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„ Effects of increased protein intake and resistance training on metabolites of the citrate cycle and urea cycle in older adults “

verfasst von / submitted by

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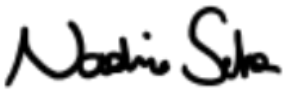
Affirmation

I hereby declare that the master's thesis submitted was in all parts exclusively fabricated on my own, and that other sources or aids than those explicitly referred to have not been utilised. All passages quoted from publications or paraphrased from these sources are properly cited and attributed. The thesis was not previously submitted in same or similar form to another examination committee and was not published elsewhere.

Vienna, July 2022

Nadine Setka



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Preface

First of all, the project partners of the NutriAging study should be mentioned, which are the University of Vienna and the Comenius University Bratislava. The project was funded by Interreg Slovakia - Austria (European Regional Development Fund).

The preparations for this master thesis were done with Sabine Trettenhahn, BSc, who was a great and ambitious lab partner and thanks to whom the lab work went so fast. While my master thesis focuses on the citrate cycle and urea cycle, Sabine Trettenhahn's thesis “Gender-specific differences in the metabolome of older adults” takes a closer look at the gender differences.

The realization of this work is due to the great support of our supervisors Univ.-Prof. Mag. Dr. Karl-Heinz Wagner (Department of Nutritional Sciences) and Mag. Dr. Bernhard Franzke, Bakk. (Department of Nutritional Sciences) and Univ.-Prof. Dr. Wolfram Weckwerth (Functional and Evolutionary Ecology).

I would also like to thank the entire department Molecular Systems Biology of the University of Vienna (mosys) for their cooperation and help and for allowing Sabine Trettenhahn and me to work in their laboratory.

As for the lab work and evaluation, we got great help from Mag. Lena Fragner (Functional and Evolutionary Ecology) and Martin Brenner, BSc MSc. (Functional and Evolutionary Ecology, Department of Pharmaceutical Sciences). I would like to thank them for their time and patience and for giving us the chance to work on this interesting topic.

At last, I would like to thank Dr. Ammar Tahir, MSc, who introduced Sabine Trettenhahn and me to further basics for the analysis of metabolomics data.

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List of abbreviations

GC-MS	Gas Chromatography-Mass Spectrometry.
LC-MS	Liquid Chromatography-Mass Spectrometry.
PE	Pentaerythritol.
PGP	Phenyl-ß-D-glucopyranoside.
TMS	Tetramethylsilane

Intervention:

CON	Control group = intervention group 1
RP+T	Recommended protein plus resistance training group = intervention group 2
HP+T	High protein plus resistance training group = intervention group 3
T1	Measurement time point 1/ testing day 1
T2	Measurement time point 2/ testing day 2
T3	Measurement time point 3/ testing day 3

Metabolites:

ALA	Alanine
ARG	Arginine
ASN	Asparagine
ASP	Aspartic acid
a-TOC	Alpha-Tocopherol
b-ALA	Beta-Alanine
CIT	Citric acid
CRE	Creatinine
CYS	Cysteine
CYT	Cystine

FUM	Fumaric acid
GABA	4-Amino-butyrate
GLN	Glutamine
GLU	Glutamic acid
GLY	Glycine
GLYC	Glycolic acid
GR	Glyceric acid
HIS	Histidine
HYP	4-Hydroxyproline
IAA	Indole-3-Acetate
ILE	Isoleucine
K-LEU	Keto-Leucine
KYN	Kynurenine
KYNA	Kynurenic acid
LAC	Lactic acid
LEU	Leucine
LYS	Lysine
MAL	Malic acid
MET	Methionine
OAA	Oxaloacetic acid
OMV	2-Oxo-3 Methyl-valerate
ORN	Ornithine
OXA	Oxalic acid
OXO	2- Oxoglutaric acid
PHE	Phenylalanine

PRO	Proline
PYR	Pyruvic acid
SER	Serine
SUC	Succinic acid
THR	Threonine
TRA	Threonic acid
TRP	Tryptophan
TYR	Tyrosine
VAL	Valine

1. Introduction

Physical activity and adequate protein intake in the older population are important topics, which will be considered in more detail in this master thesis. The citrate cycle and urea cycle are closely linked to energy metabolism as well as protein metabolism. Therefore, the present work focuses on the metabolites of these metabolic pathways.

1.1. Scientific background

1.1.1. Metabolomics

Metabolomics is a widely used method which can increase the probability to detect diseases. With this method metabolites which are involved in biochemical pathways can be identified and evaluated. Depending on the substance class, some metabolic products can be detected by GC-MS (Gas Chromatography-Mass Spectrometry) others by LC-MS (Liquid Chromatography-Mass Spectrometry). Therefore GC-MS and LC-MS are often applied in combination (Zeki, et al., 2020).

For our study we only used GC-MS. This method can determine substances smaller than 650 daltons. Toxins, fatty acids, alcohols, hydroxyl acids, catecholamines, amino acids, sugars, sterols, drugs, fatty acids and sterols are the main substances used. The substances are usually derivatized because this allows them to be more volatile and thus more suitable for measurement (Fiehn, 2016). Moreover derivatized samples are thermally stable (Papadimitropoulos, et al., 2018). Quantification can be approached in two ways: targeted and untargeted. Targeted metabolomics can be used to quantify known substances by comparing with a reference substance measured with the same device and the same method, while untargeted metabolomics can be used to discover unknown components. Assistance in annotation and quantification is provided by database annotations and spectral libraries (Fiehn, 2016).

1.1.2. Protein Supplementation

Protein turnover during and immediately after physical activity differs depending on the nutrients and total energy provided. The type, time, and intensity of the workout also play a crucial role. Age, gender, and physiological modifications are other important factors (Colonetti, et al., 2016).

A high protein intake might have a positive effect on the muscles in older people (Durainayagam, et al., 2019). Protein consumption is relevant for protein synthesis in the muscle and might catalyse protein degradation, which can be harmful for the DNA (Draxler, et al., 2021). The entire metabolism has not yet been fully explored (Durainayagam, et al., 2019). Increased protein intake together with exercise can also positively affect the muscle breakdown and strength in older adults (Mertz, et al., 2021).

The Recommended Daily Allowances (RDA) for people older than 19 years is 0.8 g protein per kg body weight per day. For older adults this value might be not enough. For the prevention of sarcopenia and other consequences of muscle mass loss, a higher consumption of protein in older adults (subjects > 60 years) seems to be important. Therefore, the review by Traylor, et al., (2018) recommends a protein intake of about 1.2 g/kg/bw/d. Other experimental and epidemiological studies seem to confirm this view (Franzke, et al., 2018). The D-A-CH reference values for nutrient intake recommend an intake of 1 g/kg/bw/d for adults aged 65 years and older (D-A-CH, 2021).

Muscle protein synthesis has been considered in more detail in experimental trials and showed that a higher relative protein supply appears to be useful for a total induction of protein synthesis in older adults. There is reason to believe that steady exercise might possibly maintain improve skeletal muscle reactivity to protein supply, even at very old age (Franzke, et al., 2018).

1.1.3. Exercise

Exercise and sitting activity are possibly responsible for various aspects of physical and mental well-being (Mañas, et al., 2019). Repeated exercise can reduce the risk of diseases in older adults (Maurer, et al., 2021). There are some data showing that

exercise in older adults may have a positive effect on frailty. In the review of Puts, et al. (2017) interventions with training workouts had a positive impact on indicators for frailty compared with interventions without exercise.

Results of the study from Mañas, et al. (2019) indicate a better physical ability and a lower risk of frailty in an intervention group with corporeal fit and short sedentary time and an intervention group with corporeal fit and a lot of sedentary time in contrast to an intervention group with insufficient fitness and a lot of sedentary time. Furthermore, physical activity and additionally high protein intake can reduce the loss of muscle mass at old age (Deutz, et al., 2014).

Physical activity leads to a higher proteolysis and amino acid degradation. Protein synthesis is downregulated while training and amino acids are metabolized and transformed in the muscle. Furthermore, amino acids are affected by stress, as well as stress due to training. A single bout of physical activity enhances the concentration of tryptophan and taurine in plasma, alanine and glutamine are elevated in the muscle. The concentration of glutamate in the muscle and glutamine in plasma are diminished. Long-term training affects muscle and causes glutamine and glutamate concentrations to decrease, whereas tyrosine and phenylalanine concentrations are enhanced. Glutamate and taurine values in muscle and phenylalanine, leucine, isoleucine, and tyrosine values in plasma are greater in individuals who prefer exercise regularly than in individuals who do not exercise (Ishikura, et al., 2013).

Age also seems to be a decisive factor, as Sabine Trettenhahn explains more detailed in her master's thesis. In the study from Zhou, et al. (2019), plasma metabolites were examined in rats subjected to strenuous physical activity. In male rats, fatty acid metabolism, amino acid breakdown, energy metabolism and glucose metabolism were elevated, while in female rats lipid oxidation, carbohydrate and protein metabolism and disturbances in the urea cycle were enhanced (Zhou, et al., 2019).

1.1.4. Urea Cycle and Citrate Cycle

As mentioned above, this thesis takes a closer look at the urea cycle and the citrate cycle. Plasma concentrations of metabolites of the citrate cycle and metabolites closely connected to it alter in response to physical activity (Hara, et al., 2021). Me-

tabolites of the citrate cycle are transformed inside the muscle during physical activity. Physiological changes due to physical activity are most apparent in relation to succinate (Maurer, et al., 2021). Amino acid degradation is enhanced during physical activity, as hepatic glucose production rises and the citrate cycle becomes more active. Protein synthesis, in turn, is inhibited during physical activity. Plasma concentrations of glutamic acid decrease during short-term exercise, while glutamine concentrations rise. During long-term exercise, amino acids are metabolized more in different organs than in the muscle, so that plasma amino acid levels are probably exhausted (Henriksson, 1991). To better understand the exact metabolism and effects of citrate cycle metabolites on physical activity, further research in this area needs to be conducted (Maurer, et al., 2021).

Regarding metabolic processes, Nitrogen is primarily discarded via the urea cycle (Lee, et al., 2018). Surplus Nitrogen is transformed into urea. Metabolites are variously exposed apart from the liver. Thus, nitrogen can produce necessary intermediates of the urea cycle (Keshet, et al., 2018). Urea is produced in the liver by metabolites of the urea cycle. The urea cycle is mainly influenced by the diet, the endocrine system, but also diseases. The excretion of urea occurs via the urine (Wang, et al., 2014).

