	483	2,112	175	2,193	554	4,689	445	3,924	802	3,684	544	6,241	257	1,651	57	3,674	236
C_0042	*S*-Adenosylhomocysteine	50	7.7	23	8.4	48	5.5	23	11	48	4.6	22	2.4	17	2.4	59	30	20	8.4	47	28
C_0043	Spermine	546	99	406	66	473	107	295	43	412	26	327	36	315	38	432	61	335	22	378	27
C_0044	Trp	426	131	695	79	224	131	225	90	584	61	1,027	149	955	109	1,525	68	233	32	852	100
C_0045	Cystathionine	3,237	109	735	67	2,997	180	2,023	319	3,425	476	789	374	518	61	1,995	42	6,724	1,385	3,088	117
C_0046	Adenosine	0.5	N.A.	N.A.	N.A.	N.A.	N.A.	0.6	N.A.	4.7	2.3	0.7	0.8	0.7	N.A.	3.5	1.1	4.0	3.7	N.A.	N.A.
C_0047	Inosine	11	9.4	14	3.5	27	12	20	8.6	154	93	115	35	95	28	103	29	92	112	25	7.2
C_0048	Guanosine	12	4.4	14	2.6	14	4.7	10	4.8	59	42	56	13	51	15	54	11	24	10	16	5.0
C_0049	Argininosuccinic acid	171	33	101	15	118	31	100	25	145	15	90	21	83	9.5	169	3.7	103	9.6	86	2.0
C_0050	Glutathione (GSSG)	13,581	3,609	12,298	3,958	11,741	1,788	11,222	4,868	9,739	1,664	10,242	5,734	5,666	863	12,385	4,015	11,849	4,661	12,498	902
C_0051	Glutathione (GSH)	31,277	7,679	8,116	7,790	28,789	3,634	8,097	10,907	31,869	3,662	13,419	4,459	13,584	1,393	37,862	7,871	10,199	11,424	26,949	2,593
C_0052	*S*-Adenosylmethionine	464	11	278	18	369	7.4	273	11	456	66	273	79	247	12	555	41	441	44	562	70

ID consists of analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficiance of the data.
§ The ratio is of computed by using averaged detection values. The latter was used as denominator.
† The p-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

Table 4: Absolute Concentration of 116 Compounds

ID	HMT DB Compound name	Concentration (pmol/10^6 cells) AR-C-MD Mean	AR-C-MD S.D.	AZD-MD Mean	AZD-MD S.D.	Syrosingopine-MD Mean	Syrosingopine-MD S.D.	Enza-AR-C-MD Mean	Enza-AR-C-MD S.D.	Enza-AZD-MD Mean	Enza-AZD-MD S.D.	Enza-Syro-MD Mean	Enza-Syro-MD S.D.	Control-Rv1 Mean	Control-Rv1-MD Mean	Comparative Analysis: Control-MD vs Control Ratio[¶]	Control-MD vs Control p-value[‖]		Enzalutamide vs Control Ratio[¶]	Enzalutamide vs Control p-value[‖]		AR-C vs Control Ratio[¶]	AR-C vs Control p-value[‖]	
A_0061	Fumaric acid	821	276	800	911	199	95	571	148	392	43	87	86	858	N.A.	0.4	0.285		0.03	N.A.		0.5	0.160	
A_0062	Citric acid	23,149	7,244	14,763	2,186	9,424	825	21,262	2,172	16,621	1,149	10,755	2,988	25,582	11,340	0.5	0.001	**	0.9	0.441		1.0	0.872	
A_0063	cis-Aconitic acid	413	140	251	46	126	9.0	464	42	359	33	150	43	422	174	0.5	5.5E-05	***	1.1	0.165		1.0	0.992	
A_0064	Isocitric acid	1,541	592	1,121	407	556	154	1,511	144	1,194	148	555	191	2,025	513	0.5	0.015	*	0.9	0.425		0.8	0.398	
C_0001	Urea	23,793	10,780	16,621	3,114	16,668	1,689	18,553	3,549	17,565	2,875	16,399	1,721	42,492	15,868	0.4	0.003	**	0.7	0.141		0.6	0.012	*
C_0002	Gly	256,203	87,412	236,292	70,827	202,736	19,650	141,104	24,488	105,244	15,733	208,538	53,383	199,496	28,841	0.9	0.034	*	0.6	0.005	**	1.4	0.257	
C_0003	Putrescine	2.7	N.A.	N.A.	N.A.	4.4	2.9	N.A.	N.A.	N.A.	N.A.	24	16	N.A.	64	0.12	0.014	*	<1	N.A.		1.3	0.678	
C_0004	β-Ala	6,569	2,672	4,261	291	3,923	213	2,949	830	2,485	401	3,707	261	14,660	14,342	0.6	0.002	**	0.9	0.232		0.9	0.370	
C_0005	Sarcosine	1,531	754	1,177	171	823	60	620	138	497	65	424	64	813	248	0.5	0.050		0.6	0.003	**	2.6	0.015	*
C_0006	Ala	126,469	46,854	104,345	8,788	72,198	8,381	94,291	14,614	67,976	10,647	79,803	10,111	157,453	28,455	0.7	0.002	**	0.4	8.5E-05	***	1.0	0.884	
C_0007	*N,N*-Dimethylglycine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.		N.A.	N.A.	
C_0008	γ-Aminobutyric acid	3,617	1,298	2,040	755	1,412	75	1,028	110	729	32	2,398	753	2,361	3,317	0.5	0.006	**	0.3	0.001	**	0.7	0.121	
C_0009	Choline	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		1<	N.A.		N.A.	N.A.	
C_0010	Ser	33,804	15,821	50,900	15,514	33,308	2,613	12,431	2,495	9,663	1,737	46,219	9,254	8,351	2,026	0.8	0.071		0.3	0.005	**	1.1	0.627	
C_0011	Carnosine	2,719	1,105	1,651	95	1,506	184	587	131	474	63	2,196	237	659	340	4.2	0.005	**	0.4	0.002	**	1.1	0.326	
C_0012	Creatinine	673	356	500	38	431	43	346	106	298	36	369	32	678	406	0.5	0.030	*	0.7	0.083		0.9	0.260	
C_0013	Pro	120,624	48,645	69,855	4,840	62,576	6,749	66,494	11,748	54,128	9,298	63,487	3,958	328,721	78,565	0.5	0.009	**	0.7	0.003	**	1.0	0.609	
C_0014	Val	6,112	2,113	3,005	1,577	4,820	251	6,282	1,376	5,539	1,041	7,045	1,374	N.A.	1,602	0.8	0.603		1.8	0.077		0.5	0.240	
C_0015	Betaine	633	788	N.A.	N.A.	272	74	N.A.	N.A.	N.A.	N.A.	399	101	3,979	N.A.	0.6	N.A.		1.1	0.841		0.3	0.012	*
C_0016	Thr	13,236	4,215	8,077	1,499	7,896	162	11,848	1,788	9,297	1,259	8,736	1,225	29,702	8,070	0.4	0.004	**	0.7	0.013	*	0.6	0.006	**
C_0017	Homoserine	N.A.	N.A.	22	N.A.	16	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	118	33	1<	N.A.		N.A.	N.A.		N.A.	N.A.	
C_0018	Betaine aldehyde	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.		N.A.	N.A.	
C_0019	Cys	1,958	1,356	1,192	603	715	759	274	362	297	171	884	948	370	422	6.5	0.446		0.4	0.165		1.6	0.384	
C_0020	Hydroxyproline	60,873	27,354	38,195	2,754	32,360	2,011	18,392	3,873	15,605	2,233	27,615	2,439	181,112	18,845	0.6	0.003	**	0.3	8.0E-04	***	0.9	0.371	
C_0021	Creatine	59,347	16,697	47,857	2,856	40,306	2,485	34,562	9,220	26,540	4,173	49,353	8,629	40,519	38,759	0.7	0.010	*	0.8	0.040	*	0.8	0.051	
C_0022	Leu	12,912	5,090	7,740	2,115	9,370	476	10,754	2,197	9,067	1,493	11,571	1,369	6,312	6,696	0.7	0.074		1.2	0.339		0.7	0.074	
C_0023	Ile	11,299	5,229	6,663	1,804	7,841	494	8,383	1,983	7,166	1,093	7,901	459	9,604	8,373	0.6	0.020	*	1.1	0.467		0.6	0.034	*
C_0024	Asn	77,497	33,268	55,036	3,369	43,450	4,975	32,741	7,107	25,902	3,905	40,730	2,591	109,611	25,072	0.6	0.012	*	0.4	4.7E-04	***	0.8	1.2E-04	***
C_0025	Ornithine	7,672	3,373	8,960	749	4,238	329	3,210	545	2,587	421	3,714	760	13,806	2,268	0.4	0.004	**	0.7	0.070		0.8	0.040	*
C_0026	Asp	34,080	17,066	40,918	4,543	38,265	3,265	8,068	2,394	6,852	1,596	34,875	12,167	12,201	1,542	1.6	0.036	*	0.12	8.2E-04	***	0.9	0.165	
C_0027	Homocysteine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.		N.A.	N.A.	
C_0028	Adenine	8.0	N.A.	8.5	6.9	13	N.A.	0.02	N.A.	6.2	N.A.	10	8.2	N.A.	N.A.	1<	N.A.		N.A.	N.A.		N.A.	N.A.	
C_0029	Hypoxanthine	1,630	438	1,710	301	1,379	154	1,421	360	1,167	196	1,644	248	N.A.	N.A.	1.4	0.377		1.0	0.944		1.4	0.178	
C_0030	Spermidine	119	27	85	4.2	92	3.1	77	13	59	11	97	14	353	241	0.4	0.002	**	0.6	0.019	*	0.9	0.360	
C_0031	Gln	11,320	6,623	9,143	1,113	7,686	2,640	3,591	622	3,280	70	9,616	1,317	35,946	812	2.6	0.081		0.4	0.005	**	1.1	0.833	
C_0032	Lys	8,163	2,481	5,091	1,409	6,025	606	8,203	1,406	7,052	1,098	8,459	1,632	1,558	1,622	0.7	0.289		1.4	0.146		0.6	0.157	
C_0033	Glu	140,370	57,153	150,163	14,395	93,477	8,935	81,026	18,218	64,589	10,734	108,050	15,122	238,435	49,126	1.2	0.002	**	0.4	6.5E-05	***	1.0	0.975	
C_0034	Met	2,911	1,128	1,735	123	2,422	155	2,254	113	1,839	186	2,456	773	579	2,235	0.8	0.321		1.6	0.021	*	0.8	0.304	
C_0035	Guanine	194	82	225	7.8	137	9.7	135	32	109	26	168	25	N.A.	N.A.	0.9	0.653		1.0	0.895		1.3	0.244	
C_0036	His	5,310	2,019	3,801	45	3,885	290	3,438	772	3,055	455	4,046	391	26,964	2,644	0.7	0.003	**	0.9	0.483		0.9	0.009	**
C_0037	Carnitine	105	N.A.	251	182	177	83	N.A.	N.A.	N.A.	N.A.	591	363	3,341	480	1.2	0.393		<1	N.A.		1.0	0.974	
C_0038	Phe	3,544	1,128	1,979	642	2,562	149	3,406	684	2,978	480	3,592	739	2,379	1,651	0.6	0.114		1.6	0.048	*	0.6	0.131	
C_0039	Arg	4,083	3,174	2,956	1,524	2,276	790	1,581	267	1,578	550	1,787	321	44,612	15,276	0.4	0.188		0.7	0.476		0.5	0.237	
C_0040	Citrulline	91	50	42	3.3	30	12	66	21	66	5.2	16	14	637	354	0.2	0.022	*	1.5	0.075		0.8	0.271	
C_0041	Tyr	4,580	1,772	3,224	266	3,456	48	3,572	756	3,206	496	3,869	411	16,472	3,027	0.6	0.027	*	1.1	0.415		0.8	0.075	
C_0042	*S*-Adenosylhomocysteine	78	49	25	1.7	28	4.8	39	13	15	2.6	26	6.9	63	78	0.4	0.011	*	0.5	0.016	*	1.0	0.821	
C_0043	Spermine	613	160	527	63	447	45	507	75	408	23	519	88	917	721	0.6	0.060		0.7	0.124		0.9	0.437	
C_0044	Trp	969	279	486	166	696	20	949	211	801	149	946	192	766	482	0.5	0.118		1.6	0.050	*	0.5	0.131	
C_0045	Cystathionine	6,704	2,702	7,802	472	5,251	148	1,539	444	1,205	216	5,542	466	4,658	399	2.1	0.048	*	0.2	2.4E-05	***	0.9	0.134	
C_0046	Adenosine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	7.7	N.A.		<1	N.A.		<1	N.A.	
C_0047	Inosine	66	22	79	10	70	21	69	14	74	33	66	26	N.A.	N.A.	8.7	0.334		1.3	0.631		2.6	0.139	
C_0048	Guanosine	17	8.4	31	11	21	8.1	23	7.0	21	7.6	21	1.3	N.A.	N.A.	2.0	0.174		1.2	0.608		1.2	0.661	
C_0049	Argininosuccinic acid	201	60	162	50	120	12	121	6.0	89	9.2	106	7.6	2,104	258	0.6	0.061		0.6	0.049	*	0.7	0.109	
C_0050	Glutathione (GSSG)	13,928	4,135	13,291	835	12,175	1,892	8,408	1,919	9,219	1,093	15,257	3,862	18,652	10,750	0.9	0.639		0.9	0.700		0.9	0.488	
C_0051	Glutathione (GSH)	37,049	15,348	31,867	10,619	12,894	5,922	25,669	5,919	14,193	3,129	14,369	11,935	233,848	107,954	0.3	0.065		0.3	0.021	*	0.9	0.649	
C_0052	*S*-Adenosylmethionine	661	196	571	60	448	22	522	100	445	79	502	102	698	498	1.0	0.463		0.6	3.0E-04	***	0.8	5.9E-04	***

ID consists of analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficience of the data.
[¶] The ratio is of computed by using averaged detection values. The latter was used as denominator.
[‖] The p-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

3-2. Statistical Analysis

3-2-1. *Principal Component Analysis*

Principal component analysis (PCA) was conducted in order to compare the overall metabolomic profiles in prostate cancer cells (Fig.1).

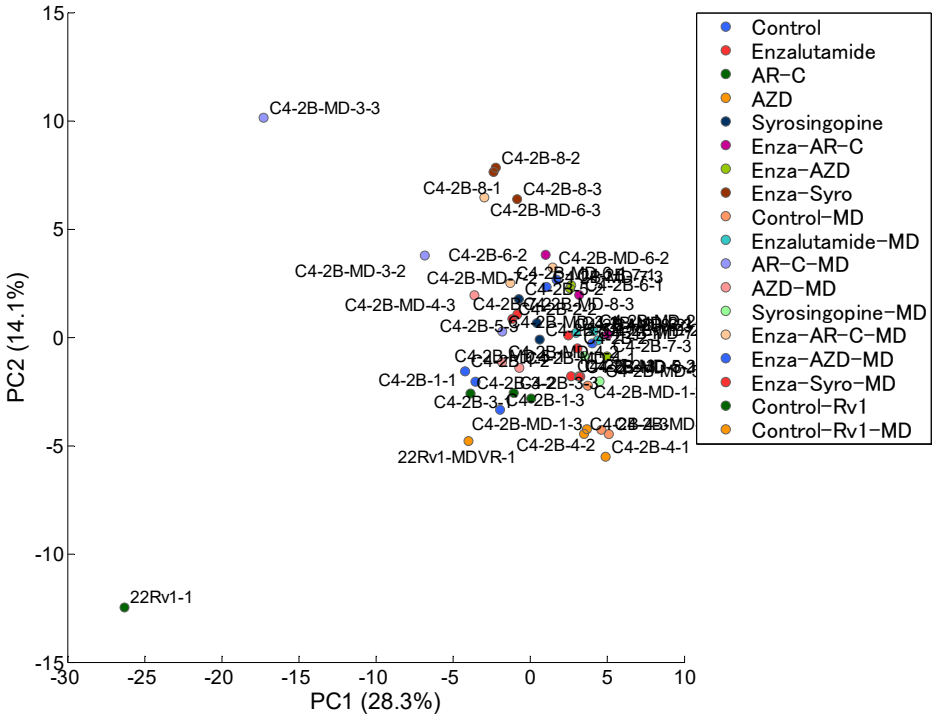


Fig. 1: PC1 *vs* PC2 plot based on the result of principal component analysis

3-2-2. *Hierarchical Cluster Analysis*

Hierarchical clustering analysis was conducted in order to compare the overall metabolomic profiles in prostate cancer cells (Fig. 2).