To ensure an adequate level of glucose in the muscle tissue, the glucose-alanine cycle is adjusted. In this cycle, amino acids are converted, and alanine is transported out of the muscle. In the liver, this amino acid is integrated into the urea cycle, where it is converted to ammonia (Schranner, et al., 2020).

However, it should also be noted that the changes in each metabolite may vary depending on the body fluid. For example, the changes in alanine, valine and tryptophan are not identical in different body fluids (Schranner, et al., 2020).

Oxidation of amino acids plays a minor role in temporary strenuous physical activity. During prolonged physical activity, 3-6 % of the adenosine triphosphate produced is consumed (Gibala, 2001).

Valine, leucine, isoleucine, glutamic acid, and alanine affect other metabolites and the citrate cycle. At the initiation of physical activity, metabolites of the citrate cycle are elevated in muscular tissue. Alanine aminotransferase (glutamic acid +

pyruvate ↔ alanine + 2-oxoglutaric acid) and glutamic acid especially appear to be responsible. The chemical equilibrium of the reaction glutamic acid + pyruvate ↔ alanine + 2-oxoglutaric acid is shifted to the latter (Gibala, 2001). Metabolites of the citrate cycle are not just elevated in muscular tissue, but also in blood and urine. Higher values of the metabolites in blood can be primarily seen at the beginning of physical activity (< 30 Minutes after start). It does not seem to make a difference whether endurance or strength training is performed (Schranner, et al., 2020).

One possible reason for the increase in metabolites of the citrate cycle during physical activity could be the need to cover the increased energy intake. The metabolites possibly diminish again when the muscle is exhausted (Gibala, 2001).

1.1.5. Aging

Many aspects are involved in human aging, including way of living, genetic or epigenetic aspects, stress vulnerability, nutrition, and sociocultural milieu. Different markers are necessary to evaluate the biological age (Kondoh, et al., 2020). The consequences of biological aging include impairments in repair and recovery processes in the organism. These changes are variously advanced in subjects of the same age. This also indicates that a distinction must be made between biological age and chronological age (Khan, et al., 2017).

Aging induces changes in the body composition. There is a reduction in muscle and bone mass and body fat increases or is reallocated (JafariNasabian, et al., 2017). Maldevelopments of the skeletal musculature lead to impairments in older people that make daily life more difficult. Sarcopenia is a common syndrome in aging individuals and is manifested by a decrease in muscle mass and muscle strength. Deficient metabolic build-up and breakdown and higher levels of oxidative stress can lead to a decrease in muscle mass. Sarcopenia can have a negative effect on mortality and frailty. The development of sarcopenia is promoted by an unhealthy lifestyle (insufficient physical exercise, malnourishment), endocrine imbalance, increased insulin levels in combination with increased blood glucose levels, lack of appetite and inherited predisposition. Pysical activity and dietary advice are prefered to prevent muscular maldevelopments, although pharmaceutical methods are also available (Gomes, et al., 2017).

The gut microbiote is also involved in the process of anabolic resistence and the resulting higher protein intake in older adults. There is a link between aging, consumed protein, muscle function and the microbiota (Lochlainn, et al., 2018).

There is an interaction between the aging organism and imbalanced metabolic processes. The mTOR or AMPK metabolisms, rapamycin, metformin, and physical activity are associated with aging and are the main aims of anti-aging interventions. Nicotinamide adenine dinucleotide reduced nicotinamide dinucleotide phosphate, α-ketoglutarate, and β-hydroxybutyrate play an important role in the aging metabolism (Sharma & Ramanathan, 2020).

Furthermore, the water balance changes with age. The feeling of thirst decreases, and the hormone systems related to fluid and electrolyte balance also change. For example, the productivity of the renin-angiotensin system is decreased. The exact cause of this reduced feeling of thirst has not yet been fully elucidated (Begg, 2017). Changes also occur in appetite regulation with age, and decreased food intake can lead to malnutrition (Volkert, 2013).

In rodents a reduction of methionine may slow down the aging process and the supply of glycine is able to imitate methionine limitation. Limited tryptophan levels might have a positive effect on aging, despite that it might impairs mental competencies (Canfielda & Bradshaw, 2019).

To further understand metabolite changes in the aging population, this master thesis addressed the research question "What is the effect of physical activity and increased protein intake in the older population?" more closely.

2. Material and Methods

2.1 Study Design

The NutriAging Protein study started in July 2018. It was designed randomised, controlled, and observer blind. The baseline number of participants was 155, while 117 people between 60 and 85 years finished the Study in December 2018. The study population was divided into 3 groups: the control group ((CON) intervention group 1), the recommended protein plus resistance training group ((RP+T) intervention group 2) and the high protein plus resistance training group ((HP+T) intervention group 3). Group 1 had a protein intake of about 0.8 – 1 g protein/kg/day, group 2 consumed 1 g protein/kg/day the first 6 weeks and 1 g protein/kg/day and resistance training twice a week the next 8 weeks. The last group was aimed to have a protein intake of 2 g protein/kg/day the first 6 weeks and an intake of 2 g protein/kg/day and strength training twice a week the next 8 weeks (**Figure 1**).

Furthermore, the study consisted of 3 measurement time points where blood, anthropometric measurements, faeces, urine, and fitness parameters (functional fitness testing, handgrip strength, leg extension power) were quantified. The first testing (T0) occurred 4 weeks before the intervention started, the second testing was on day 1 (T1), the third testing (T2) took part after the first 6 weeks of intervention and the last measurements (T3) were noted at the end of the intervention (week 17) (Figure 1). The exclusion criteria for this study were participants with diseases like heart diseases, diabetes, or cancer. After the exclusion of the participants who did not meet the required criteria, the youngest participant was 65 and the oldest 85.

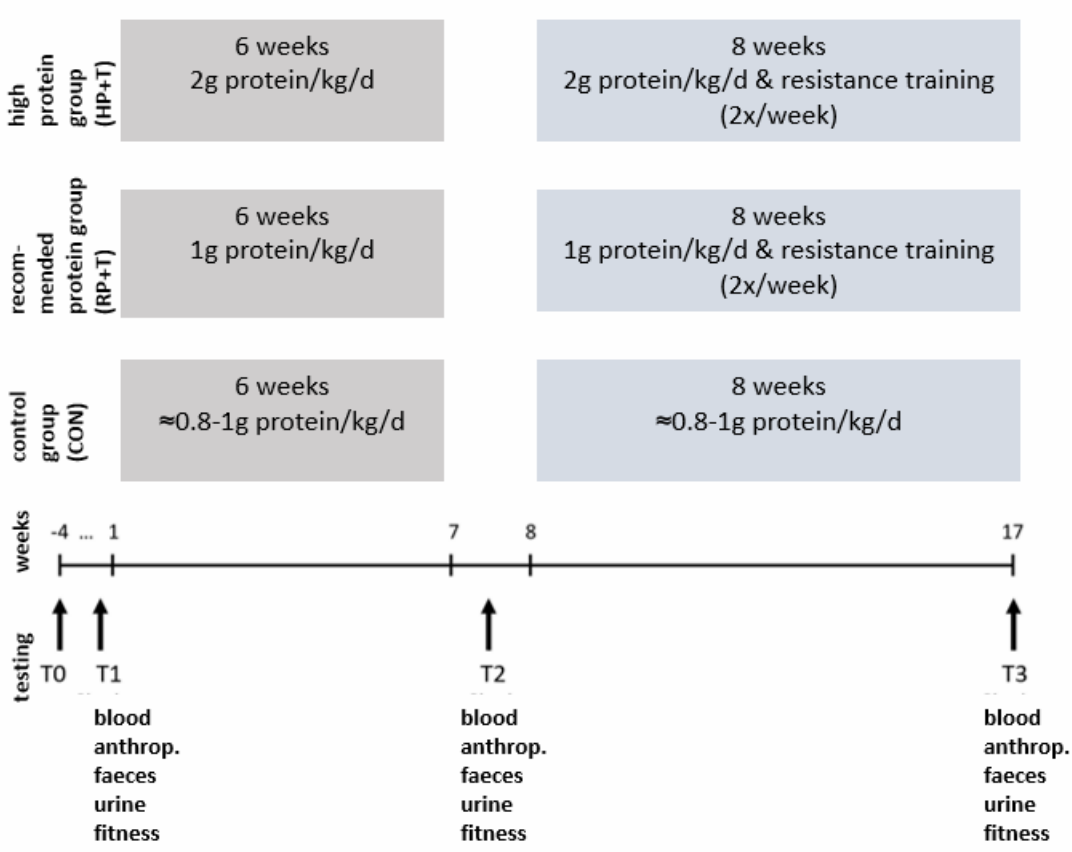

Figure 1 - Study design showing the course from intervention start to study finish in the three groups CON, RP+T, and HP+T

Further information of nutrition, resistance training and the required criteria of the NutriAging Protein study can be found in the paper "The Effect of Elevated Protein Intake on DNA Damage in Older People: Comparative Secondary Analysis of Two Randomized Controlled Trials" from Draxler A, et al. (2021) and in the paper "Effects of an increased habitual dietary protein intake followed by resistance training on fitness, muscle quality and body composition of seniors: A randomised controlled trial" from Unterberger S., et al. (2022).

In our study we took a closer look at the plasma samples of the participants which were collected in heparin tubes. Our prelaboratory tests started in October 2020 and our final laboratory experiment in March 2021 and finished in June 2021.

2.2. Participants

A total of 384 samples were measured from 155 older adults. For the statistical analysis, all subjects who were not present at all measurement time points and who ter-

minated participation prematurely were excluded, which corresponds to 13 participants. 35 plasma samples, from a total of 25 subjects did not meet the recommended criteria and were excluded from the analysis. Furthermore, 1 participant, corresponding to 3 samples, was subsequently excluded because the inclusion criteria were not achieved. The internal standard phenyl-ß-D-glucopyranoside (PGP) showed a deviation of -46.39 % from the mean in one sample, so all 3 samples from that subject were not included in the statistical analysis. 4 further samples from 2 subjects also had to be removed, as one testing from each of these subjects was missing. After the measurement, we found that 6 samples had been prepared with EDTA (Ethylenediaminetetraacetic acid) as anticoagulant. For our experiment, we selected only samples treated with heparin, so we had to exclude 18 other samples.

In conclusion, of the 142 participants and 384 samples measured, 107 participants (CON: n = 40; RP: n = 31; HP: n = 36) and 321 samples were used for statistical analysis. A more detailed overview is given in **Figure 2**. In the study by Unterberger S, et al. (2022) with the same subjects, 9 additional participants could be analyzed (CON: n = 1; RP: n = 4; HP: n = 4).

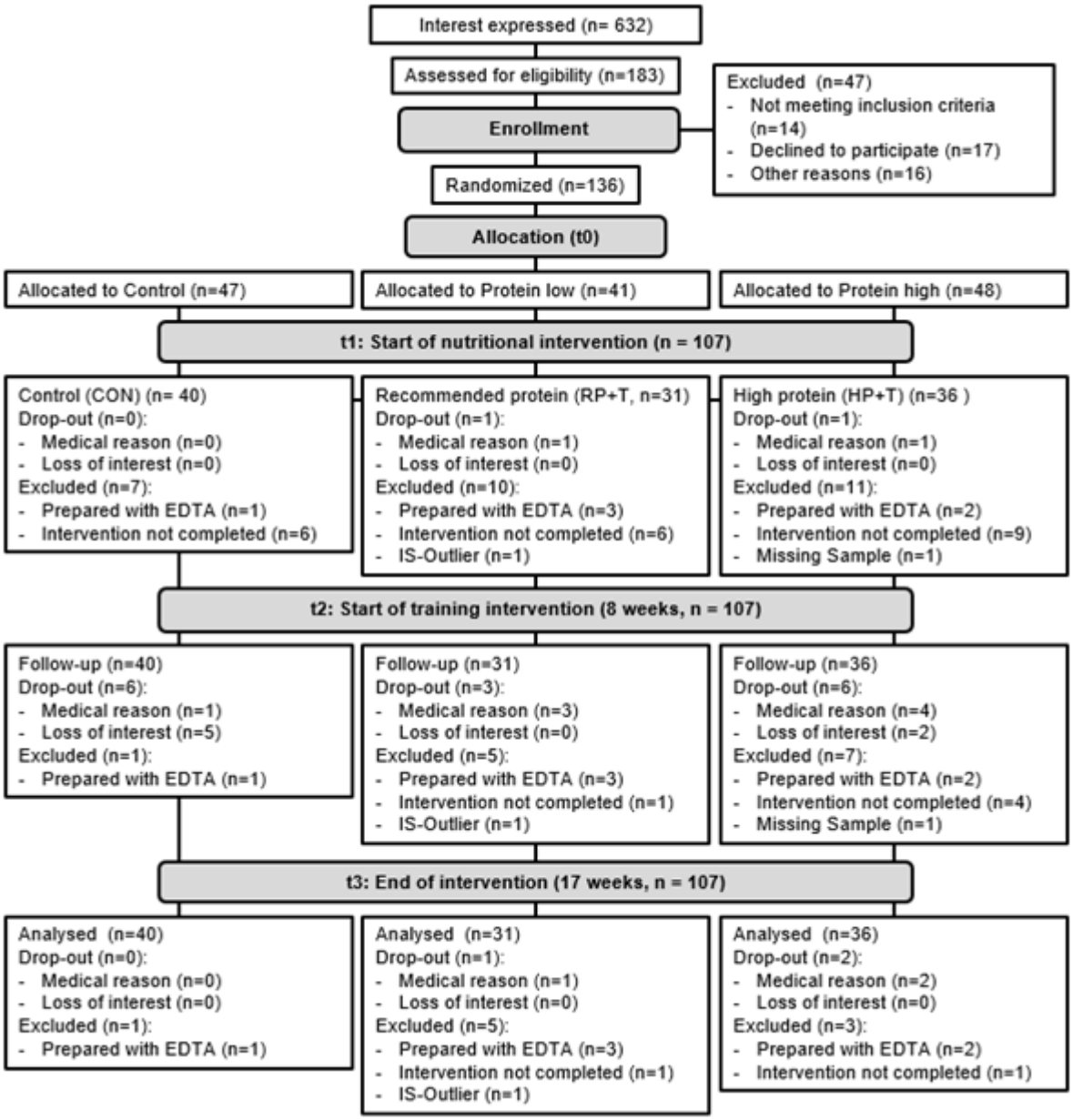

Figure 2 - Flow-chart of the participants of the NutriAging Study

2.3. Materials

2.3.1. Biological Samples

For our experiment we analysed human plasma samples which had been prepared in the NutriAging study from 2018 (Unterberger, et al., 2022) and were stored at -80 °C at the Department of Nutritional Sciences. The collected human blood was previously treated with heparin as anticoagulant to prevent blood clotting.

2.3.2. Plasma Pool

A plasma pool was prepared to check the quality of the measurement. The pool samples we used for our experiment were produced in a preliminary study for method determination. For the pool creation we defrosted human plasma samples which were stored at -80 °C and also prepared with heparin as anticoagulant from the Active Aging-study (see paper "Age and the effect of exercise, nutrition and cognitive training on oxidative stress – The Vienna Active Aging Study (VAAS), a randomized controlled trial" from Franzke, et al., (2018)).

For aliquoting, 400 µL of each defrosted sample was pipetted into a 50 mL tube. This mixture was vortexed for 10 seconds and had a total volume of 33 mL. Aliquots of 200 µL were then transferred to 2 mL safe-lock tubes (Eppendorf, Hamburg, Germany). For the entire laboratory tests, the plasma samples were embedded in ice and after aliquoting were placed back in the freezer at -80 °C.

2.3.3. Internal Standards

As internal standard pentaerythritol (PE), phenyl-ß-D-glucopyranoside (PGP) and D-(+)-raffinose pentahydrate were used. PE had a molar mass of 136.15 g/mol and the purity of 98 %. The molar mass of PGP was 256.25 g/mol and purity 97 % raffinose was 98 % pure and had a molar mass of 594.51 g/mol. The following amounts (mg) were dissolved to obtain various concentrations (mM):

Table 1 - Internal Standard Composition

Internal Standard	**H_2O** ***[mL]***	***PE [mg]***	***c [mM]***
PE	5.326	18.5	25
PGP	4.954	163.6	125
Raffinose	5.282	60.2	50

After the preparation of the standard solutions, the stocks were stored in the freezer at -20 °C.

2.3.4. Quality control mix

The next two tables (**Table 2** and **Table 3**) list the substances which were mixed in the QC-mix. The concentrations of QC compounds were matched to the mean concentrations of metabolites in the adult human blood plasma.

Table 2 - GC-MS Human Quality Control Composition

Components	***c [mM]***
Organic Acids	
Citric acid	2
Fumaric acid	0.02
Gluconic acid	0.075
Malic acid	0.04
Pyruvic acid	1
Succinic acid	0.25
2-Oxoglutaric acid	0.1
Lactic acid	1
Oxaloacetic acid	0.1
Glycolic acid	0.1
Pantothenic ac	0.04
GABA	0.008
Extra	
Hypoxanthine	0.1
Urea	1

Components	***c [mM]***
Amino Acids	
Asparagine	0.45
Glutamine	6
Tryptophan	0.48
Commercial Mix	1
Ornithine	0.6
b-Alanine	0.03
Polyamines	
Spermidine	0.05
Sugar & Alcohols	
Sucrose	0.02
Glucose	0.8
Fructose	0.1
Threitol	0.02
myo-Inositol	0.3
Galactose	0.56

Table 3 - Commercial mix: Amino acid standard solution for calibrating amino acid analyzers, stock No. AA-S-18 (Sigma-Aldrich, Inc.)

Components	***c [mM]***	***Components***	***c [mM]***
Amino acids		**Amino Acids**	
L-Alanine	2.5	L-Leucine	2.5
Ammonium Chloride	2.5	L-Lysine	2.5
L-Arginine	2.5	L-Methionine	2.5
L-Aspartic acid	2.5	L-Phenylalanine	2.5
Cystine	2.5	L-Proline	2.5
L-Glutamic acid	2.5	L-Serine	2.5
Glycine	2.5	L-Threonine	2.5
L-Histidine	2.5	L-Tyrosine	2.5
L-Isoleucine	2.5	L-Valine	2.5

The dilution series of the quality control mix are explained in **Table 4**. The QC-Mix was diluted with distillated water (Milli-Q® H_2O) 1:5 and the IS-Mix (internal standard mix) 1:100. Per Sample: 0.15 µL IS PE, PGP and raffinose from the stocks were added. In our preliminary study we optimized the best dilution factors for the main experiment.

Table 4 - QC-Mix-Mix assembly

	QC-Mix (µl)	H_2O (µl)	IS-Mix (µl)
QC0	0	200	15
QC1	5	195	15
QC3	15	185	15
QC5	40	160	15
QC16	80	120	15
QC40	200	0	15

All QCs (0-40) were dried in a SpeedVac with the setting “quick gradient” (Error! Reference source not found.). This gradient reaches 0 bar faster (after 48 min) than the standard gradient (after about 2 hours), which we set for our plasma samples, plasma pools, and blanks. The duration depends on how many samples are in the SpeedVac at the same time.

After completion, samples were preserved at -20 °C and the derivatization was handled in the same way as the human plasma samples.

2.4. Methods

2.4.1. Preliminary experiments

The preliminary tests started in October 2020. Our final laboratory experiment began in March 2021 and finished in June 2021.

Before the main experiment, several tests were carried out to determine the most suitable method for obtaining the metabolites in the plasma samples. The preliminary study was carried out using the plasma pool as described in chapter 2.3.2.. We used different amounts of plasma (20 µL, 50 µL or 100 µL plasma), two various solvents for extraction (methanol or methanol and chloroform) and three dilution rates (split less (without dilution), split 2 (a dilution of 1:2) and split 5 (a dilution of 1:5)) for

GC-MS. Additionally we adapted the quality control mix to the human metabolism and calculated the optimal amount of our internal standards PE, PGP and raffinose.

We found that a plasma volume of 20 µL was unsuitable for metabolites that occur in low concentrations in human blood (e.g., 2-oxoglutaric acid and oxaloacetic acid) as they had not been detected. The amount of 100 µL as a volume caused an overload of metabolites present in larger amounts in the blood (e.g., urea and glucose), which resulted in surrounding metabolites being masked and unidentifiable.