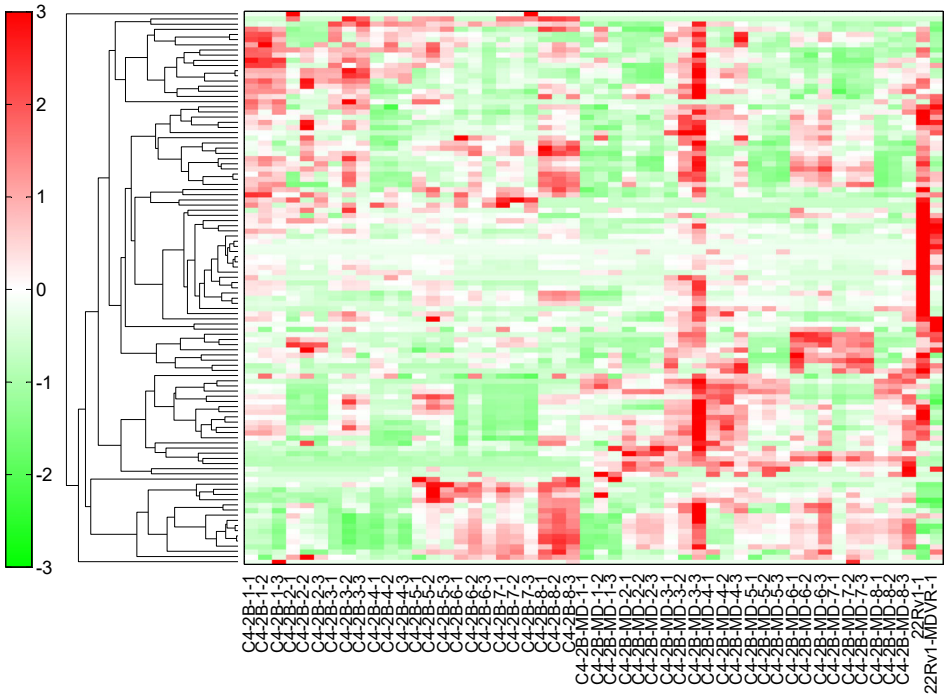


Fig. 2: Heat map representation of metabolome profiles analyzed by hierarchical clustering analysis

3-3. Metabolome Data Pathway Maps

Graphs of the obtained metabolome data were created and superimposed on the following metabolic pathway maps: energy metabolism overview, glycolysis and pentose phosphate pathway, TCA cycle, urea cycle and polyamine and creatine metabolism, purine metabolism, methionine cycle and glutathione metabolism, branched-chain amino acid metabolism, lysine, tryptophan, and nicotinamide metabolism, and choline and lipid metabolism, respectively (Fig. 3-11).

3-3-1. *Energy Metabolism Overview*

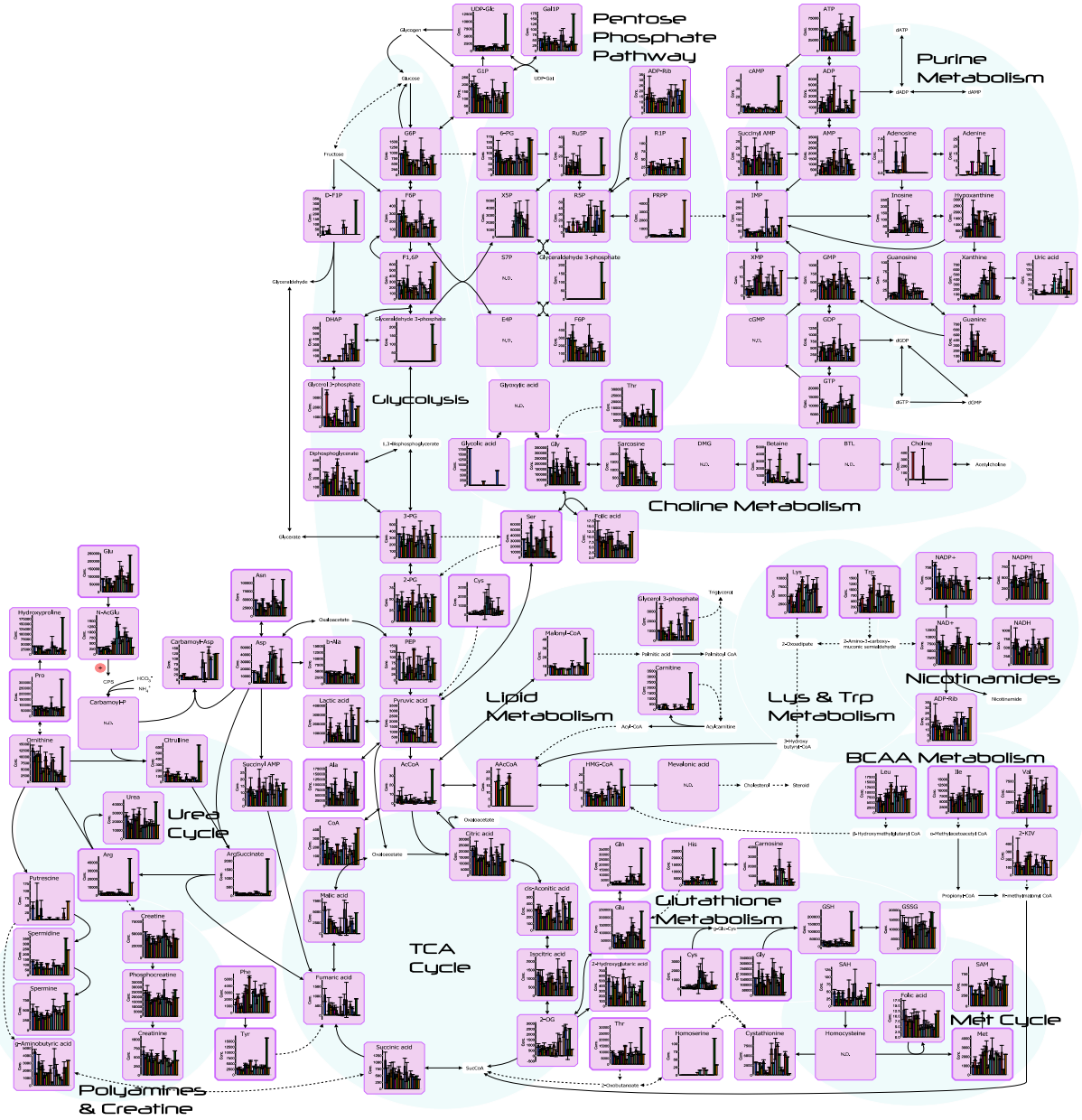


Fig. 3: Energy metabolism overview

Graphs of all the 116 target metabolites were superimposed on the pathway map of overall energy metabolism. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines show the omission of multiple reactions between the connected metabolites.

3-3-2. *Glycolysis and Pentose Phosphate Pathway*

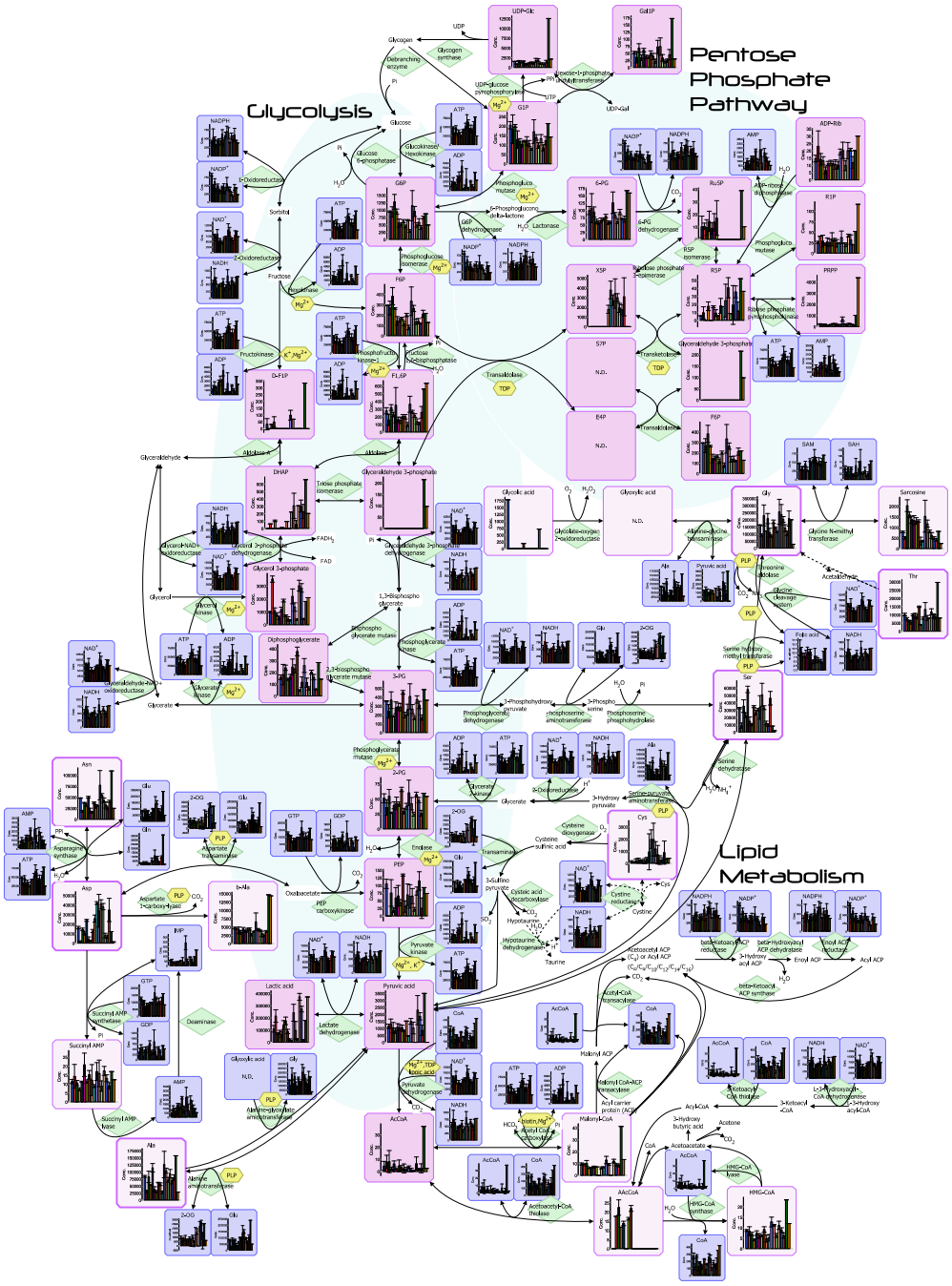


Fig. 4: Glycolysis and pentose phosphate pathway

Graphs of metabolites involved in glycolysis and pentose phosphate pathway (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-3. *TCA Cycle*

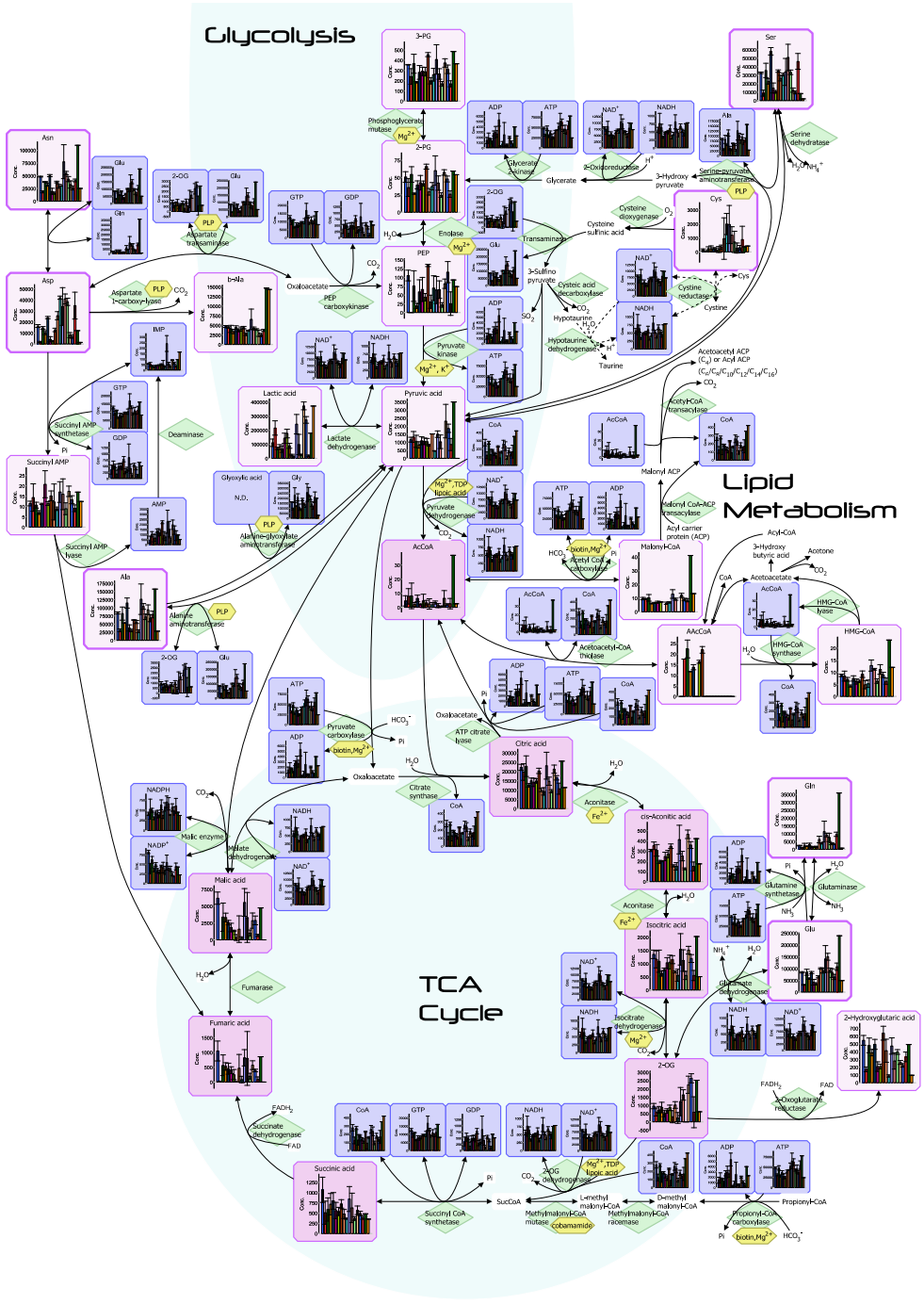


Fig. 5: TCA cycle

Graphs of metabolites involved in TCA cycle (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-4. *Urea Cycle and Polyamine and Creatine Metabolism*

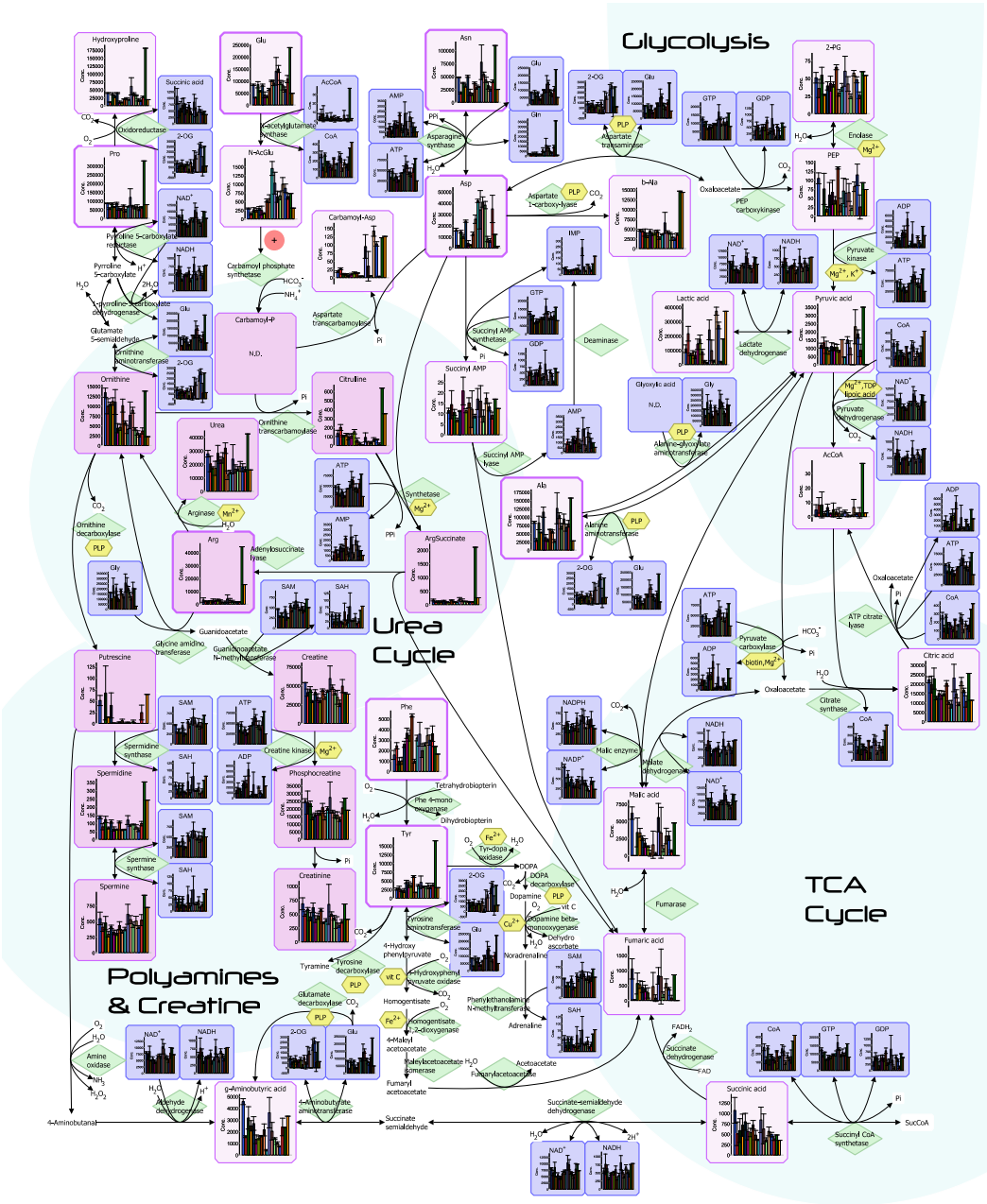


Fig. 6: Urea cycle and polyamine and creatine metabolism

Graphs of metabolites involved in urea cycle, polyamine metabolism and creatine metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-5. *Purine Metabolism*