In our study, the effects on increased protein intake and exercise were considered in more detail and we were interested in muscle and energy metabolism. Therefore, changes in the polar region were important for us and apolar metabolites, such as the lipidome, were filtered out.

To determine the optimal solvent, experiments were conducted with methanol and chloroform. Extraction with only methanol or only chloroform was tested, and extraction with methanol and chloroform. Chloroform was used to look more closely to the solubility of metabolites. The best results were found for extraction with methanol only. For the additional extractions with chloroform, we could observe negative effects on the internal standards PGP and raffinose, which is why we did not choose this method.

The PGP internal standard was shown to be most robust for the methanol extraction method. PE and raffinose standards showed greater variation between samples, QCs, and controls (plasma pool and blanks).

Splitless and split 2 tended to overload with metabolites of higher concentrations, which made annotation of the surrounding metabolites difficult or not possible. For this reason, we chose split 5.

2.4.2. Main laboratory analysis

In the final laboratory experiment we used 50 µL plasma. As extraction solvent we used methanol, chloroform wasn`t utilized. The split rate 5 for GC-MS was set and a quality control mix was prepared. Despite the noticeable fluctuations in the PE and raffinose standards, we added all three internal standards to the samples.

2.4.2.1. Extraction

The first step was to slowly thaw the frozen plasma samples on ice for 30 minutes, then they were vortexed for 10 seconds every 10 minutes until they were entirely thawed.

Methanol, previously stored at -20 °C, was used as the solvent for the extraction. All internal standards were pipetted to the methanol. To keep the terminal IS concentration, 2.5 µL of the dissolved PE stock, 2.5 µL of the dissolved PGP stock, and 2.5 µL of the dissolved raffinose stock were added to the 5 mL of methanol.

From each sample, 50 µL of plasma was pipetted into 2 mL Safe-Lock tubes (Eppendorf, Hamburg, Germany), then 300 µL of the ice-cold methanol/IS mix was added and immediately vortexed for 10 seconds. Samples were then incubated for 15 minutes at 4 °C in a thermal shaker and then centrifuged at 18,000 g for 4 minutes at 4 °C. All steps were performed on ice and for each transmission we utilized RPT Graduated Filter sterile tips.

The next step was to transfer the supernatant into new 2 mL Safe-Lock tubes. After the transfer the samples were frozen at -20 °C for 17-25 hours and then dried by SpeedVac vacuum centrifugation for 5 hours using the standard gradient. Finally, the dried samples were stored at -20 °C until derivatization started.

With each batch we extracted a blank (50 µL Milli-Q® H2O) and a plasma pool (50 µL plasma from the preliminary study) following the same method.

2.4.2.2. Derivatisation

For the derivatization a concentration of 40 mg/mL MEOX (methoxamine hydrochloride; 98 % purity) was solved with pyridine and 20 µL were pipetted to each dried sample, pool, blank, and QC. After this step the samples were vortexed for 90 min with 700 rpm and a temperature of 30 °C.

Samples were removed from the thermal shaker and 80 µL of MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) was added to each sample. In the next step, the samples were again placed in the thermal shaker for 30 minutes at 750 rpm and a temperature of 37 °C.

Then the samples were put in the centrifuge for 4 minutes with 14,000 g and a temperature of 24 °C. Afterwards the supernatant was transferred into GC-vials and accurately closed.

As a last step the samples were measured by Gas Chromatography Time-of-Flight Mass Spectrometer (GC-MS) with LECO Pegasus BT. Before the derivatized samples were placed in the instrument, any contaminated outer areas of the instrument were cleaned with isopropanol. Pyridine for HPLC (High-performance liquid chromatography (≥ 99.9 %)) was taken as wash solution and helium was the carrier gas. The liner that we used was Agilent 5183-4647 Split Liner 4 mm. A new liner was applied for each measurement. The measuring was initiated after 276 seconds (MS Method SL_DV-opt_20Specs_276sec) to avoid an overload of our data with pyridine. Starting the measurement after 270 seconds would have been better, since we missed the first alkane (C10) of the alkane standard series.

As already described, a dilution of 1:5 was selected for the flow-through (split rate 5). The whole measurement procedure included 2 washes with pyridine, 1 alkane standard mixture for functional tests of GC systems (C10 - C40, 50 mg/L each), 1 QC dilution series, 15 samples, 2 blank samples, 4 plasma pools and 2 replicates of QC8 from the dilution series. One batch/measurement had a duration of nearly 24 hours. 25 batches were necessary to measure all samples.

2.5. Evaluation and statistical analysis

The results of GC-MS were analysed with the software programs called ChromaTOF for BT (version 5.31.16) and MS-Dial (version 4.48). Data processing and annotation was done using the alkane standard series and the library of the Fiehn laboratory for untargeted metabolomics. Statistical analysis was accomplished with SPSS (SPSS Statistics 28 for Windows.

2.5.1. Preparation of data

When the metabolites were annotated, the peak area was narrowed down using the MS-Dial program and these values were used for statistical analysis. ChromaTOF was only used to assist in the search for metabolites and in the annotation process.

For the selected metabolites, the different Tetramethylsilane (TMS) derivatives of the peak areas were summed up, and the peak areas of the PGP standards were subtracted from each sum. By these steps, batch differences after derivatisation and measurement can be minimised. The variability of the PE and raffinose standards was too high and therefore not included in the analysis.

The metabolites we annotated are semi-quantified (relative quantification) and are expressed as chromatographic peak areas. The area by itself cannot be used to make a statement about the absolute quantity, but differences between the subjects can be detected. It would be feasible to obtain absolute quantification of our target metabolites based on the co-measured QC.

2.5.2. Statistical analysis

IBM SPSS (version 28) and Excel statistical software were used for data preparation and statistical analysis. One-way ANOVA of general linear models with Bonferroni-corrected post-hoc tests and two-way ANOVA with Bonferroni-corrected post-hoc tests were conducted to determine significant differences. Standardized means (95 % confidence interval) were used for statistical analysis. The significance level was defined as p-value ≤ 0.05, missing values were excluded.

Those subjects whose area of the standard internal PGP deviated from the mean by more than 16 % were excluded from the statistical analysis (N = 1).

2.6. Metabolite selection

The aim of this evaluation was to find metabolites that are involved in energy and muscle metabolism. Therefore, we searched for substances involved in the citric acid cycle and the urea cycle, as well as metabolites relevant to muscle metabolism. Finally, we annotated 45 metabolites, that are shown in a metabolic pathway map in **Table 5**. Of these 45 metabolites, 33 were present in the QC and 11 were untargeted metabolites.

Table 5 - all Metabolites of interesting Pathways for evaluation

Metabolites present in QC		
2-Oxoglutaric acid	Glutamine	Oxaloacetic acid
Alanine	Glycine	Phenylalanine
Arginine	Glycolic acid	Proline
Asparagine	Histidine	Pyruvic acid
Aspartic acid	Isoleucine	Serine
beta-Alanine	Lactic Acid	Succinic acid
Citric acid	Leucine	Threonine
Cystine	Lysine	Tryptophan
Fumaric acid	Malic acid	Tyrosine
GABA	Methionine	Urea
Glutamic acid	Ornithine	Valine
untargeted Metabolites		
3-Methyl-2-oxopentanoic-acid	Glyceric acid	Kynurenine
4-Hydroxyproline	Indole-3-acetate	Oxalic acid
Creatinine	Ketoleucine	Threonic acid
Cysteine	Kynurenic acid	Alpha-Tocopherol

There were 50 metabolites that could not be found because either there was no literature on them, or the metabolites were not suitable for GC-MS measurement due to their chemical properties. To annotate substances in ChromaTOF and MS-Dial, a reference for the retention index and quantum mass, as well as the quantum spectrum of the substance is needed.

For reference we used Fiehn Laboratory for Untargeted Metabolomics, the Human Metabolome Database (HMDB), PubChem, NIST MS Search version 2.3, and validated data and libraries from the MOSYS laboratory.

During the evaluation, there was one metabolite for which we were not certain whether it is GABA or 4-hydroxyproline since the retention times of the metabolites were quite close to each other and the peak areas overlapped. GABA was found in the QC at the same position as 4-hydroxyproline in the individual plasma samples. The other listed 4-hydroxyproline in **Table 5** is the trans-2 TMS derivative.

Annotation was not completely confirmed for the following metabolites: indole-3-acetate, kynurenic acid and kynurenine. Alignment of the peak areas of indole-3-acetate and kynurenine was not possible on MS-Dial-targeted because no ideal QM was nearby. For kynurenic acid, the spectrum fitted at MS-Dial, but not at ChromaTOF. For evaluation and statistical analysis indole-3-acetate, kynurenic acid and kynurenine were integrated.

For urea, there were problems with overloading surrounding metabolites because urea was present in fairly large amounts in the plasma of some subjects. Because of the overloading, annotation of the area was difficult. The area could still be used to measure the differences in the amounts of urea. Serine derivative 2 TMS had a close retention time to urea. Therefore, in the subjects with overloaded urea peaks, this serine derivate could not be annotated accurately and was therefore not included in the analysis. Only the serine derivate 3 TMS was used for the statistical analysis.

The next figure (**Figure 3**) shows the selected metabolites as a metabolic pathway map. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites are not included into the analysis. Alpha-tocopherol was aligned and significant, but it was not added to **Figure 3**, because it belongs to a different metabolic pathway. Metabolites of the citrate cycles that could

be annotated and quantified were 2-oxoglutaric acid, succinic acid, citric acid, fumaric acid, malic acid and oxaloacetic acid. From urea cycle urea, ornithine, arginine, aspartate and fumaric acid were found and included into statistical analysis. Moreover, metabolites which have influence on these two cycles were also considered.