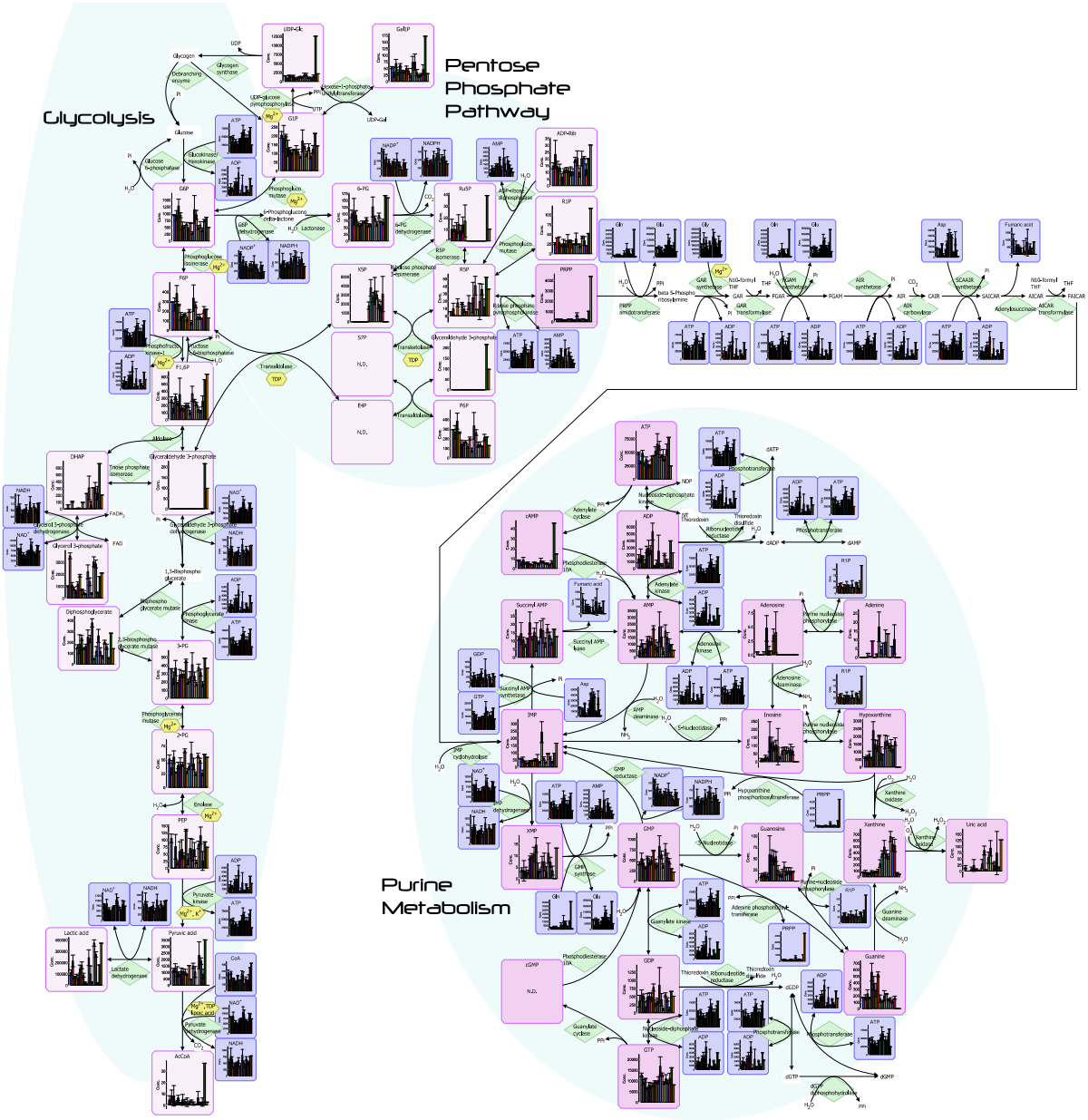


Fig. 7: Purine metabolism

Graphs of metabolites involved in purine metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-6. *Methionine Cycle and Glutathione Metabolism*

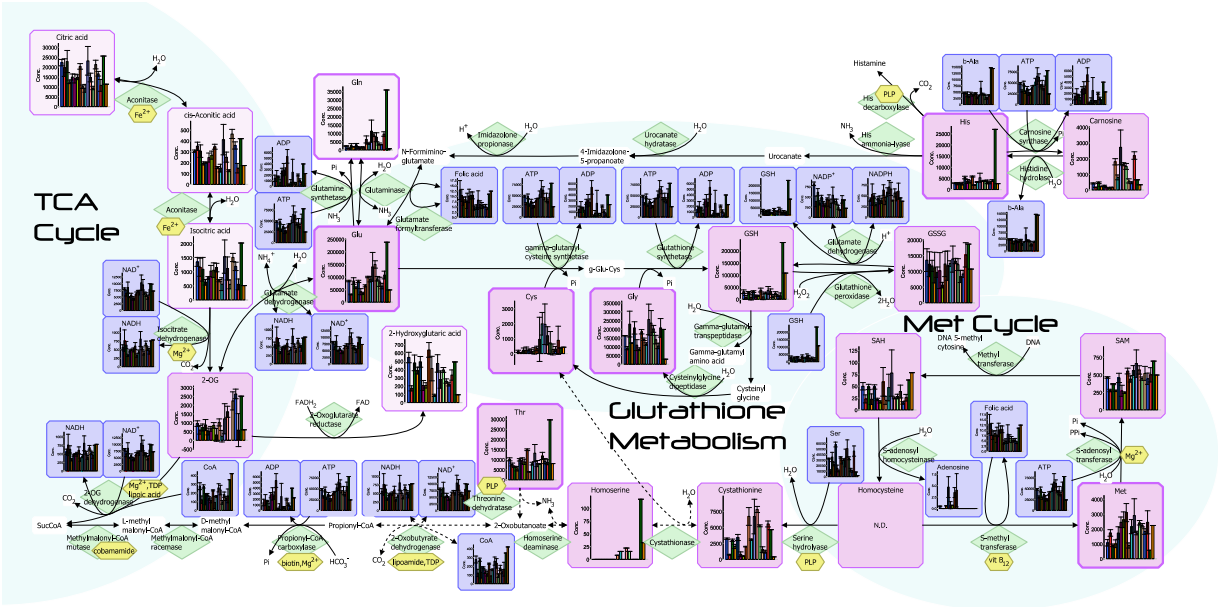


Fig. 8: Methionine cycle and glutathione metabolism

Graphs of metabolites involved in methionine cycle and glutathione metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-7. *Branched-chain Amino Acid Metabolism*

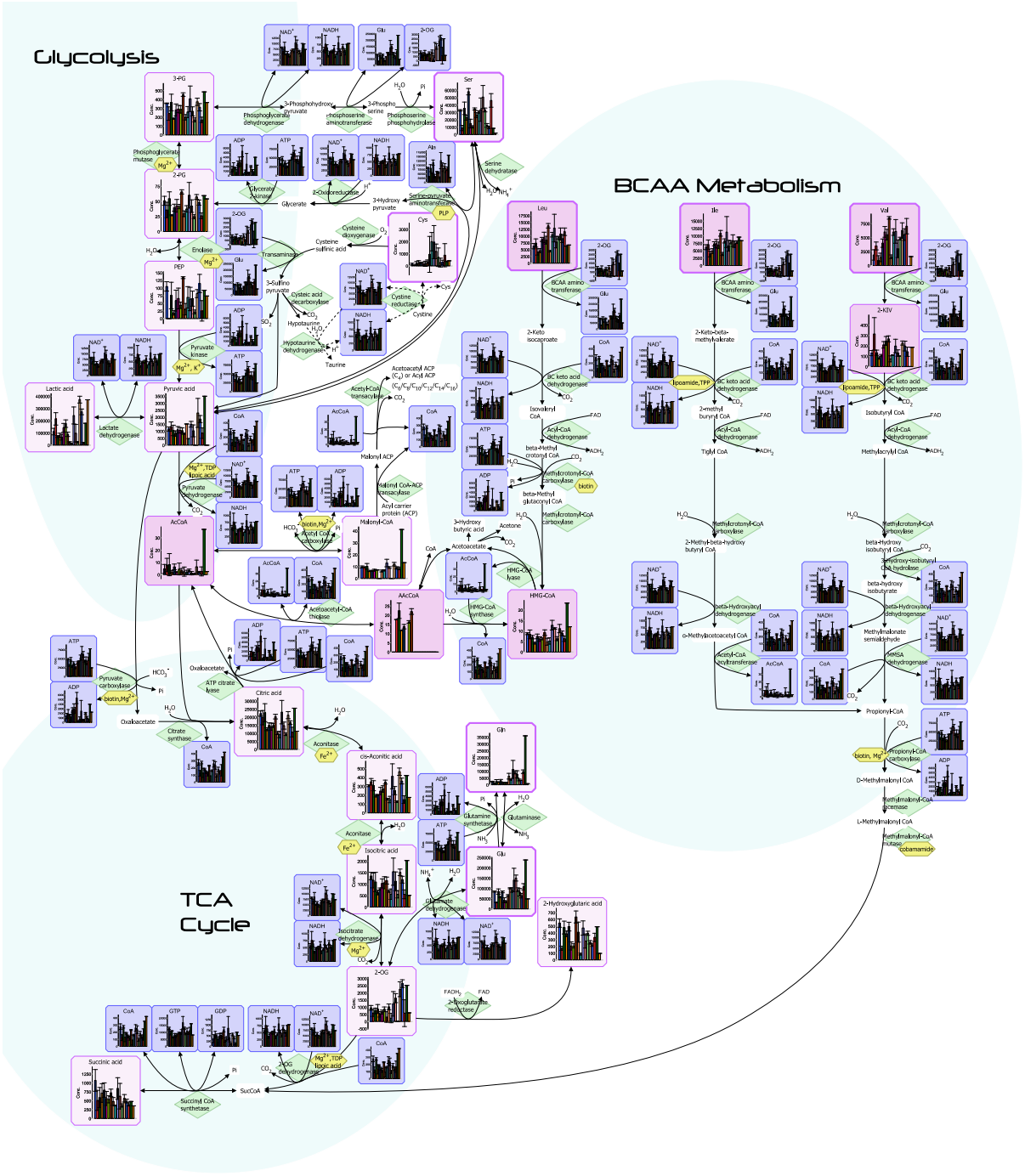


Fig. 9: Branched-chain amino acid metabolism

Graphs of metabolites involved in branched-chain amino acid metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-8. *Lysine, Tryptophan, and Nicotinamide Metabolism*

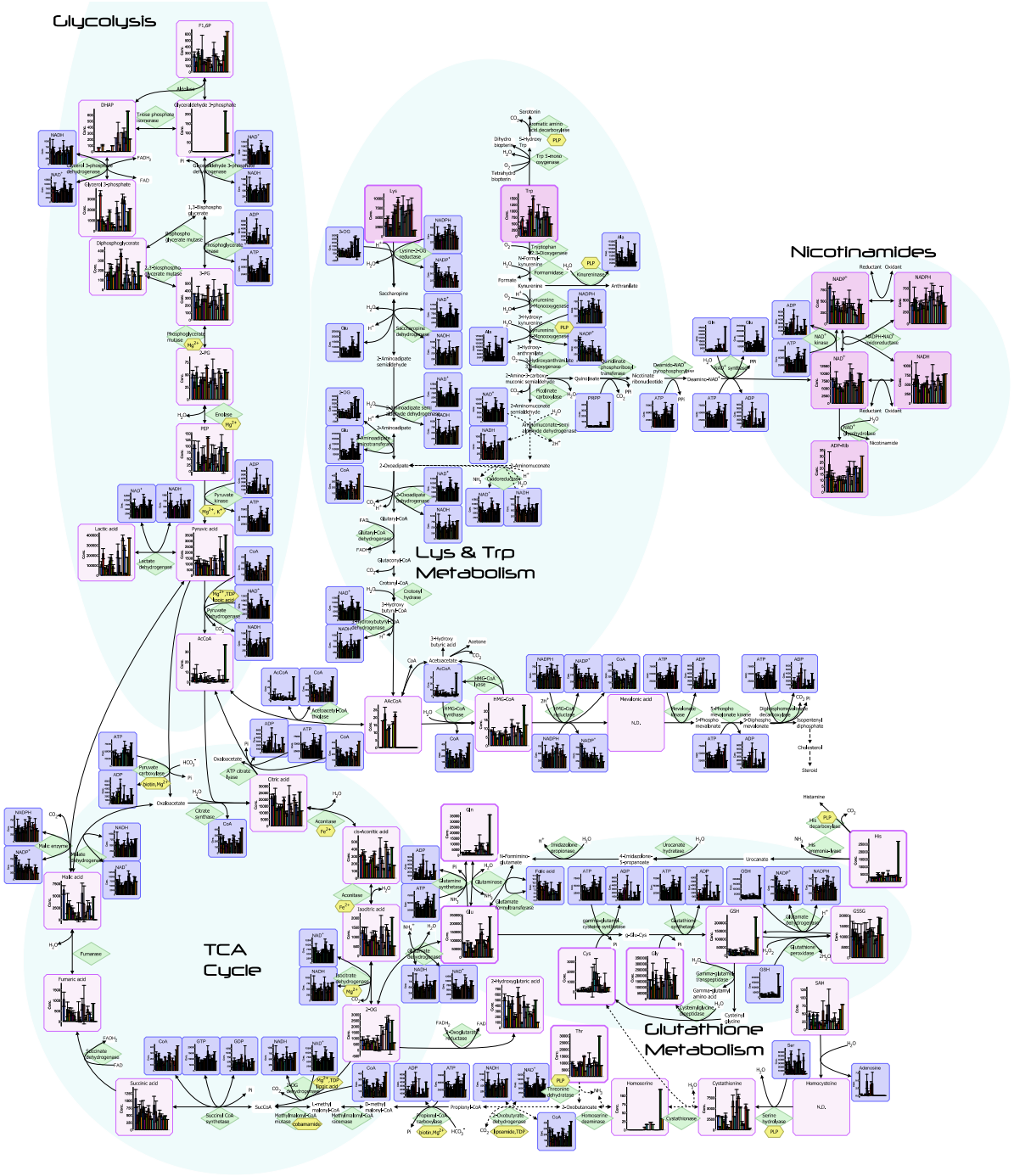


Fig. 10: Lysine, tryptophan, and nicotinamide metabolism

Graphs of metabolites involved in lysine, tryptophan, and nicotinamide metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-3-9. *Choline and Lipid Metabolism*

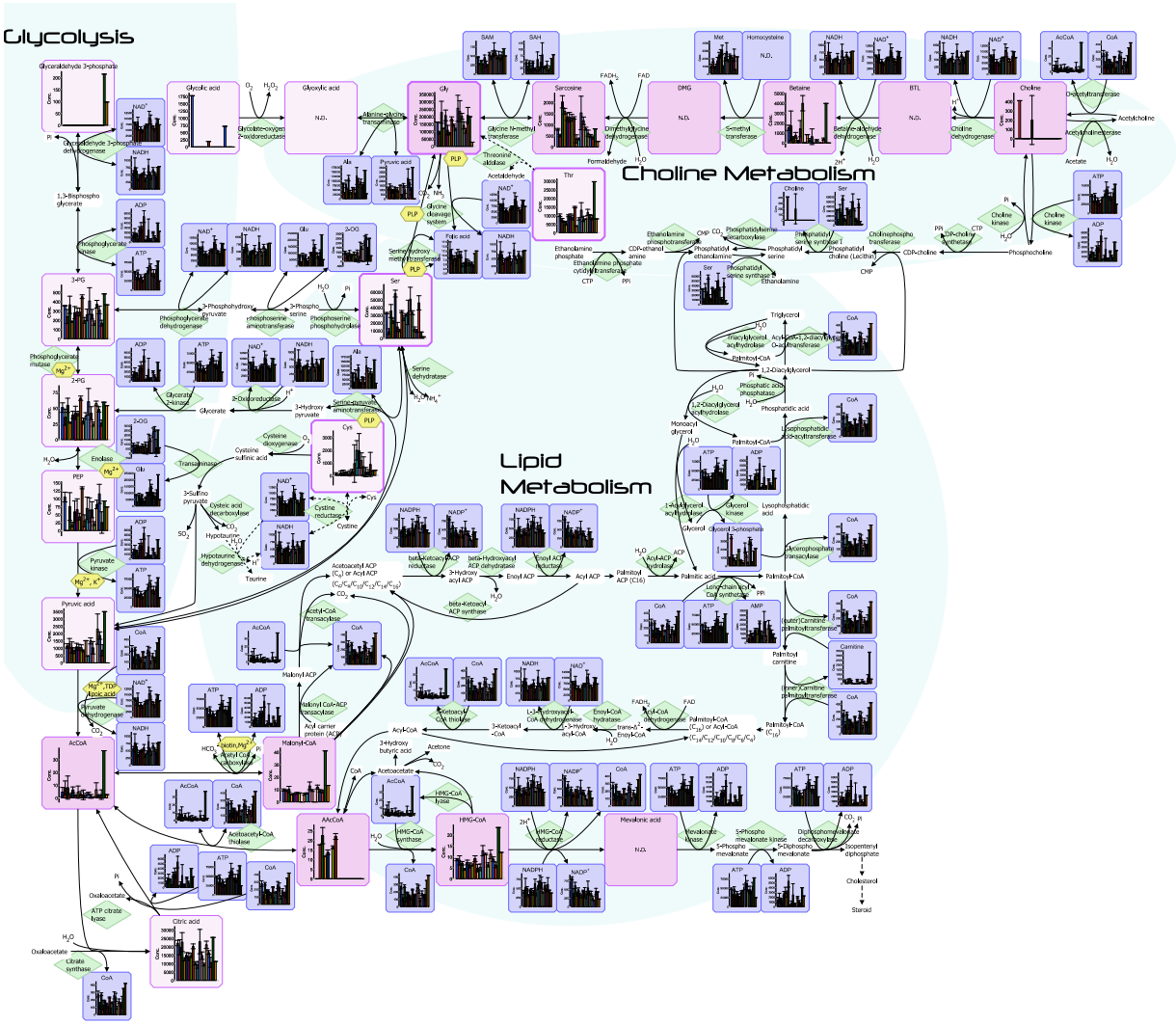


Fig. 11: Choline and lipid metabolism

Graphs of metabolites involved in choline and lipid metabolism (background in red) and their related metabolites (background in pink) were superimposed on the pathway map. Bold frames are used to indicate amino acids for a better viewability. The bars/lines represent absolute concentrations of each metabolite in Control (blue), Enzalutamide (red), AR-C (green), AZD (orange), Syrosingopine (mazarine), Enza-AR-C (purple), Enza-AZD (grass green), Enza-Syro (brown), Control-MD (pale red), Enzalutamide-MD (jade green), AR-C-MD (lilac), AZD-MD (pink), Syrosingopine-MD (light green), Enza-AR-C-MD (flesh color), Enza-AZD-MD (blue), Enza-Syro-MD (red), Control-Rv1 (green), Control-Rv1-MD (orange), respectively. In addition, “N.D.” indicates that the targeted metabolites were not detected, and dashed lines represent the reactions with enzymes that have not been identified in humans.

3-4. Metabolic Parameters

Metabolic parameters such as energy charge, lactate-to-pyruvate ratio (LP ratio), glutathione redox ratio, and total amino acids are extremely useful for gaining further insights into the obtained metabolome data and facilitating understandings of physiological states such as energy and redox statuses of cells and tissues. Metabolic parameters are evaluated using absolutely quantified data, which are useful for comparing the results among different study projects, while relatively quantified data tend to be greatly varied among studies and are unsuitable for inter-study comparisons. Equations to calculate each metabolic parameter, its relevance to cell metabolism and physiological states are listed in Table 5.

Table 5: Metabolic Parameters and Their Relevance to Cell Metabolism and Physiology

Chapter	Metabolic Parameters	Equation	Relevance
3-4-1	Adenylate Energy Charge	([ATP] + 0.5 x [ADP]) / ([ATP] + [ADP] + [AMP])	Energy status
3-4-1	Total Adenylate	[ATP] + [ADP] + [AMP]	Purine synthesis / degradation
3-4-2	Guanylate Energy Charge	([GTP] + 0.5 x [GDP]) / ([GTP] + [GDP] + [GMP])	Energy status
3-4-2	Total Guanylate	[GTP] + [GDP] + [GMP]	Purine synthesis / degradation
3-4-3	Glutathione Redox Ratio	[GSH] / [GSSG]	Oxidative stress
3-4-3	Total Glutathione	[GSH] + 2 x [GSSG]	Glutathione synthesis / degradation
3-4-4	NADPH / $NADP^+$	[NADPH] / [$NADP^+$]	Oxidative stress, Fatty acid synthesis
3-4-5	NADH / NAD^+	[NADH] / [NAD^+]	Redoxpotential
3-4-6	Lactate / Pyruvate (LP ratio)	[Lactic acid] / [Pyruvic acid]	Anaerobic glycolysis, Redoxpotential
3-4-7	Glycerol 3-phosphate / DHAP	[Glycerol 3-phosphate] / [DHAP]	Redoxpotential
3-4-8	Total Amino Acids	Sum of all 20-proteinogenic amino acids	Amino acid synthesis / degradation, influx / efflux
3-4-9	Total Essential Amino Acids	Sum of [His], [Ile], [Leu], [Lys], [Met], [Phe], [Thr], [Trp] and [Val]	Essential amino acid degradation, influx / efflux
3-4-10	Total Non-essential Amino Acids	Sum of [Ala], [Arg], [Asn], [Asp], [Cys], [Gln], [Glu], [Gly], [Pro], [Ser] and [Tyr]	Non-essential amino acid synthesis / degradation, influx / efflux
3-4-11	Total Glucogenic Amino Acids	Sum of all 20-proteinogenic amino acids except [Leu] and [Lys]	Glucogenic amino acid degradation, influx / efflux, Gluconeogenesis
3-4-12	Total Ketogenic Amino Acids	Sum of [Ile], [Leu], [Lys], [Phe], [Thr], [Trp] and [Tyr]	Ketogenic amino acid degradation, influx / efflux, Ketogenesis
3-4-13	Total Branched-chain Amino Acids (BCAA)	Sum of [Ile], [Leu] and [Val]	Branched-chain amino acid degradation, influx / efflux
3-4-14	Total Aromatic Amino Acids (AAA)	Sum of [Phe], [Trp] and [Tyr]	Aromatic amino acid degradation, influx / efflux
3-4-15	Fischer's Ratio (BCAA / AAA)	[BCAA] / [AAA]	Liver failure, BCAA and AAA metabolism
3-4-16	Total Glu-related Amino Acids	Sum of [Arg], [Gln], [Glu], [His] and [Pro]	Amino acids that are catabolized to Glu
3-4-17	Total Pyr-related Amino Acids	Sum of [Ala], [Cys], [Gly], [Ser], [Thr] and [Trp]	Amino acids that are catabolized to pyruvate
3-4-18	Total Acetyl CoA-related Amino Acids	Sum of [Ile], [Leu], [Lys] and [Trp]	Amino acids that are catabolized to acetyl CoA
3-4-19	Total Fumarate-related Amino Acids	Sum of [Phe] and [Tyr]	Amino acids that are catabolized to fumarate
3-4-20	Total Succinyl CoA-related Amino Acids	Sum of [Ile], [Met] and [Val]	Amino acids that are catabolized to Succinyl CoA
3-4-21	Total Oxaloacetate-related Amino Acids	Sum of [Asn] and [Asp]	Amino acids that are catabolized to oxaloacetate
3-4-22	Malate / Asp	[Malic acid] / [Asp]	Malate-Asp shuttle, Redoxpotential
3-4-23	Citrulline / Ornithine	[Citruline] / [Ornithine]	Arg consumption for NOS or arginase
3-4-24	Glu / 2-Oxoglutarate	[Glu] / [2-Oxoglutaric acid]	Amino acid synthesis / degradation
3-4-25	Glucose 6-phosphate / Ribose 5-phosphate	[Glucose 6-phosphate] / [Ribose 5-phosphate]	Glycolysis, pentose phosphate pathway
3-4-26	*S*-Adenosylmethionine (SAM) / *S*-Adenosylhomocysteine (SAH)	[SAM] / [SAH]	Methylation status, Met metabolism
3-4-27	Putrescine / Spermidine	[Putrescine] / [Spermidine]	Aging, Carcinogenesis

3-4-1. *Adenylate Energy Charge and Total Adenylate*

Adenylate energy charge indicates the energy status of cells, and total adenylate is the sum of adenylate phosphates, ATP, ADP and AMP, which are evaluated by the following equations:

$$\text{Adenylate Energy Charge} = \frac{[\text{ATP}] + 0.5 \times [\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}, \quad \text{Total Adenylate} = [\text{ATP}] + [\text{ADP}] + [\text{AMP}]$$

According to Atkinson, D.E. (7), adenylate energy charge is maintained between 0.8 and 0.95 in most cells, and a high energy charge may slow down the metabolism (or ATP production) and a low energy charge may signal upregulation of metabolism. The following figures show the evaluated adenylate energy charge, ATP, ADP and AMP. Total adenylate is often affected by the rate of *de novo* purine synthesis. In addition, total adenylate tends to decrease under stressed conditions, such as treatment with H_2O_2, oligomycin, and 2-deoxyglucose (8), whereas it appears to be relatively stable against the change in medium glucose and glutamine concentration (9).

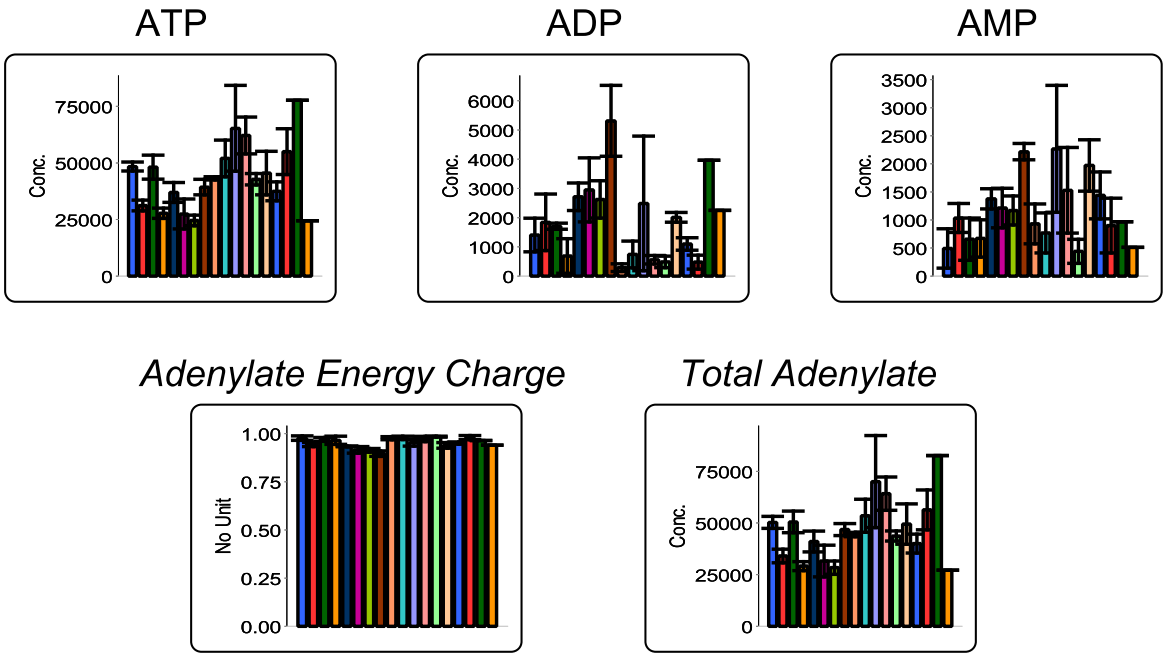


Fig. 12: Adenylate Energy Charge and Total Adenylate

3-4-2. *Guanylate Energy Charge and Total Guanylate*

Guanylate energy charge also indicates the energy status of cells as adenylate energy charge does, and total guanylate is the sum of guanylate phosphates, GTP, GDP and GMP, which are evaluated by the following equations:

$$\text{Guanylate Energy Charge} = \frac{[\text{GTP}] + 0.5 \times [\text{GDP}]}{[\text{GTP}] + [\text{GDP}] + [\text{GMP}]}, \quad \text{Total Guanylate} = [\text{GTP}] + [\text{GDP}] + [\text{GMP}]$$

According to Derr, R.F. (10), changes in guanylate energy charge often parallel with those in adenylate energy charge, while the guanylate energy charge changes more slowly than the adenylate energy charge against certain stresses such as phenobarbital, ether, ammonia, and insulin coma. In addition, in leukemia patients, total guanylate content and synthesis are lower, and total adenylate-to-total guanylate ratio is higher in lymphocytes of patients than controls (11). The following figures show the evaluated guanylate energy charge, GTP, GDP and GMP.

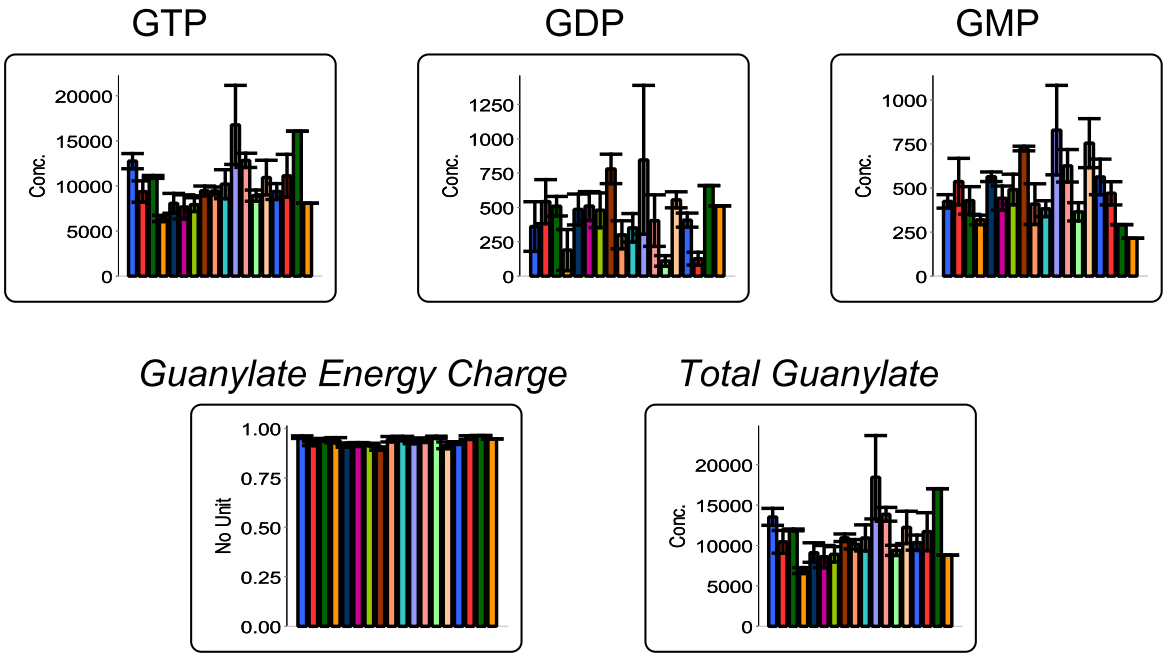


Fig. 13: Guanylate Energy Charge and Total Guanylate

3-4-3. *Glutathione Redox Ratio and Total Glutathione*

Glutathione redox ratio, or reduced glutathione (GSH) over oxidized glutathione (GSSG), indicates the oxidative stress status of cells and total glutathione is the sum of GSH and GSSG, which are evaluated by the following equations:

$$\text{Glutathione Redox Ratio} = \frac{[GSH]}{[GSSG]}, \text{ Total Glutathione} = [GSH] + 2 \times [GSSG]$$

Functions of glutathione have been well-documented. Glutathione exists in almost all cells in the body and is produced from glutamate, cysteine, and glycine by two ATP-consuming steps. More than 90% of glutathione is known to be in the form of GSH in healthy cells and tissues and a decrease of [GSH]/[GSSG] ratio is considered to be an indication of oxidative stress. Besides its property as an antioxidant, glutathione exhibits multiple functions such as regulation of nitric oxide cycle, involvement in ion metabolism, DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation (12). In relation to cancer, glutathione removes and detoxifies carcinogens, whereas it confers resistance to chemotherapeutic drugs and protects cancer cells (13). Note that the quantified glutathione concentrations need to be evaluated with care, as it is highly unstable in solution and reliability of the data is relatively low.

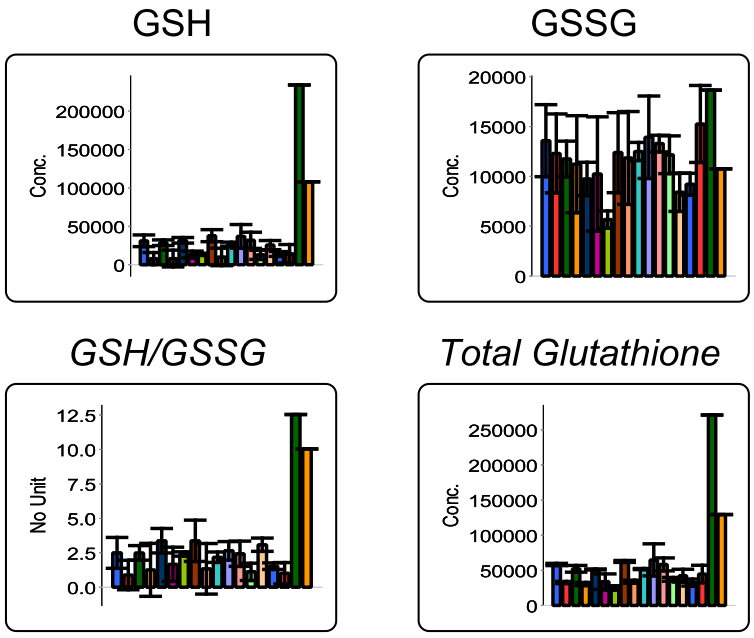


Fig. 14: Glutathione Redox Ratio and Total Glutathione

3-4-4. *NADPH / NADP+*

NADPH is consumed in the production of fatty acids, isoprenoids, and aromatic amino acids and is important in protecting cells against oxidative stress by transferring its reductive power to oxidized glutathione (GSSG) via glutathione disulphide reductase (14). $NADP^+$-to-NADPH conversion is mainly conducted in the pentose phosphate pathway, NADPH-to-$NADP^+$ ratio shows a positive linear relationship with glucose 6-phosphate dehydrogenase activity, which is a key regulatory enzyme in the pentose phosphate pathway. Note that the quantified NADPH and $NADP^+$ concentrations need to be evaluated with care, as these compounds are highly unstable in solution and reliability of the data is relatively low.