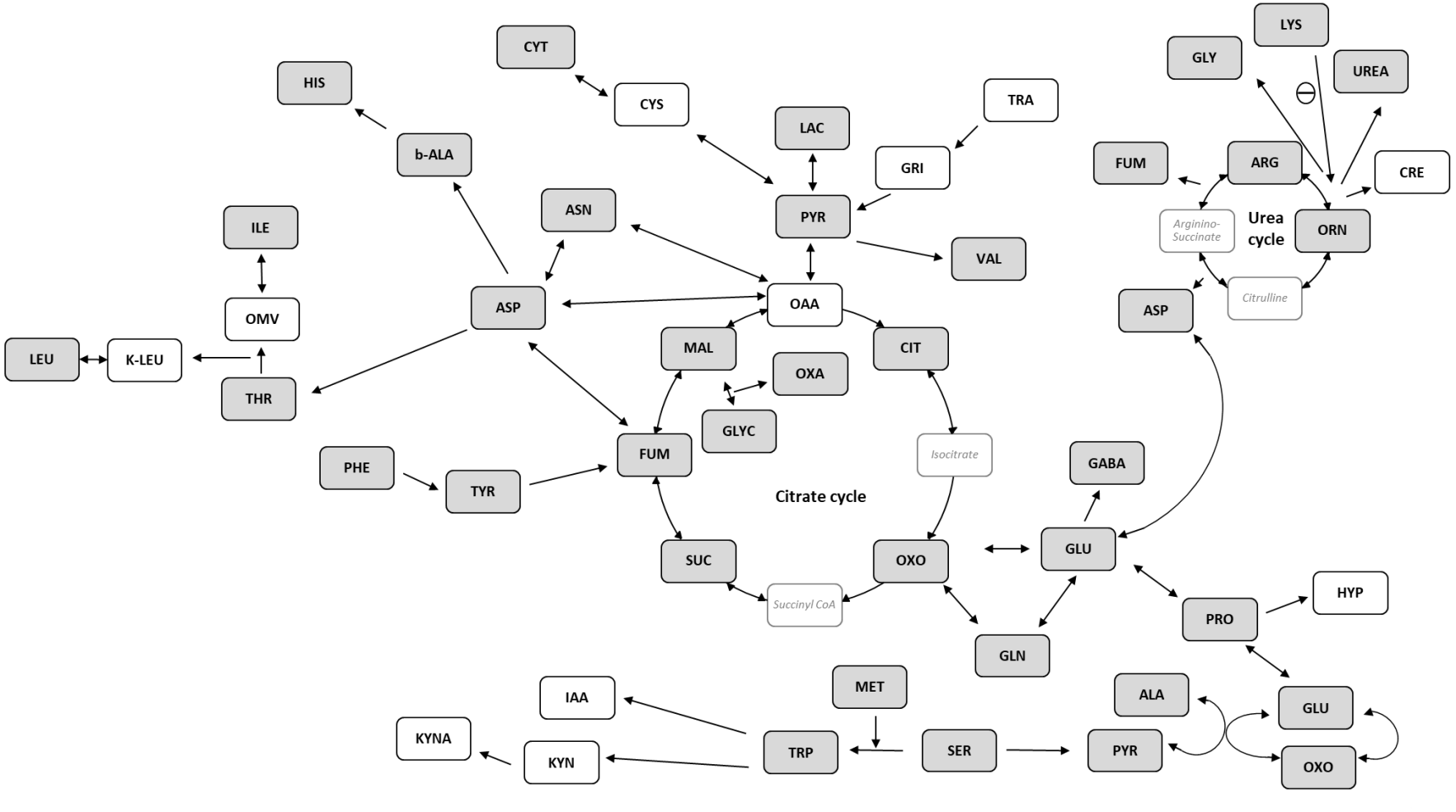

Figure 3 - Annotated and quantified metabolites. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis.

Table 6 - List of Abbreviations from Figure 3

Abbreviation	Amino Acid
ALA	Alanine
ARG	Arginine
ASN	Asparagine
ASP	Aspartic acid
a-TOC	Alpha-Tocopherol
b-ALA	Beta-Alanine
CIT	Citric acid
CRE	Creatinine
CYS	Cysteine
CYT	Cystine
FUM	Fumaric acid
GABA	4-Amino-butyrate
GLN	Glutamine
GLU	Glutamic acid
GLY	Glycine
GLYC	Glycolic acid
GRI	Glyceric acid
HIS	Histidine
HYP	4-Hydroxyproline
IAA	Indole-3-Acetate
ILE	Isoleucine
K-LEU	Keto-Leucine

Abbreviation	Amino Acid
KYN	Kynurenine
KYNA	Kynurenic acid
LAC	Lactic acid
LEU	Leucine
LYS	Lysine
MAL	Malic acid
MET	Methionine
OAA	Oxaloacetic acid
OMV-	2-Oxo-3 Methyl-valerate
ORN	Ornithine
OXA	Oxalic acid
OXO	2- Oxoglutaric acid
PHE	Phenylalanine
PRO	Proline
PYR	Pyruvic acid
SER	Serine
SUC	Succinic acid
THR	Threonine
TRA	Threonic acid
TRP	Tryptophan
TYR	Tyrosine
VAL	Valine

3. Results and Discussion

The primary purpose of the statistical analysis was to determine whether a pattern could be discerned between altered metabolic pathways and changes in individual metabolites by and over the course of the interventions and what might be the reason for this.

3.1. MANOVA - Multivariate analysis of variance

At first, we analysed the differences between the intervention groups and time effects and examined possible group-time effects with multivariate significance analysis. **Table 7** lists all these outcomes.

In general, we found a significant difference between groups for 5 of the 45 plasma metabolites: glutamic acid p = 0.025, proline p = 0.013, pyruvic acid p = 0.034, urea p = 0.000, and kynurenine p = 0.042. As for differences in times in general, the following 11 metabolites were significant: tyrosine p = 0.008, 2-oxoglutaric acid p = 0.000, citric acid p = 0.006, cystine p = 0.020, fumaric acid p = 0.000, lactic acid p = 0.000, malic acid p = 0.000, pyruvic acid p = 0.000, tryptophan p = 0.005, urea p = 0.001, and cysteine p = 0.000. A group-time effect was detected for the 3 metabolites: GABA/4-hydroxyproline p = 0.013, 4-hydroxyproline p = 0.039, and alpha-tocopherol p = 0.043.

Table 7 - MANOVA of metabolites with significant group and time effects

Metabolite	Group	T1		T2		T3		group	time	group x time
		MW	SD	MW	SD	MW	SD	p-value	p-value	p-value
2-Oxoglutaric acid	CON	0.0210 ±	0.0099	0.0189 ±	0.0097	0.0156 ±	0.0063			
	RP+T	0.0194 ±	0.0079	0.0193 ±	0.0080	0.0165 ±	0.0068	0.581	0.000	0.847
	HP+T	0.0203 ±	0.0092	0.0176 ±	0.0094	0.0145 ±	0.0061			
4-Hydroxyproline	CON	0.0349 ±	0.0189	0.0362 ±	0.0156	0.0350 ±	0.0175			
	RP+T	0.0410 ±	0.0194	0.0428 ±	0.0297	0.0336 ±	0.0167	0.291	0.226	0.039
	HP+T	0.0419 ±	0.0179	0.0288 ±	0.0094	0.0364 ±	0.0202			
alpha-Tocopherol	CON	0.2978 ±	0.0778	0.2944 ±	0.0648	0.2986 ±	0.0614			
	RP+T	0.2736 ±	0.0509	0.3149 ±	0.0716	0.2854 ±	0.0773	0.076	0.623	0.043
	HP+T	0.2953 ±	0.0627	0.2691 ±	0.0613	0.2682 ±	0.0550			
Citric acid	CON	0.5495 ±	0.1597	0.5335 ±	0.1487	0.5021 ±	0.1145			
	RP+T	0.5499 ±	0.1391	0.5494 ±	0.1669	0.4918 ±	0.1287	0.084	0.006	0.908
	HP+T	0.5434 ±	0.1456	0.4893 ±	0.1174	0.4599 ±	0.1002			
Cysteine	CON	0.1371 ±	0.0182	0.1431 ±	0.0283	0.1240 ±	0.0254			
	RP+T	0.1334 ±	0.0236	0.1377 ±	0.0161	0.1225 ±	0.0257	0.115	0.000	0.480
	HP+T	0.1434 ±	0.0236	0.1386 ±	0.0239	0.1330 ±	0.0291			
Cystine	CON	0.4155 ±	0.1421	0.4484 ±	0.1272	0.3510 ±	0.1821			
	RP+T	0.3793 ±	0.1340	0.4478 ±	0.0994	0.3810 ±	0.1506	0.578	0.020	0.088
	HP+T	0.4100 ±	0.1144	0.4199 ±	0.1129	0.4320 ±	0.1305			
Fumaric acid	CON	0.0181 ±	0.0043	0.0169 ±	0.0040	0.0152 ±	0.0026			
	RP+T	0.0181 ±	0.0033	0.0165 ±	0.0030	0.0148 ±	0.0033	0.147	0.000	0.972
	HP+T	0.0173 ±	0.0031	0.0156 ±	0.0037	0.0146 ±	0.0030			
GABA/ 4-Hydroxyproline	CON	0.0035 ±	0.0020	0.0036 ±	0.0016	0.0042 ±	0.0030			
	RP+T	0.0042 ±	0.0024	0.0047 ±	0.0040	0.0036 ±	0.0017	0.436	0.635	0.013
	HP+T	0.0046 ±	0.0027	0.0029 ±	0.0011	0.0039 ±	0.0025			
Glutamic acid	CON	0.4296 ±	0.1440	0.3948 ±	0.1571	0.4075 ±	0.1434			
	RP+T	0.3581 ±	0.1174	0.3419 ±	0.1176	0.3784 ±	0.1173	0.025	0.295	0.898
	HP+T	0.3946 ±	0.1025	0.3770 ±	0.1358	0.4096 ±	0.1653			
Kynurenine	CON	0.0020 ±	0.0007	0.0022 ±	0.0009	0.0020 ±	0.0006			
	RP+T	0.0018 ±	0.0005	0.0020 ±	0.0005	0.0019 ±	0.0006	0.042	0.552	0.202
	HP+T	0.0020 ±	0.0006	0.0018 ±	0.0005	0.0019 ±	0.0005			
Lactic acid	CON	16.9228 ±	2.1171	16.3957 ±	2.2710	15.7007 ±	1.7976			
	RP+T	17.2606 ±	2.3266	15.8105 ±	1.6893	15.7144 ±	2.1809	0.769	0.000	0.768
	HP+T	17.3281 ±	2.6974	16.1577 ±	2.0548	15.9498 ±	2.1441			
Malic acid	CON	0.0097 ±	0.0035	0.0090 ±	0.0032	0.0080 ±	0.0019			
	RP+T	0.0094 ±	0.0026	0.0085 ±	0.0025	0.0080 ±	0.0026	0.063	0.000	0.980
	HP+T	0.0093 ±	0.0030	0.0079 ±	0.0027	0.0075 ±	0.0021			
Proline	CON	6.3803 ±	2.6766	7.1246 ±	3.1301	5.9742 ±	1.9181			
	RP+T	6.2118 ±	2.6666	6.1278 ±	1.8852	6.2012 ±	2.1639	0.013	0.339	0.557
	HP+T	6.4922 ±	2.5092	7.4326 ±	2.2495	7.1960 ±	2.5275			
Pyruvic acid	CON	0.6081 ±	0.2138	0.5195 ±	0.1856	0.4508 ±	0.1774			
	RP+T	0.5667 ±	0.2053	0.4842 ±	0.2107	0.3979 ±	0.1924	0.034	0.000	0.827
	HP+T	0.6113 ±	0.2695	0.5455 ±	0.1701	0.5173 ±	0.1879			
Tryptophan	CON	2.2092 ±	0.5572	2.4019 ±	0.4184	2.3061 ±	0.3736			
	RP+T	2.0802 ±	0.5011	2.3783 ±	0.3911	2.2853 ±	0.5245	0.598	0.005	0.667
	HP+T	2.2198 ±	0.4012	2.3147 ±	0.4419	2.3735 ±	0.4290			
Tyrosine	CON	2.5949 ±	0.4694	2.7056 ±	0.5059	2.6853 ±	0.4579			
	RP+T	2.5351 ±	0.3939	2.7496 ±	0.5378	2.6419 ±	0.4312	0.956	0.008	0.786
	HP+T	2.5395 ±	0.4537	2.7545 ±	0.5329	2.7248 ±	0.4552			
Urea	CON	23.1635 ±	7.4424	24.1035 ±	6.3469	24.4778 ±	7.9400			
	RP+T	23.1660 ±	5.4895	24.3996 ±	5.5580	24.8292 ±	6.5297	0.000	0.001	0.055
	HP+T	23.7221 ±	6.9448	29.9908 ±	7.5640	30.4464 ±	6.6783			