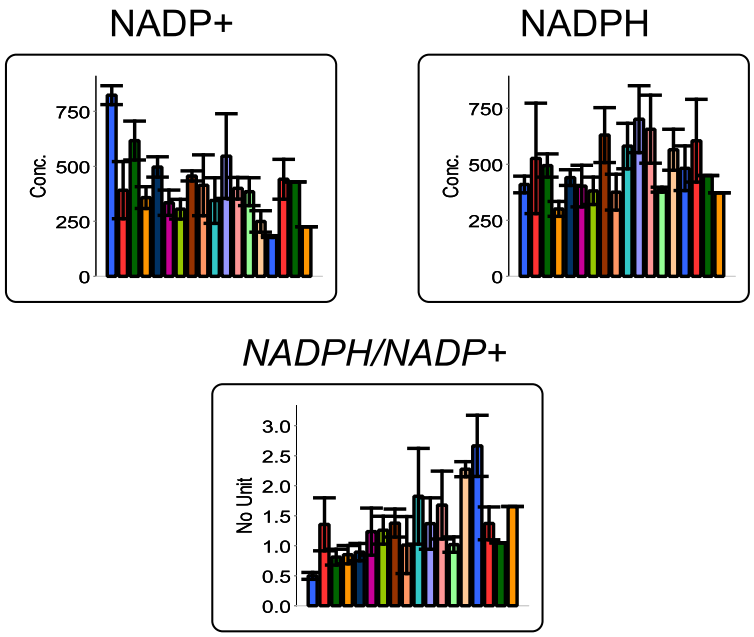


Fig. 15: NADPH / NADP+

3-4-5. *NADH / NAD+*

NAD^+ is required as cofactors in numerous reactions involved in glycolysis, TCA cycle, and β-oxidation of fatty acids and NADH is produced as a byproduct. In contrast, NADH is required in the electron transport chain for generating ATP via oxidative phosphorylation, and thus, the $NADH/NAD^+$ ratio is often disturbed by a stress that affects the central carbon metabolism such as hypoxia (15). Note that the quantified NADH and NAD^+ concentrations need to be evaluated with care, as these compounds are highly unstable in solution and reliability of the data is relatively low.

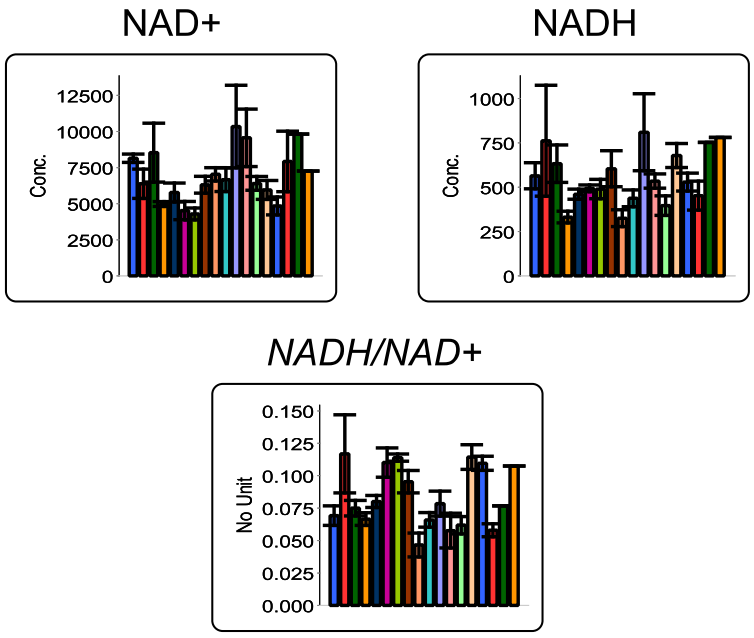


Fig. 16: NADH / NAD+

3-4-6. *Lactate / Pyruvate (LP ratio)*

Lactate is the primary product of anaerobic glycolysis. In addition, pyruvate-to-lactate conversion couples with NADH-to-NAD^+ conversion. Therefore, the lactate-to-pyruvate ratio, or LP ratio, serves not only as a marker of hypoxic metabolism and ischemia (16) but also as an indirect parameter for NADH/NAD^+ ratio.

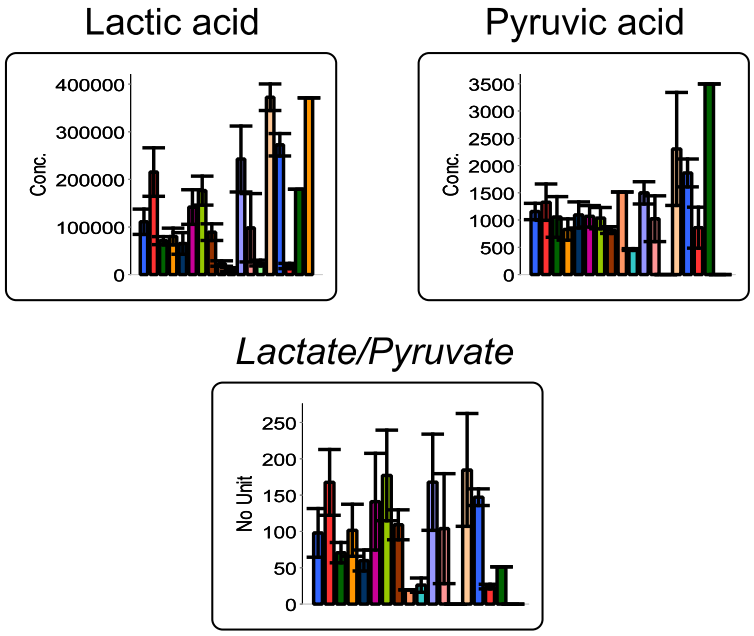


Fig. 17: Lactate / Pyruvate (LP ratio)

3-4-7. *Glycerol 3-phosphate / DHAP*

DHAP-to-glycerol 3-phosphate conversion couples with NADH-to-NAD^+ conversion in the cytosol, while glycerol 3-phosphate is then converted back to DHAP, coupled with FAD-to-$FADH_2$ conversion, in the intermembrane space of mitochondria, thereby contributing to ATP synthesis via oxidative phosphorylation. This is the so-called glycerol phosphate shuttle and it is particularly important in insect metabolism. Therefore, the glycerol 3-phosphate/DHAP ratio serves as an indirect parameter for NADH/NAD^+ ratio and energy status.

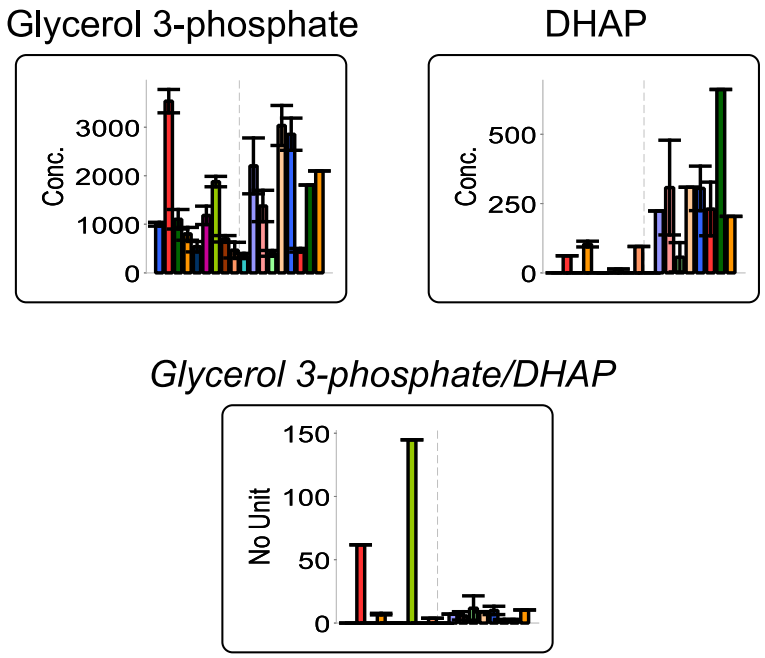


Fig. 18: Glycerol 3-phosphate / DHAP

3-4-8. *Total Amino Acids*

Total amino acids are the summation of all the 20 proteinogenic amino acids and are evaluated by the following equation:

Total Amino Acids = [Ala] + [Arg] + [Asn] + [Asp] + [Cys] + [Gln] + [Glu] + [Gly] + [His] + [Ile] + [Leu] + [Lys] + [Met] + [Phe] + [Pro] + [Ser] + [Thr] + [Trp] + [Tyr] + [Val]

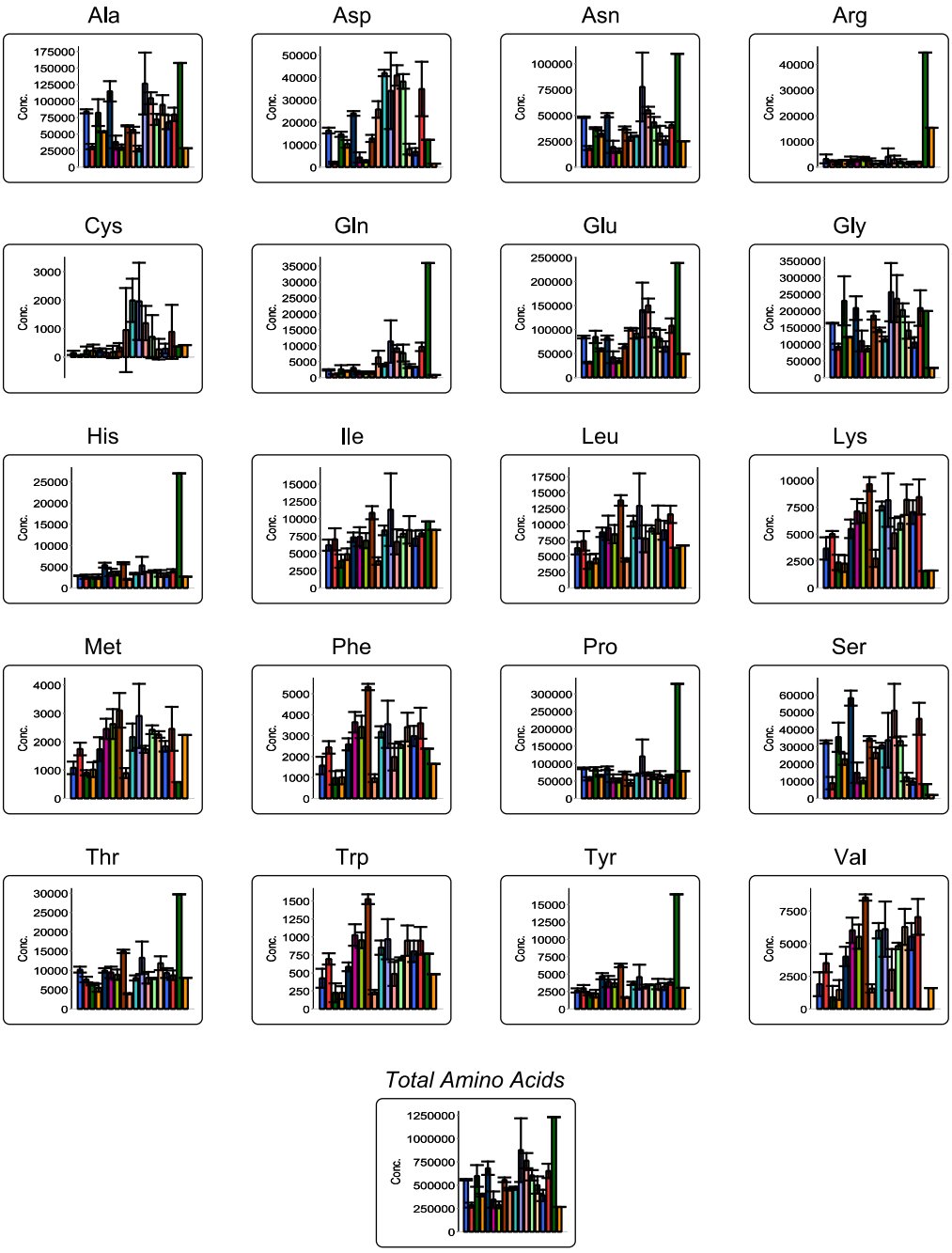


Fig. 19: Total Amino Acids

3-4-9. *Total Essential Amino Acids*

Total essential amino acids are the summation of all the 9 essential amino acids and are evaluated by the following equation:

Total Essential Amino Acids = [His] + [Ile] + [Leu] + [Lys] + [Met] + [Phe] + [Thr] + [Trp] + [Val]

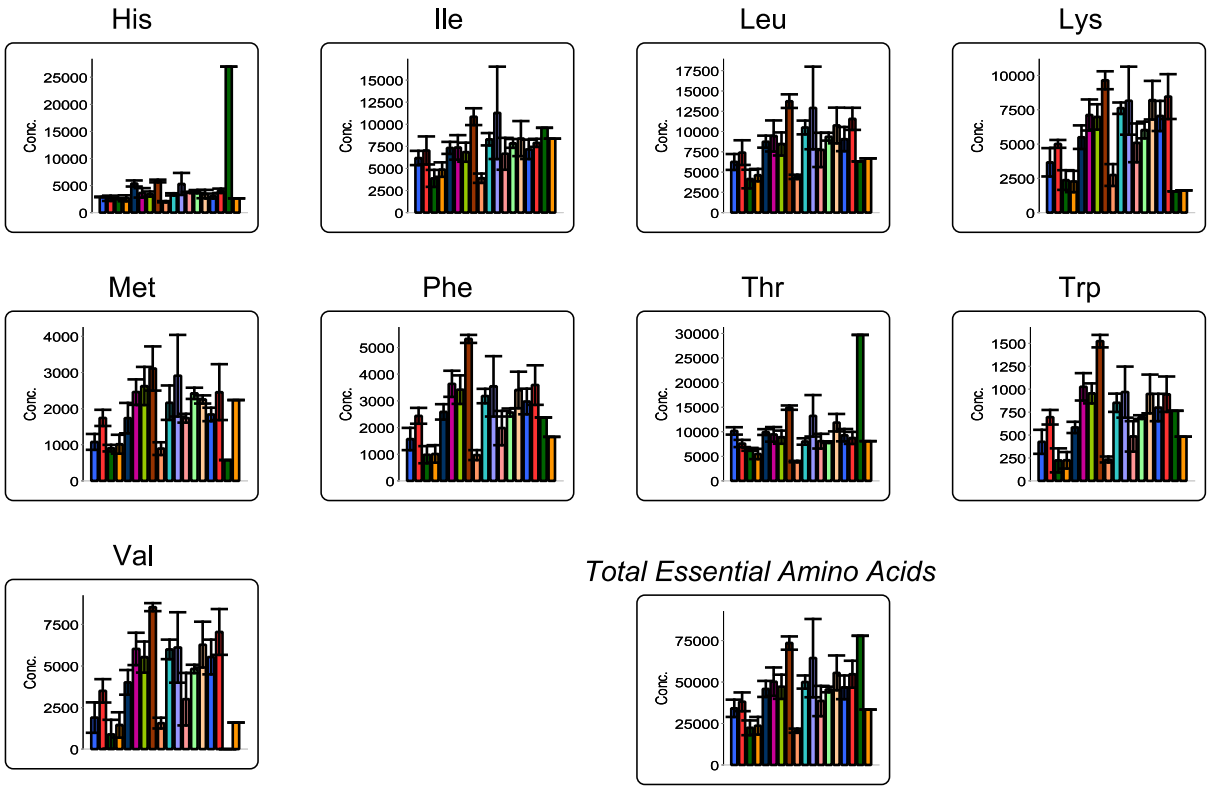


Fig. 20: Total Essential Amino Acids

3-4-10. *Total Non-essential Amino Acids*

Total non-essential amino acids are the summation of all the 11 non-essential amino acids and are evaluated by the following equation:

Total Non-essential Amino Acids = [Ala] + [Arg] + [Asn] + [Asp] + [Cys] + [Gln] + [Glu] + [Gly] + [Pro] + [Ser] + [Tyr]

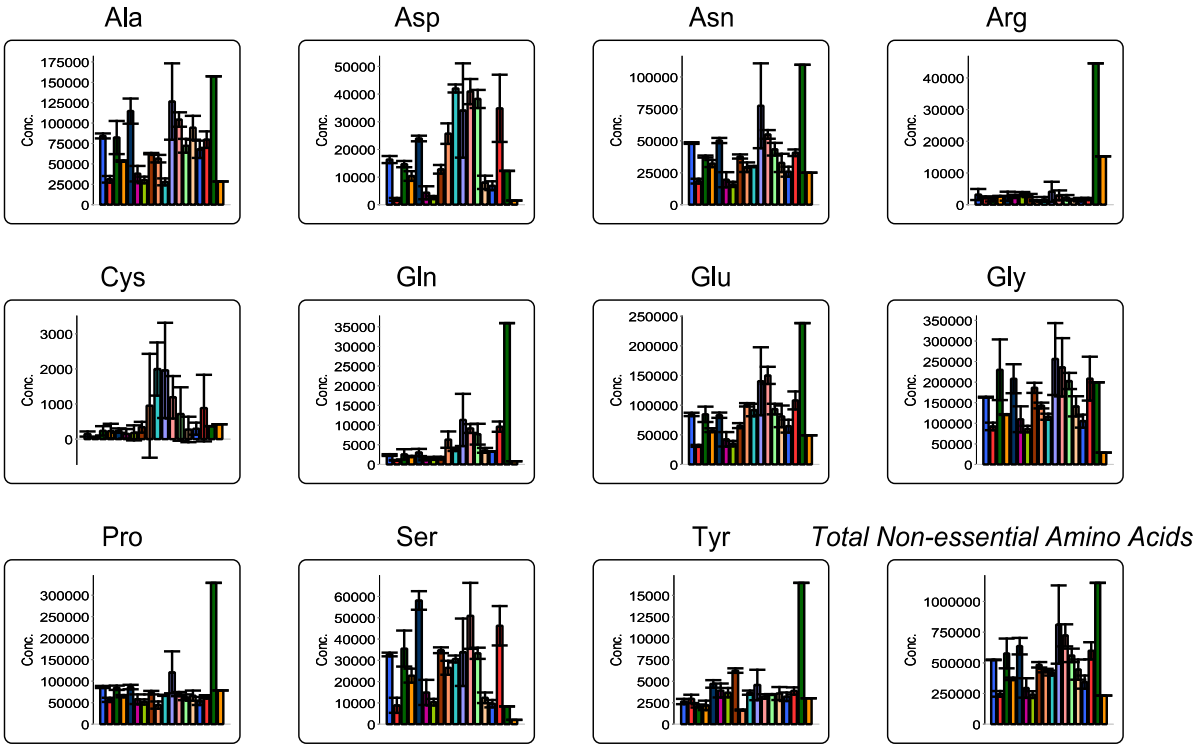


Fig. 21: Total Non-essential Amino Acids

3-4-11. *Total Glucogenic Amino Acids*

Total glucogenic amino acids are the summation of all the 18 amino acids, which can be used for gluconeogenesis, and evaluated by the following equation:

Total Glucogenic Amino Acids = [Ala] + [Arg] + [Asn] + [Asp] + [Cys] + [Gln] + [Glu] + [Gly] + [His] + [Ile] + [Met] + [Phe] + [Pro] + [Ser] + [Thr] + [Trp] + [Tyr] + [Val]

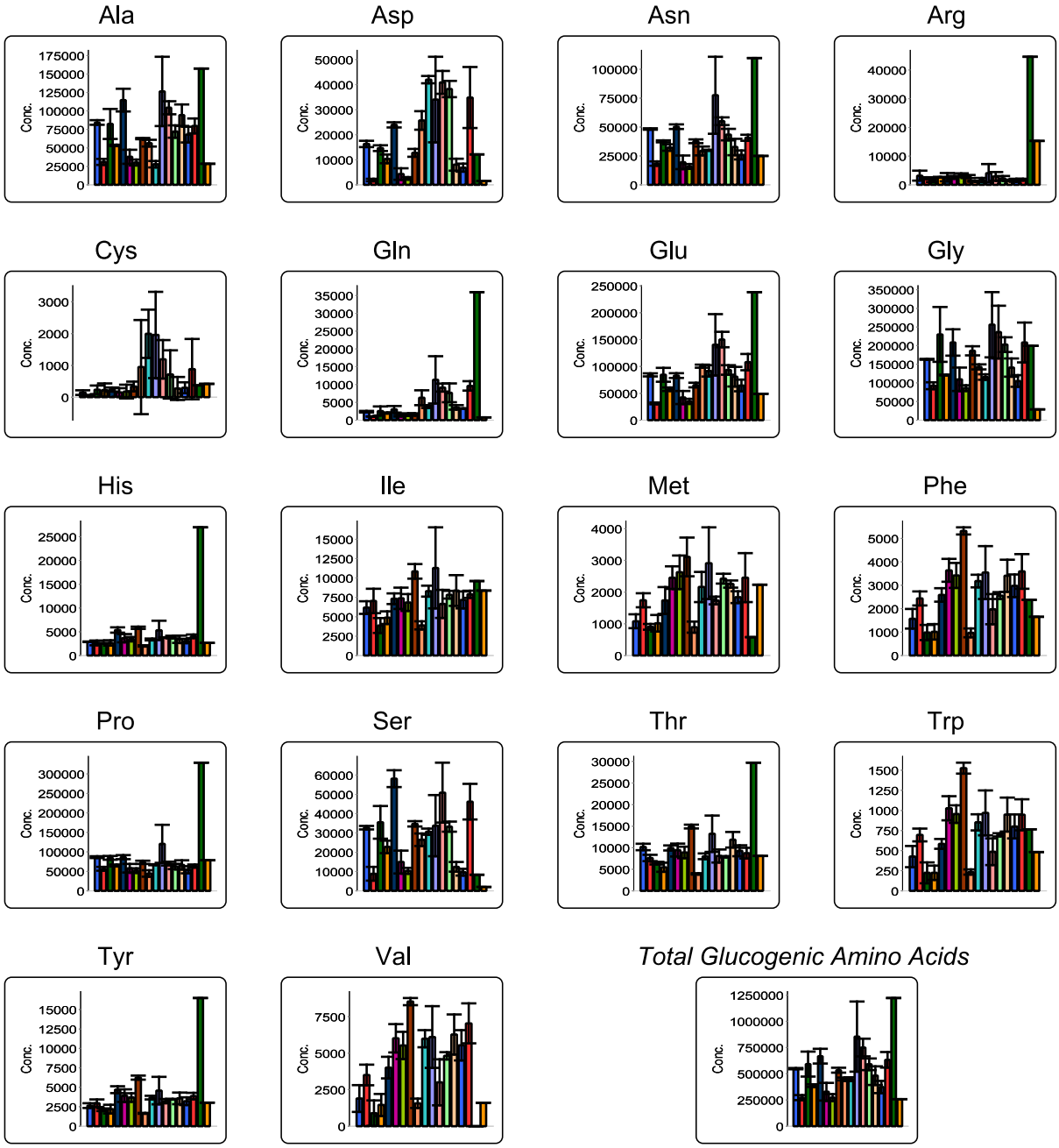


Fig. 22: Total Glucogenic Amino Acids

3-4-12. *Total Ketogenic Amino Acids*

Total ketogenic amino acids are the summation of all the 7 amino acids, which can be used for ketogenesis, and evaluated by the following equation:

Total Ketogenic Amino Acids = [Ile] + [Leu] + [Lys] + [Phe] + [Thr] + [Trp] + [Tyr]

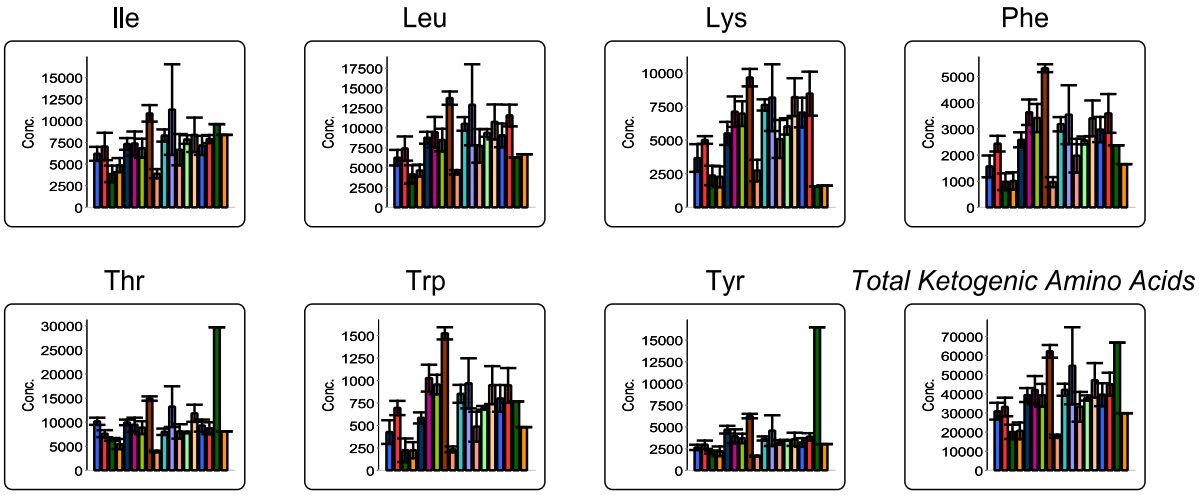


Fig. 23: Total Ketogenic Amino Acids

3-4-13. *Total Branched-chain Amino Acids*

Total branched-chain amino acids are the summation of all the 3 branched-chain amino acids and are evaluated by the following equation:

Total Branched-chain Amino Acids = [Ile] + [Leu] + [Val]

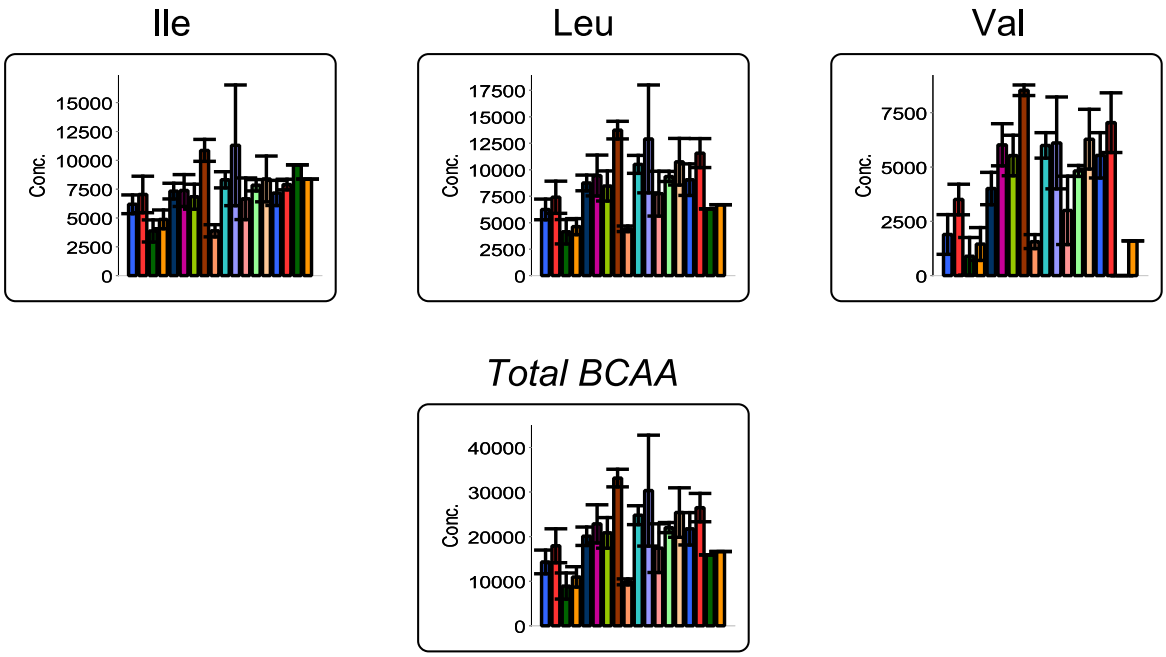


Fig. 24: Total Branched-chain Amino Acids

3-4-14. *Total Aromatic Amino Acids*

Total aromatic amino acids are the summation of all the 3 aromatic amino acids and are evaluated by the following equation:

Total Aromatic Amino Acids = [Phe] + [Trp] + [Tyr]

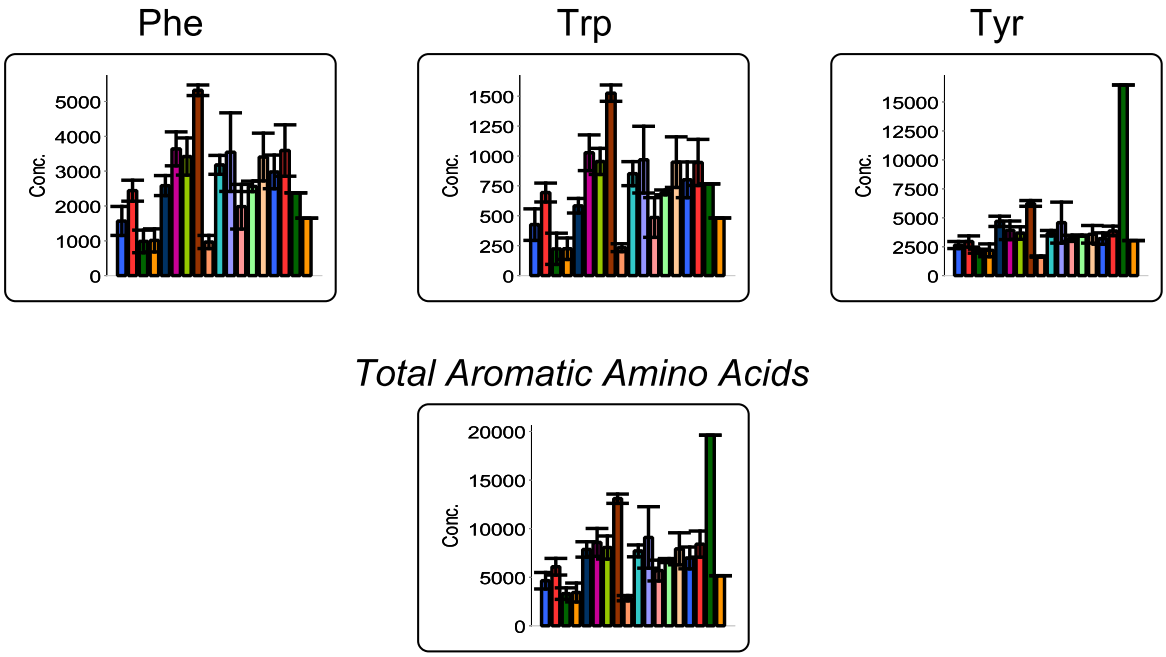


Fig. 25: Total Aromatic Amino Acids

3-4-15. *Fischer's Ratio (BCAA/AAA)*

Fischer's ratio is the total branched-chain amino acids over the total aromatic amino acids and is often used as an indicator for hepatic failure (17). The Fischer's ratio can be thus evaluated by the following equation:

$$\text{Fischer's Ratio} = \frac{[\text{Ile}]+[\text{Leu}]+[\text{Val}]}{[\text{Phe}]+[\text{Trp}]+[\text{Tyr}]} = \frac{[\text{BCAA}]}{[\text{AAA}]}$$

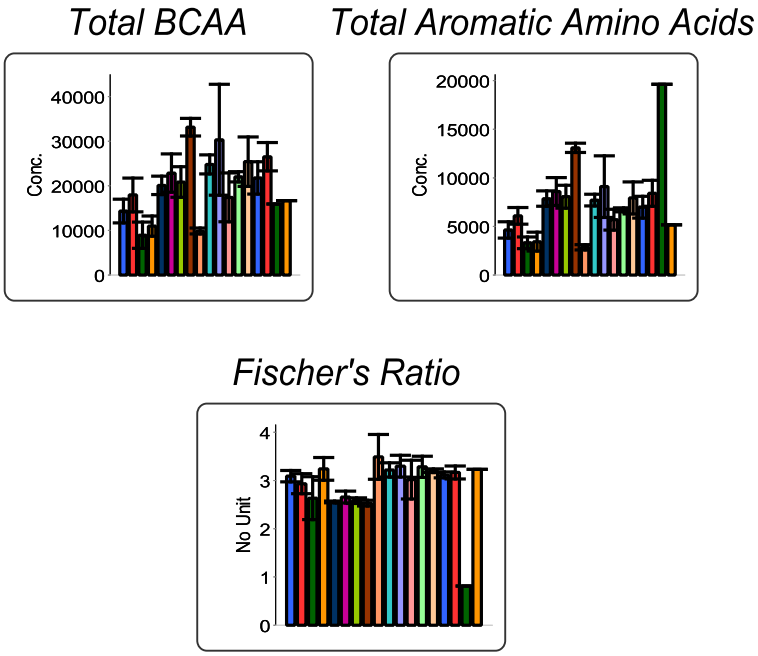


Fig. 26: Fischer's Ratio (BCAA/AAA)

3-4-16. *Total Glu-related Amino Acids*

Total Glu-related amino acids are the summation of all the 5 amino acids that are catabolized to Glu and evaluated by the following equation:

Total Glu-related Amino Acids = [Arg] + [Gln] + [Glu] + [His] + [Pro]