Values are shown as mean±stdv (95% confidence interval). Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); green cells show significant p-values; p-values refer to group, time and groupxtime effects. Significant effects p-value <0.05 (MANOVA)

3.2. MANOVA post-hoc tests

Post-hoc tests with Bonferroni correction of Multivariate and Univariate analysis of variance were performed to further investigate the changes within a group. Significant differences could be found for 16 metabolites.

The results of the variance analyses are listed in **Table 8** and **Table 9**. Fumaric acid and lactic acid were significant in all three intervention groups. 2-oxoglutaric acid, fumaric acid, lactic acid, malic acid, pyruvic acid, urea, and cysteine showed p-values < 0.001.

When looking at the individual intervention groups, significant changes could be seen especially in the HP+T: 2-oxoglutaric acid ($p = 0.012$), citric acid ($p = 0.039$), fumaric acid ($p = 0.002$) and lactic acid ($p = 0.039$) decreased from T1 to T3. Phenylalanine ($p = 0.02$) and urea ($p \leq 0.001$) increased from T1 to T3. GABA/4-hydroxyproline ($p = 0.006$), glycolic acid ($p = 0.03$), ornithine ($p = 0.032$) and 4-hydroxyproline ($p = 0.003$) decreased from T1 to T2.

In terms of intervention, most changes could be observed when comparing T1 to T3 (T1 → T3). There were 9 metabolites that showed significant changes when compared between the first and last testing: phenylalanine ($p = 0.02$), 2-oxoglutaric acid ($p = 0.012$), citric acid ($p = 0.039$), fumaric acid ($p = 0.002$), lactic acid ($p = 0.039$) and urea ($p \leq 0.001$) in HP+T as already mentioned; 2-oxoglutaric acid ($p = 0.021$), fumaric acid ($p = 0.002$), lactic acid ($p = 0.028$), malic acid ($p = 0.029$) and pyruvic acid ($p = 0.001$) decreased in CON; fumaric acid ($p \leq 0.001$), lactic acid ($p = 0.013$) and pyruvic acid ($p = 0.004$) decreased in RP+T and 2-oxoglutaric acid ($p \leq 0.001$), citric acid ($p = 0.006$), fumaric acid ($p \leq 0.001$), lactic acid ($p \leq 0.001$), malic acid ($p \leq 0.001$), pyruvic acid ($p \leq 0.001$), urea ($p = 0.002$) and cysteine ($p = 0.002$) in general, which means in all three groups together. Cysteine was nearly significant in CON with a p-value of 0.054 and tryptophan in general with a p-value of 0.053**.**

Between T2 and T3, 4 metabolites showed significant changes in the mean values. These changes were present in the CON and in total (all three groups together): Cysteine ($p = 0.002$) and Cystine ($p = 0.015$) decreased in CON, 2-oxoglutaric acid ($p = 0.021$) and fumaric acid ($p = 0.006$) were significantly different in general.

Furthermore, the analysis showed differences between groups and within groups. Significant differences within groups could be found and are due to large variations of the areas. Within CON there were significant differences in 2-oxoglutraric acid ($p = 0.025$), cysteine ($p = 0.002$), cystine ($p = 0.017$), fumaric acid ($p = 0.003$), lactic acid ($p = 0.033$), malic acid ($p = 0.034$) and pyruvic acid ($p = 0.002$). In the RP+T there were significant differences within the group in cysteine ($p = 0.025$), fumaric acid ($p < 0.001$), lactic acid ($p = 0.006$), pyruvic acid ($p = 0.006$) and tryptophan ($p = 0.046$). The HP+T showed significant differences within the group in 2-oxoglutaric acid ($p = 0.015$), 4-hydroxyproline ($p = 0.005$), citric acid ($p = 0.044$), fumaric acid ($p = 0.003$), GABA/4-hydroxyproline ($p = 0.008$), glycolic acid ($p = 0.035$), lactic acid ($p = 0.028$), ornithine ($p = 0.026$), phenylalanine ($p = 0.016$), tyrosine ($p = 0.040$) and urea ($p < 0.001$). The metabolites 2-oxoglutaric acid ($p = 0.009$), alpha-tocopherol ($p = 0.048$), citric acid ($p = 0.043$), cysteine ($p < 0.001$), cystine ($p = 0.029$), fumaric acid ($p < 0.001$), lactic acid ($p = 0.002$), malic acid ($p = 0.004$), pyruvic acid ($p < 0.001$) and urea ($p < 0.001$) were significantly different in all groups and times combined.

Glutamic acid was significantly higher ($p = 0.021$) in CON than in RP+T **Table 7**. Urea was significantly higher ($p < 0.001$) in HP+T than in CON. Proline ($p = 0.013$), pyruvic acid ($p = 0.028$) and urea ($p < 0.001$) were significantly higher in HP+T than in RP+T. Urea increased significantly more in HP+T compared to RP+T and CON (lowest values in CON).

Table 8 - Intervention effects of all significant metabolites and post-hoc 1

Metabolite	Group	T1 & T2	T1 & T3	T2 & T3	Within groups	Between groups	
		p-value	p-value	p-value	p-value		p-value
2-Oxoglutaric acid	total	0.396	<0.001	0.021			0.009
	CON	0.901	0.021	0.275	0.025	CON - RP+T	1.000
	RP+T	1.000	0.421	0.482	0.250	CON - HP+T	1.000
	HP+T	0.513	0.012	0.361	0.015	RP+T - HP+T	1.000
4-Hydroxyproline	total	0.564	0.380	1.000			0.055
	CON	1.000	1.000	1.000	0.934	CON - RP+T	0.438
	RP+T	1.000	0.608	0.347	0.247	CON - HP+T	1.000
	HP+T	0.003	0.485	0.166	0.005	RP+T - HP+T	0.583
alpha-Tocopherol	total	1.000	1.000	1.000			0.048
	CON	1.000	1.000	1.000	0.958	CON - RP+T	1.000
	RP+T	0.054	1.000	0.265	0.051	CON - HP+T	0.078
	HP+T	0.195	0.171	1.000	0.096	RP+T - HP+T	0.416
Citric acid	total	0.840	0.006	0.121			0.043
	CON	1.000	0.417	0.977	0.320	CON - RP+T	1.000
	RP+T	1.000	0.360	0.370	0.202	CON - HP+T	0.161
	HP+T	0.426	0.039	0.896	0.044	RP+T - HP+T	0.166
Cysteine	total	1.000	0.002	<0.001			<0.001
	CON	0.814	0.054	0.002	0.002	CON - RP+T	0.865
	RP+T	1.000	0.167	1.000	0.025	CON - HP+T	0.799
	HP+T	1.000	0.258	1.000	0.226	RP+T - HP+T	0.114
Cystine	total	0.169	1.000	0.017			0.029
	CON	1.000	0.182	0.015	0.017	CON - RP+T	1.000
	RP+T	0.121	1.000	0.136	0.065	CON - HP+T	1.000
	HP+T	1.000	1.000	1.000	0.736	RP+T - HP+T	1.000
Fumaric acid	total	0.005	<0.001	0.006			<0.001
	CON	0.407	0.002	0.144	0.003	CON - RP+T	1.000
	RP+T	0.153	<0.001	0.121	<0.001	CON - HP+T	0.162
	HP+T	0.104	0.002	0.544	0.003	RP+T - HP+T	0.622
GABA/4-Hydroxyproline	total	0.916	1.000	1.000			0.051
	CON	1.000	0.376	0.670	0.269	CON - RP+T	0.681
	RP+T	1.000	1.000	0.391	0.317	CON - HP+T	1.000
	HP+T	0.006	0.512	0.219	0.008	RP+T - HP+T	0.900
Glutamic acid	total	0.613	1.000	0.476			0.206
	CON	0.885	1.000	1.000	0.569	CON - RP+T	0.021
	RP+T	1.000	1.000	0.674	0.475	CON - HP+T	1.000
	HP+T	1.000	1.000	0.944	0.601	RP+T - HP+T	0.229
Glycolic acid	total	1.000	1.000	1.000			0.420
	CON	1.000	1.000	1.000	0.667	CON - RP+T	1.000
	RP+T	1.000	1.000	0.828	0.499	CON - HP+T	1.000
	HP+T	0.030	0.810	0.397	0.035	RP+T - HP+T	0.773

Values are shown as p-values. Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); green cells show significant p-values; p-values refer to differences between and within intervention groups. Significant effects p-value <0.05 (one-way ANOVA and MANOVA)