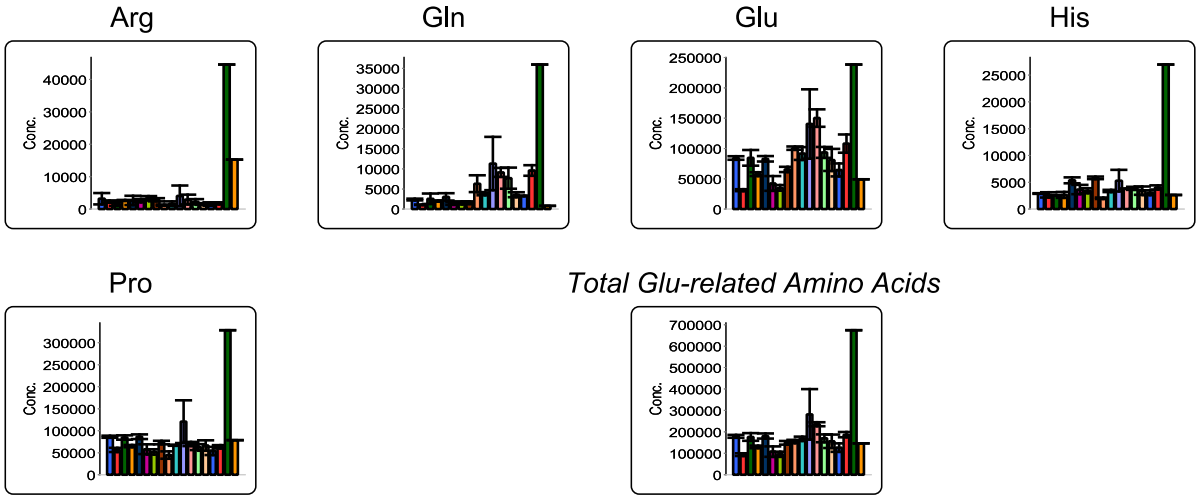


Fig. 27: Total Glu-related Amino Acids

3-4-17. *Total Pyr-related Amino Acids*

Total Pyr-related amino acids are the summation of all the 6 amino acids that are catabolized to pyruvate and evaluated by the following equation:

Total Pyr-related Amino Acids = [Ala] + [Cys] + [Gly] + [Ser] + [Thr] + [Trp]

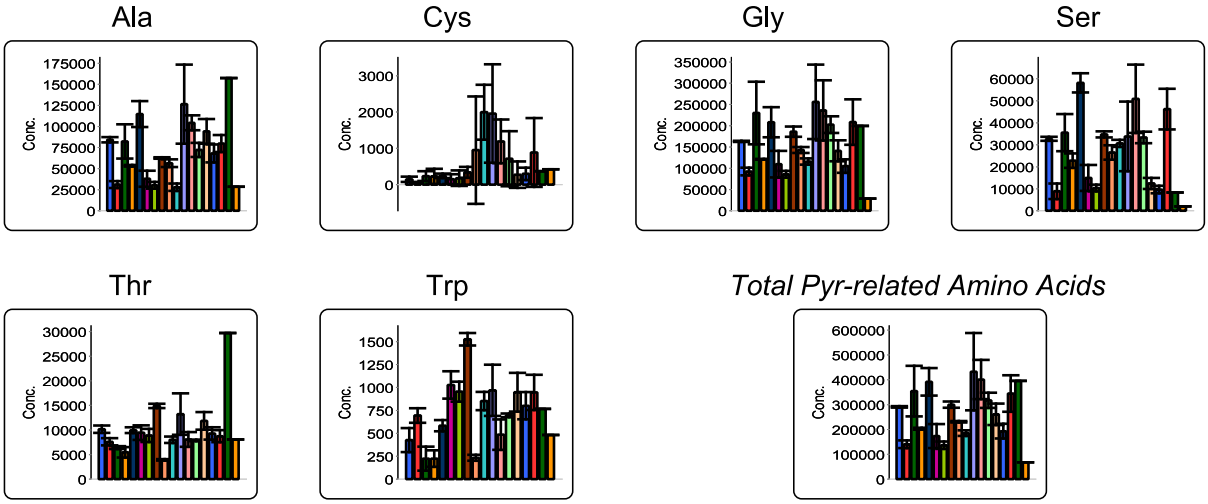


Fig. 28: Total Pyr-related Amino Acids

3-4-18. *Total Acetyl CoA-related Amino Acids*

Total acetyl CoA-related amino acids are the summation of all the 4 amino acids that are catabolized to Acetyl CoA and evaluated by the following equation:

Total acetyl CoA-related Amino Acids = [Ile] + [Leu] + [Lys] + [Trp]

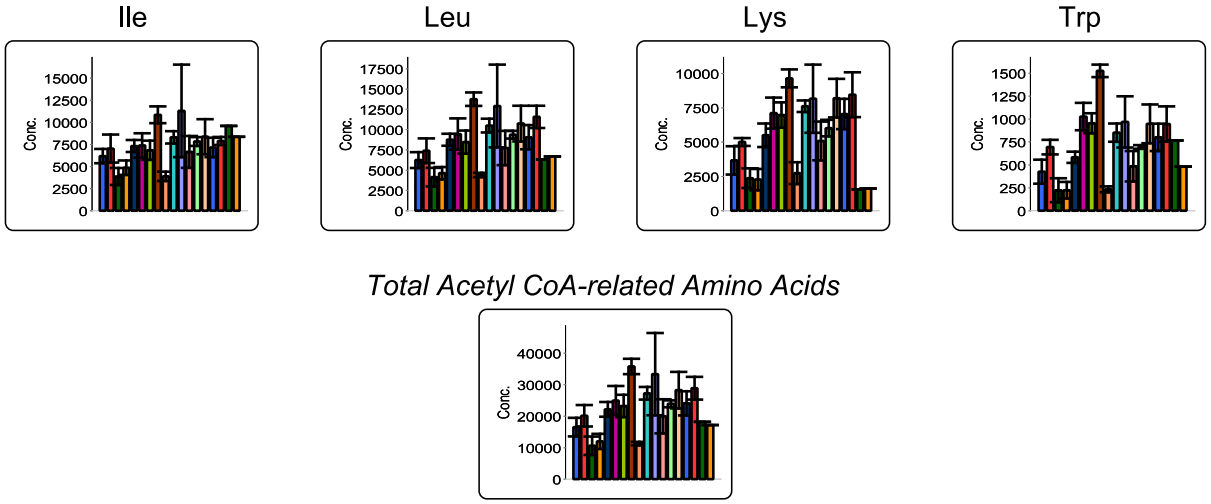


Fig. 29: Total Acetyl CoA-related Amino Acids

3-4-19. *Total Fumarate-related Amino Acids*

Total fumarate-related amino acids are the summation of all the 2 amino acids that are catabolized to fumarate and evaluated by the following equation:

Total Fumarate-related Amino Acids = [Phe] + [Tyr]

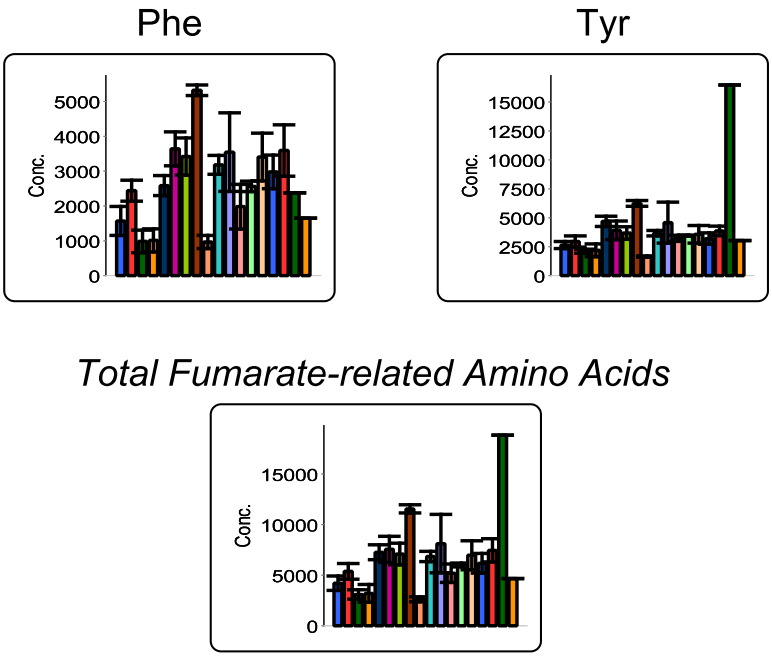


Fig. 30: Total Fumarate-related Amino Acids

3-4-20. *Total Succinyl CoA-related Amino Acids*

Total succinyl CoA-related amino acids are the summation of all the 3 amino acids that are catabolized to succinyl CoA and evaluated by the following equation:

Total Succinyl CoA-related Amino Acids = [Ile] + [Met] + [Val]

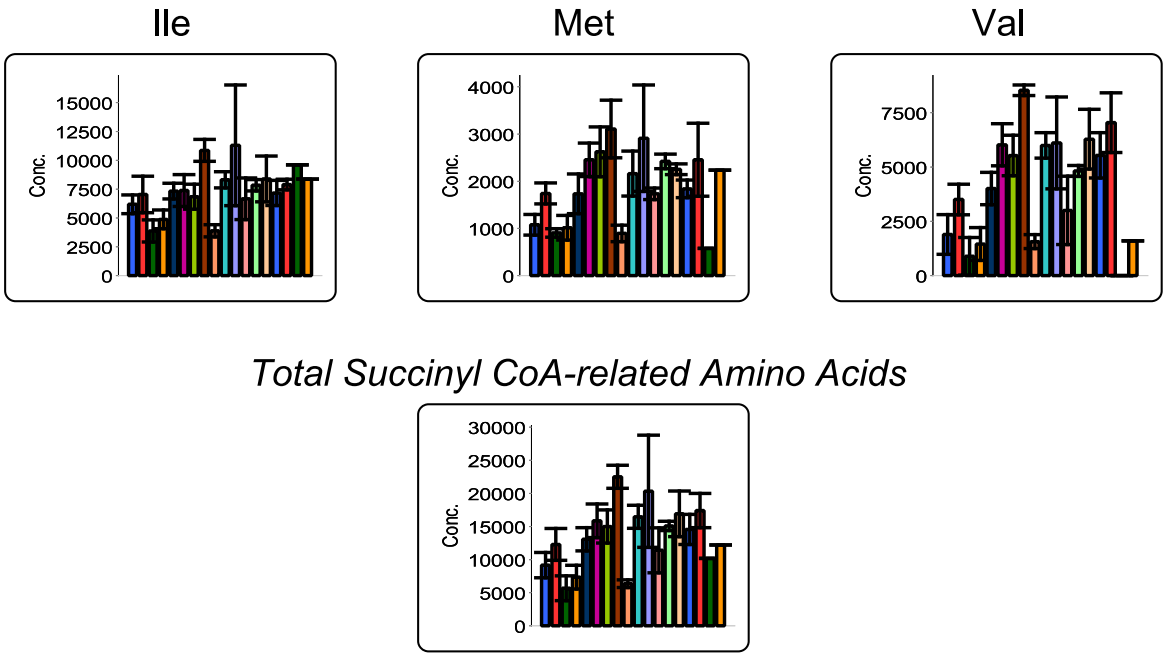


Fig. 31: Total Succinyl CoA-related Amino Acids

3-4-21. *Total Oxaloacetate-related Amino Acids*

Total oxaloacetate-related amino acids are the summation of all the 2 amino acids that are catabolized to oxaloacetate and evaluated by the following equation:

Total Oxaloacetate-related Amino Acids = [Asn] + [Asp]

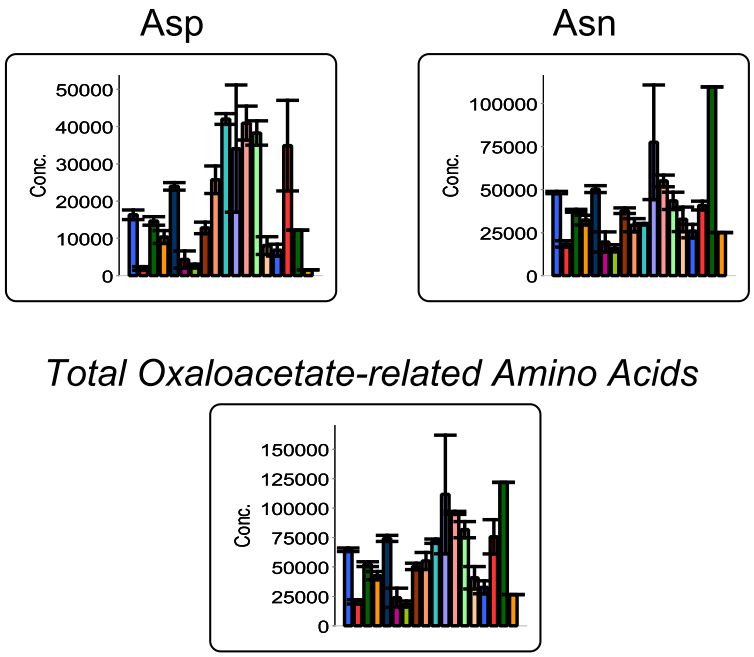


Fig. 32: Total Oxaloacetate-related Amino Acids

3-4-22. *Malate / Asp*

Malate is produced from oxaloacetate coupled with NADH-to-NAD^+ conversion in the cytosol and is then converted back to oxaloacetate coupled with NAD^+-to-NADH conversion inside the mitochondria. Oxaloacetate is subsequently converted to Asp, which is able to cross the inner mitochondrial membrane to return to the cytosol and is converted back to oxaloacetate; this cycle is known as the malate-Asp shuttle. Since this shuttle overall transfers NADH in the cytosol to the mitochondria for oxidative phosphorylation to produce ATP, malate/Asp ratio serves as an indirect parameter for NADH/NAD^+ ratio and energy status.

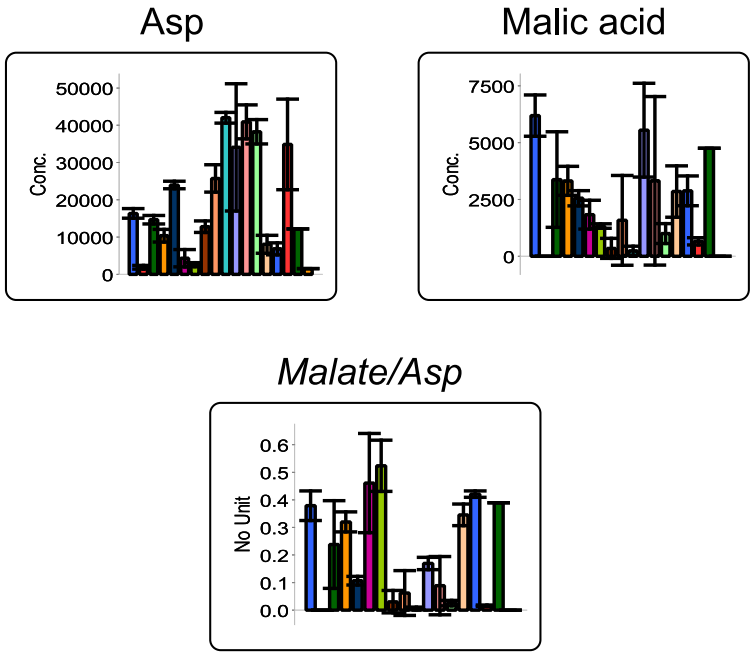


Fig. 33: Malate / Asp

3-4-23. *Citrulline / Ornithine*

Citrulline-to-ornithine ratio is known to correlate with Arg consumption. The ratio increases if Arg is metabolized via nitric oxide synthase (NOS), which produces citrulline and nitric oxide (NO), and decreases if metabolized via arginase, which breaks down Arg into ornithine and urea (18).

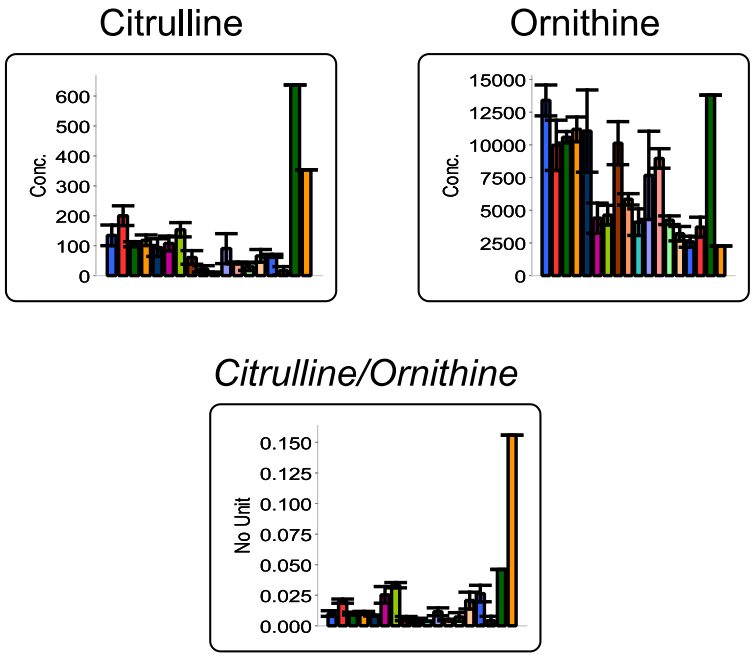


Fig. 34: Citrulline / Ornithine

3-4-24. *Glu / 2-Oxoglutarate*

Both Glu-to-2-oxoglutamate and 2-oxoglutarate-to-Glu conversions are coupled with many reactions in amino acid metabolic pathways; the former is often involved in amino acid synthesis and the latter is in amino acid breakdown pathways. Therefore, the Glu/2-oxoglutarate ratio serves as an indirect indicator to show an overall significance of amino acid synthesis or degradation.