Table 9 - Intervention effects of all significant metabolites and post-hoc 2

Metabolite	Group	T1 & T2	T1 & T3	T2 & T3	Within groups	Between groups	
		p-value	p-value	p-value	p-value		p-value
Lactic Acid	total	0.002	<0.001	0.681			0.002
	CON	0.772	0.028	0.409	0.033	CON - RP+T	1.000
	RP+T	0.022	0.013	1.000	0.006	CON - HP+T	1.000
	HP+T	0.103	0.039	1.000	0.028	RP+T - HP+T	1.000
Malic acid	total	0.060	<0.001	0.213			0.004
	CON	0.744	0.029	0.436	0.034	CON - RP+T	1.000
	RP+T	0.498	0.074	1.000	0.076	CON - HP+T	0.062
	HP+T	0.364	0.077	1.000	0.073	RP+T - HP+T	0.383
Ornithine	total	0.267	0.190	1.000			0.226
	CON	1.000	1.000	1.000	0.752	CON - RP+T	0.279
	RP+T	1.000	1.000	1.000	0.672	CON - HP+T	0.327
	HP+T	0.032	0.126	1.000	0.026	RP+T - HP+T	1.000
Proline	total	0.534	1.000	0.425			0.075
	CON	0.621	1.000	0.157	0.143	CON - RP+T	1.000
	RP+T	1.000	1.000	1.000	0.987	CON - HP+T	0.115
	HP+T	0.890	1.000	1.000	0.575	RP+T - HP+T	0.013
Pyruvic acid	total	0.014	<0.001	0.093			<0.001
	CON	0.127	0.001	0.341	0.002	CON - RP+T	0.373
	RP+T	0.338	0.004	0.293	0.006	CON - HP+T	0.709
	HP+T	0.582	0.194	1.000	0.165	RP+T - HP+T	0.028
Tryptophan	total	0.007	0.053	1.000			0.090
	CON	0.185	1.000	1.000	0.173	CON - RP+T	1.000
	RP+T	0.047	0.280	1.000	0.046	CON - HP+T	1.000
	HP+T	1.000	0.382	1.000	0.305	RP+T - HP+T	1.000
Phenylalanine	total	0.134	0.332	1.000			0.352
	CON	1.000	1.000	1.000	0.627	CON - RP+T	1.000
	RP+T	1.000	1.000	1.000	0.775	CON - HP+T	1.000
	HP+T	0.078	0.020	1.000	0.016	RP+T - HP+T	1.000
Tyrosine	total	0.009	0.076	1.000			0.180
	CON	0.908	1.000	1.000	0.546	CON - RP+T	1.000
	RP+T	0.206	1.000	1.000	0.189	CON - HP+T	1.000
	HP+T	0.062	0.116	1.000	0.040	RP+T - HP+T	1.000
Urea	total	0.008	0.002	1.000			<0.001
	CON	1.000	1.000	1.000	0.708	CON - RP+T	1.000
	RP+T	1.000	0.805	1.000	0.515	CON - HP+T	<0.001
	HP+T	<0.001	<0.001	1.000	<0.001	RP+T - HP+T	<0.001

Values are shown as p-values. Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); green cells show significant p-values; p-values refer to differences between and within intervention groups. Significant effects p-value <0.05 (one-way ANOVA and MANOVA)

3.3. Differences between intervention groups and testing phases

After examining the differences between the three intervention groups, the delta values and the percentage deviation between the different test phases were considered and interpreted.

For better illustration, the changes were inserted into pathway figures. The 19 significant metabolites are highlighted in each figure. As mentioned above, alpha-tocopherol was not included in the following figures. Blue boxes indicate a decrease between test phases and orange boxes indicate an increase. Tables with all values can be found in the Appendix. The next three figures (**Figure 4** - **Figure 6**) show the differences between test phases in the CON.

In the CON 2-oxoglutaric acid, fumaric acid, lactic acid, malic acid, and pyruvic acid decreased significantly between T1 and T3. Cysteine and cystine decreased significantly between T2 and T3 (**Table 8** and **Table 9**).

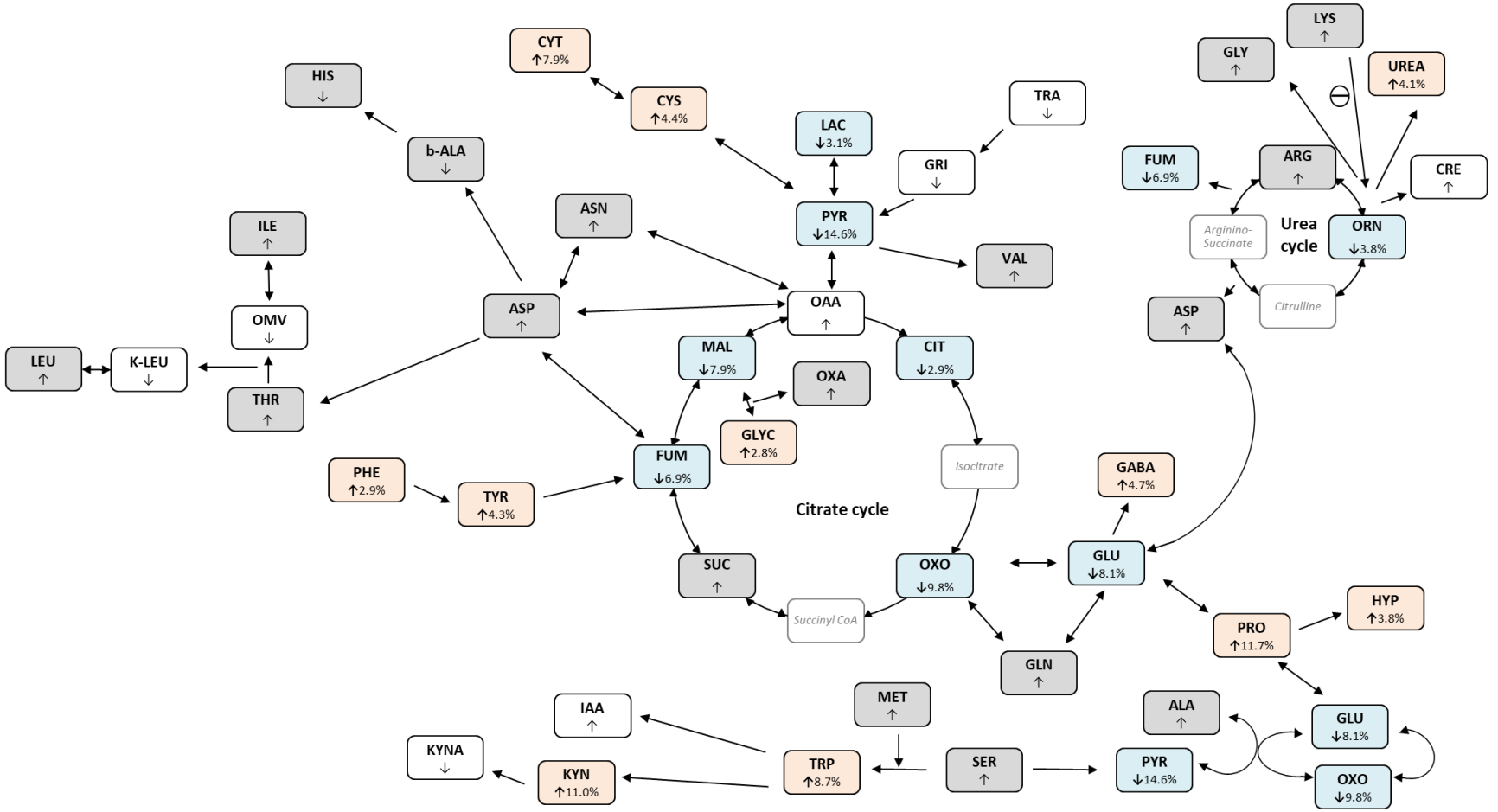

Figure 4 - Delta values and percentage deviation from Baseline (T1) to week 8 (T2) of intervention in the control group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