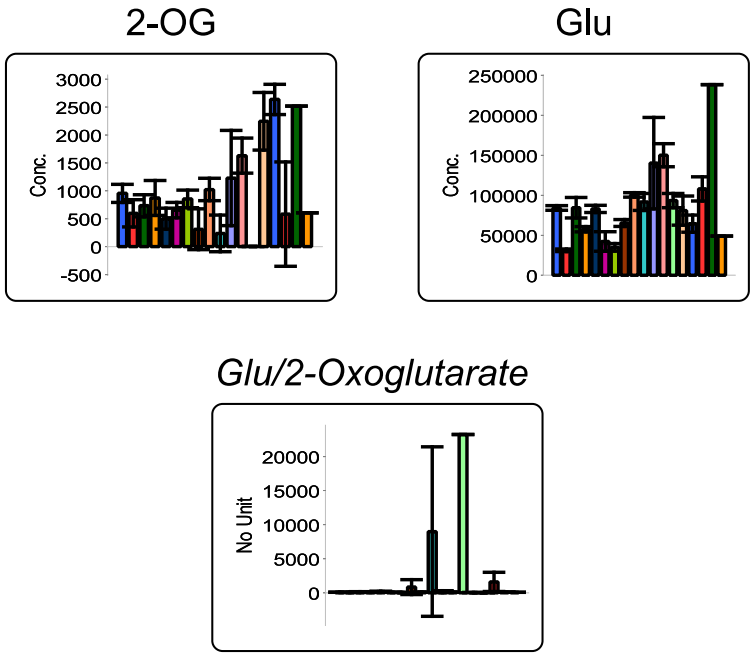


Fig. 35: Glu / 2-Oxoglutarate

3-4-25. *Glucose 6-phosphate / Ribose 5-phosphate*

Glucose 6-phosphate-to-ribose 5-phosphate conversion is the first and a bottleneck reaction in the pentose phosphate pathway. Glucose 6-phosphate is involved in glycolysis, whereas ribose 5-phosphate takes part in pentose phosphate pathway; therefore, the glucose 6-phopshate-to-ribose 5-phosphate ratio serves as an indirect parameter to show the significance in the activity of glycolysis or pentose phosphate pathway.

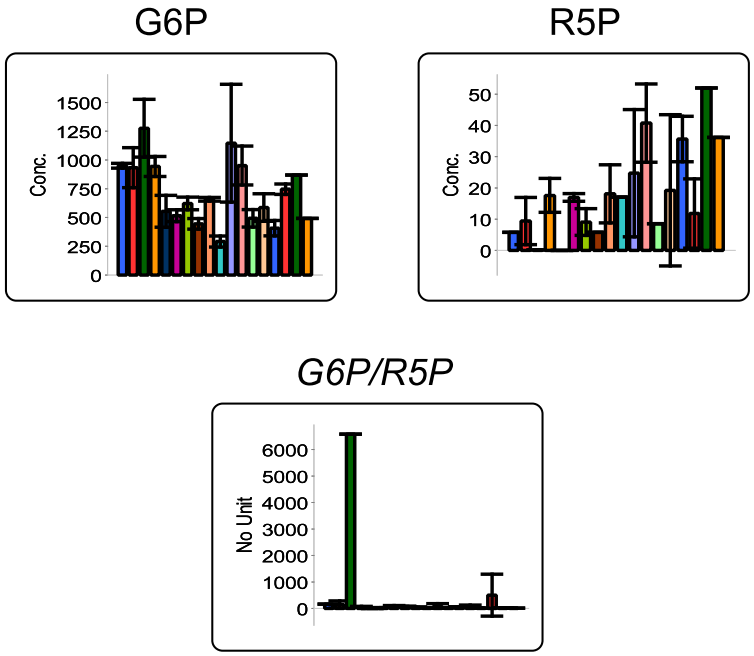


Fig. 36: Glucose 6-phosphate / Ribose 5-phosphate

3-4-26. *S-Adenosylmethionine (SAM) / S-Adenosylhomocysteine (SAH)*

More than 120 methyltransferases are known to be SAM-dependent and the SAM/SAH ratio is a key regulator for the activity of these enzymes and is thus considered to represent the cellular methylation potential (19).

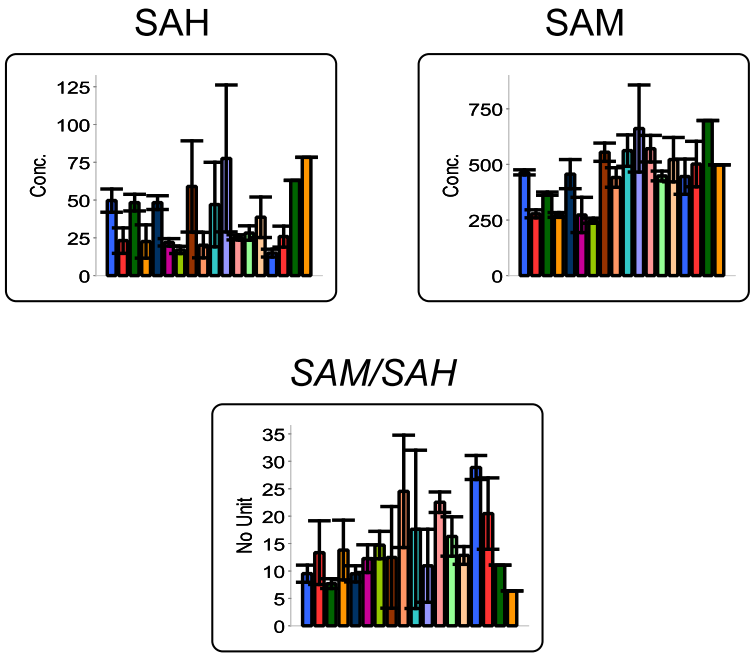


Fig. 37: *S*-Adenosylmethionine (SAM) / *S*-Adenosylhomocysteine (SAH)

3-4-27. *Putrescine / Spermidine*

Putrescine and spermidine are representative polyamines, which play crucial roles in DNA synthesis and gene expressions. In particular, the putrescine/spermidine ratio is known to increase with age in plants (20), and it increases with severity in certain types of cancer (21).

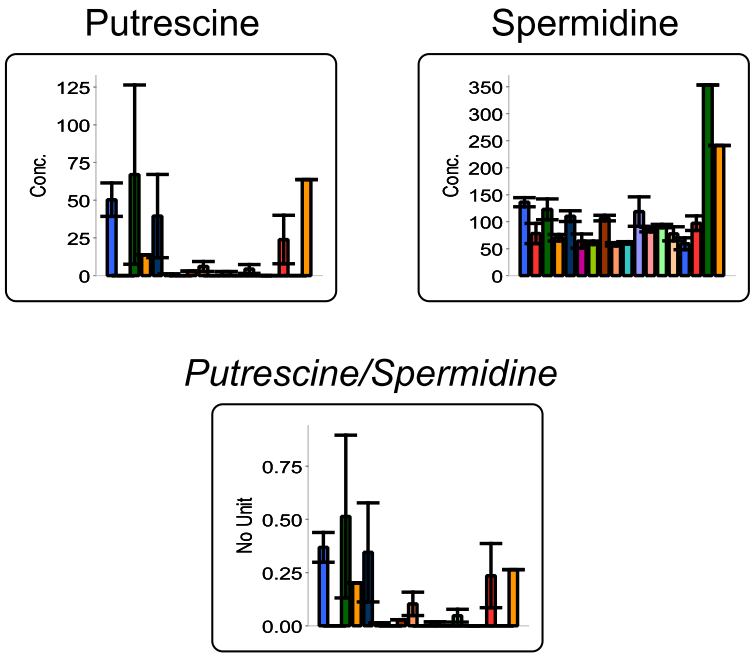


Fig. 38: Putrescine / Spermidine

4. References

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5. Storage Place for Data, Documents and Samples

Data and Documents will be stored in the following storage locations for 12 months after the submission of the final report. Samples will be stored for 6 months. However, these conditions do not apply to data re-analysis.

Storage Place for Documents

Document Depository in Human Metabolome Technologies, Inc.

Contents

- Contact Document
- Analysis Schedule
- Sample Preparation Procedure
- Analysis Condition
- Analysis Result
- Final Report

Storage Place for Electronic Files

Data Server in Human Metabolome Technologies, Inc.

Contents

- Analysis Data
- Analysis Result

Storage Place for Samples

Deep Freezer in Human Metabolome Technologies, Inc. (below -80℃)

（Contact Information） Human Metabolome Technologies, Inc.

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Fax : +81-235-25-1450

Appendix 1-1. Abbreviations in the Pathway Map (Sorted by Candidate Metabolites)

Candidates	Pathway Label‡
2,3-Diphosphoglyceric acid	Diphosphoglycerate
2-Hydroxyglutaric acid	2-Hydroxyglutaric acid
2-Oxoglutaric acid	2-OG
2-Oxoisovaleric acid	2-KIV
2-Phosphoglyceric acid	2-PG
3-Phosphoglyceric acid	3-PG
6-Phosphogluconic acid	6-PG
Acetoacetyl CoA	AAcCoA
Acetyl CoA	AcCoA
Adenine	Adenine
Adenosine	Adenosine
Adenylosuccinic acid	Succinyl AMP
ADP	ADP
ADP-ribose	ADP-Rib
Ala	Ala
AMP	AMP
Arg	Arg
Argininosuccinic acid	ArgSuccinate
Asn	Asn
Asp	Asp
ATP	ATP
Betaine	Betaine
Betaine aldehyde	BTL
cAMP	cAMP
Carbamoylphosphate	Carbamoyl-P
Carnitine	Carnitine
Carnosine	Carnosine
cGMP	cGMP
Choline	Choline
cis-Aconitic acid	cis-Aconitic acid
Citric acid	Citric acid
Citrulline	Citrulline
CoA	CoA
Creatine	Creatine
Creatinine	Creatinine
Cys	Cys
Cystathionine	Cystathionine
Dihydroxyacetone phosphate	DHAP
Erythrose 4-phosphate	E4P
Folic acid	Folic acid
Fructose 1,6-diphosphate	F1,6P
Fructose 1-phosphate	D-F1P
Fructose 6-phosphate	F6P
Fumaric acid	Fumaric acid
Galactose 1-phosphate	Gal1P
GDP	GDP
Gln	Gln
Glu	Glu
Glucose 1-phosphate	G1P
Glucose 6-phosphate	G6P
Glutathione (GSH)	GSH
Glutathione (GSSG)	GSSG
Gly	Gly
Glyceraldehyde 3-phosphate	Glyceraldehyde 3-phosphate
Glycerol 3-phosphate	Glycerol 3-phosphate
Glycolic acid	Glycolic acid
Glyoxylic acid	Glyoxylic acid
GMP	GMP
GTP	GTP
Guanine	Guanine
Guanosine	Guanosine
His	His
HMG CoA	HMG-CoA
Homocysteine	Homocysteine
Homoserine	Homoserine
Hydroxyproline	Hydroxyproline
Hypoxanthine	Hypoxanthine
Ile	Ile
IMP	IMP
Inosine	Inosine
Isocitric acid	Isocitric acid
Lactic acid	Lactic acid
Leu	Leu
Lys	Lys
Malic acid	Malic acid
Malonyl CoA	Malonyl-CoA
Met	Met
Mevalonic acid	Mevalonic acid
N,*N*-Dimethylglycine	DMG
N-Acetylglutamic acid	N-AcGlu
NAD^+	NAD+
NADH	NADH
$NADP^+$	NADP+
NADPH	NADPH
N-Carbamoylaspartic acid	Carbamoyl-Asp
Ornithine	Ornithine
Phe	Phe
Phosphocreatine	Phosphocreatine
Phosphoenolpyruvic acid	PEP
Pro	Pro
PRPP	PRPP
Putrescine	Putrescine
Pyruvic acid	Pyruvic acid
Ribose 1-phosphate	R1P
Ribose 5-phosphate	R5P
Ribulose 5-phosphate	Ru5P
S-Adenosylhomocysteine	SAH
S-Adenosylmethionine	SAM
Sarcosine	Sarcosine
Sedoheptulose 7-phosphate	S7P
Ser	Ser
Spermidine	Spermidine
Spermine	Spermine
Succinic acid	Succinic acid
Thr	Thr
Trp	Trp
Tyr	Tyr
UDP-glucose	UDP-Glc
Urea	Urea
Uric acid	Uric acid
Val	Val
Xanthine	Xanthine
XMP	XMP
Xylulose 5-phosphate	X5P
β-Ala	b-Ala
γ-Aminobutyric acid	g-Aminobutyric acid

‡ Abbreviated names in Pathway Map.

Appendix 1-2. Abbreviations in the Pathway Map (Sorted by Pathway Label Name)

Pathway Label‡	Candidates
2-Hydroxyglutaric acid	2-Hydroxyglutaric acid
2-KIV	2-Oxoisovaleric acid
2-OG	2-Oxoglutaric acid
2-PG	2-Phosphoglyceric acid
3-PG	3-Phosphoglyceric acid
6-PG	6-Phosphogluconic acid
AAcCoA	Acetoacetyl CoA
AcCoA	Acetyl CoA
Adenine	Adenine
Adenosine	Adenosine
ADP	ADP
ADP-Rib	ADP-ribose
Ala	Ala
AMP	AMP
Arg	Arg
ArgSuccinate	Argininosuccinic acid
Asn	Asn
Asp	Asp
ATP	ATP
b-Ala	β-Ala
Betaine	Betaine
BTL	Betaine aldehyde
cAMP	cAMP
Carbamoyl-Asp	*N*-Carbamoylaspartic acid
Carbamoyl-P	Carbamoylphosphate
Carnitine	Carnitine
Carnosine	Carnosine
cGMP	cGMP
Choline	Choline
cis-Aconitic acid	*cis*-Aconitic acid
Citric acid	Citric acid
Citrulline	Citrulline
CoA	CoA
Creatine	Creatine
Creatinine	Creatinine
Cys	Cys
Cystathionine	Cystathionine
D-F1P	Fructose 1-phosphate
DHAP	Dihydroxyacetone phosphate
Diphosphoglycerate	2,3-Diphosphoglyceric acid
DMG	*N*,*N*-Dimethylglycine
E4P	Erythrose 4-phosphate
F1,6P	Fructose 1,6-diphosphate
F6P	Fructose 6-phosphate
Folic acid	Folic acid
Fumaric acid	Fumaric acid
G1P	Glucose 1-phosphate
G6P	Glucose 6-phosphate
Gal1P	Galactose 1-phosphate
g-Aminobutyric acid	γ-Aminobutyric acid
GDP	GDP
Gln	Gln
Glu	Glu
Gly	Gly
Glyceraldehyde 3-phosphate	Glyceraldehyde 3-phosphate
Glycerol 3-phosphate	Glycerol 3-phosphate
Glycolic acid	Glycolic acid
Glyoxylic acid	Glyoxylic acid

Pathway Label‡	Candidates
GMP	GMP
GSH	Glutathione (GSH)
GSSG	Glutathione (GSSG)
GTP	GTP
Guanine	Guanine
Guanosine	Guanosine
His	His
HMG-CoA	HMG CoA
Homocysteine	Homocysteine
Homoserine	Homoserine
Hydroxyproline	Hydroxyproline
Hypoxanthine	Hypoxanthine
Ile	Ile
IMP	IMP
Inosine	Inosine
Isocitric acid	Isocitric acid
Lactic acid	Lactic acid
Leu	Leu
Lys	Lys
Malic acid	Malic acid
Malonyl-CoA	Malonyl CoA
Met	Met
Mevalonic acid	Mevalonic acid
N-AcGlu	*N*-Acetylglutamic acid
NAD+	NAD^+
NADH	NADH
NADP+	$NADP^+$
NADPH	NADPH
Ornithine	Ornithine
PEP	Phosphoenolpyruvic acid
Phe	Phe
Phosphocreatine	Phosphocreatine
Pro	Pro
PRPP	PRPP
Putrescine	Putrescine
Pyruvic acid	Pyruvic acid
R1P	Ribose 1-phosphate
R5P	Ribose 5-phosphate
Ru5P	Ribulose 5-phosphate
S7P	Sedoheptulose 7-phosphate
SAH	*S*-Adenosylhomocysteine
SAM	*S*-Adenosylmethionine
Sarcosine	Sarcosine
Ser	Ser
Spermidine	Spermidine
Spermine	Spermine
Succinic acid	Succinic acid
Succinyl AMP	Adenylosuccinic acid
Thr	Thr
Trp	Trp
Tyr	Tyr
UDP-Glc	UDP-glucose
Urea	Urea
Uric acid	Uric acid
Val	Val
X5P	Xylulose 5-phosphate
Xanthine	Xanthine
XMP	XMP

‡ Abbreviated names in Pathway Map.