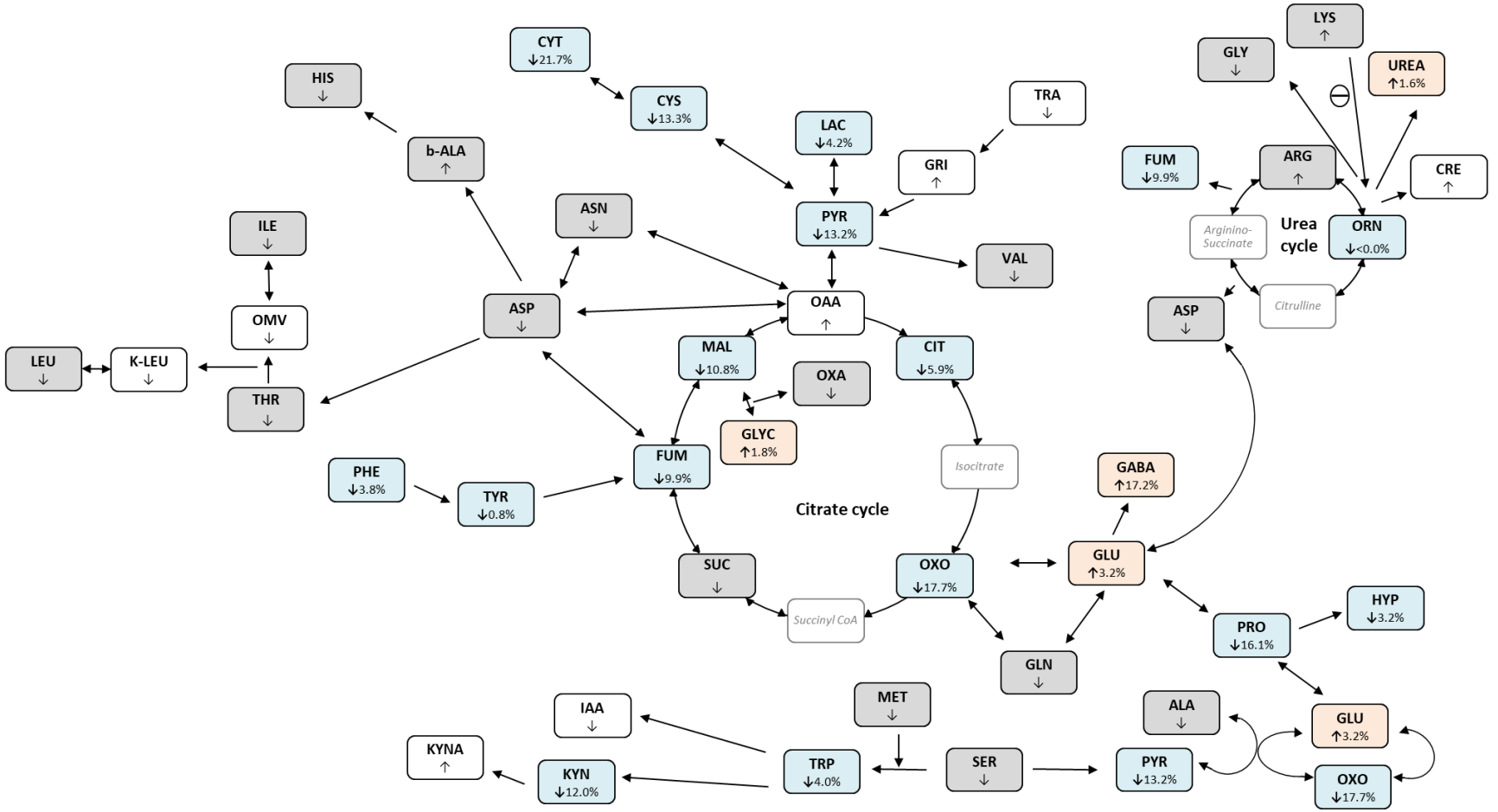

Figure 5 - Delta values and percentage deviation from week 8 (T2) to 17 weeks (T3) of intervention in the control group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

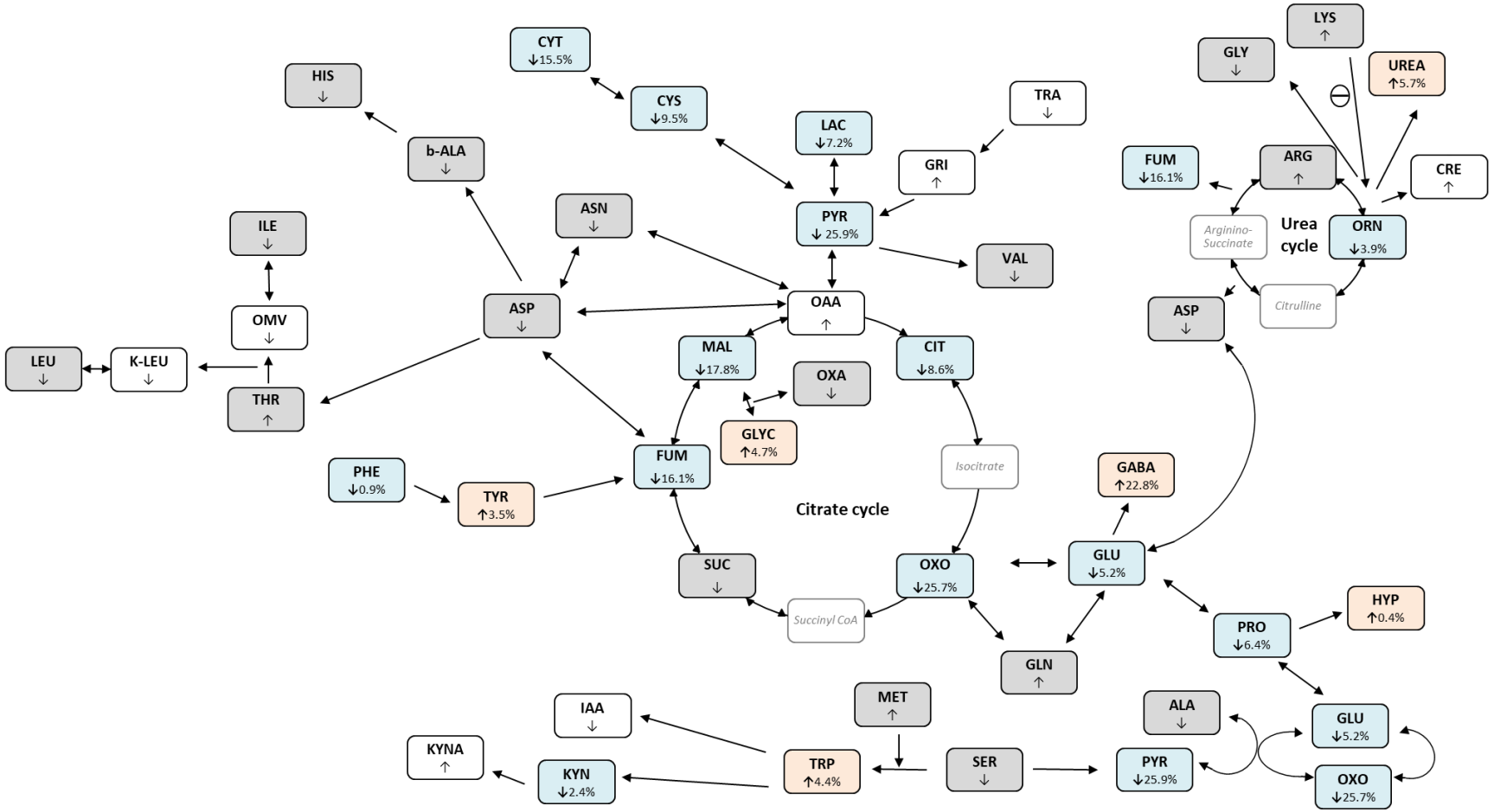

Figure 6 - Delta values and percentage deviation from Baseline (T1) to 17 weeks (T3) of intervention in the control group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

When comparing the three figures with the metabolic pathways, it is noticeable that many metabolites decreased during the duration of the intervention. While more metabolite areas increased from baseline to week 8 after intervention start, the opposite was observed between T2 and T3 and between T1 and T3. At this point, most areas of the metabolites decreased, 7 of them were significantly different, as already mentioned on page 30.

If the metabolic pathways are examined more closely, the delta values in the citrate cycle for the significant metabolites were always negative. The percentage deviations also increased during the intervention. The values of lactic acid and pyruvic acid behaved similarly. Urea, in contrast, increased from the beginning to the end of the intervention. To be able to interpret these results, figures for the two intervention groups (RP+T and HP+T) were also created: The outcomes can be seen on the next six pages (**Figure 7** - **Figure 12**).

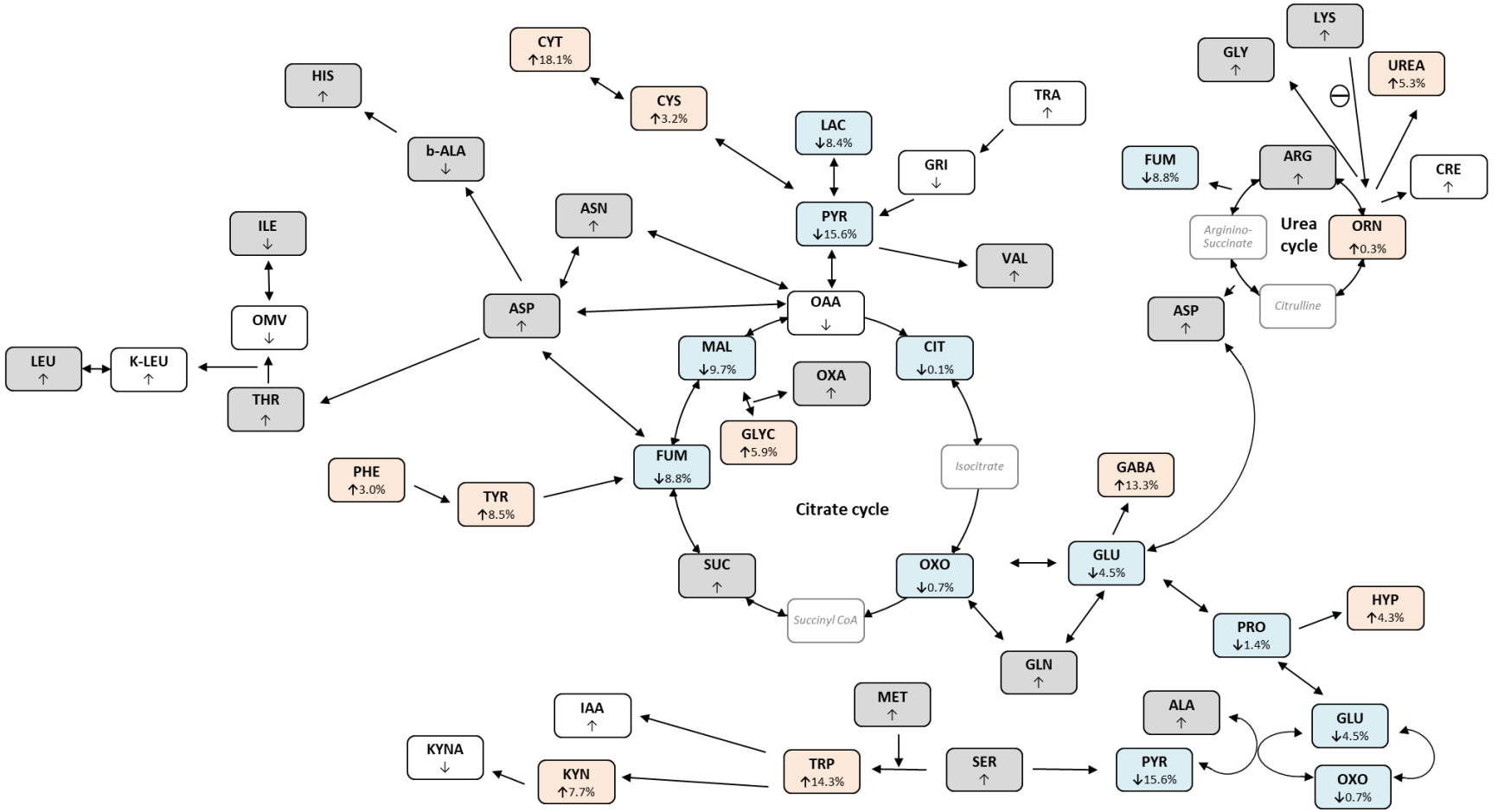

Figure 7 - Delta values and percentage deviation from Baseline (T1) to week 8 (T2) of intervention in the recommended protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

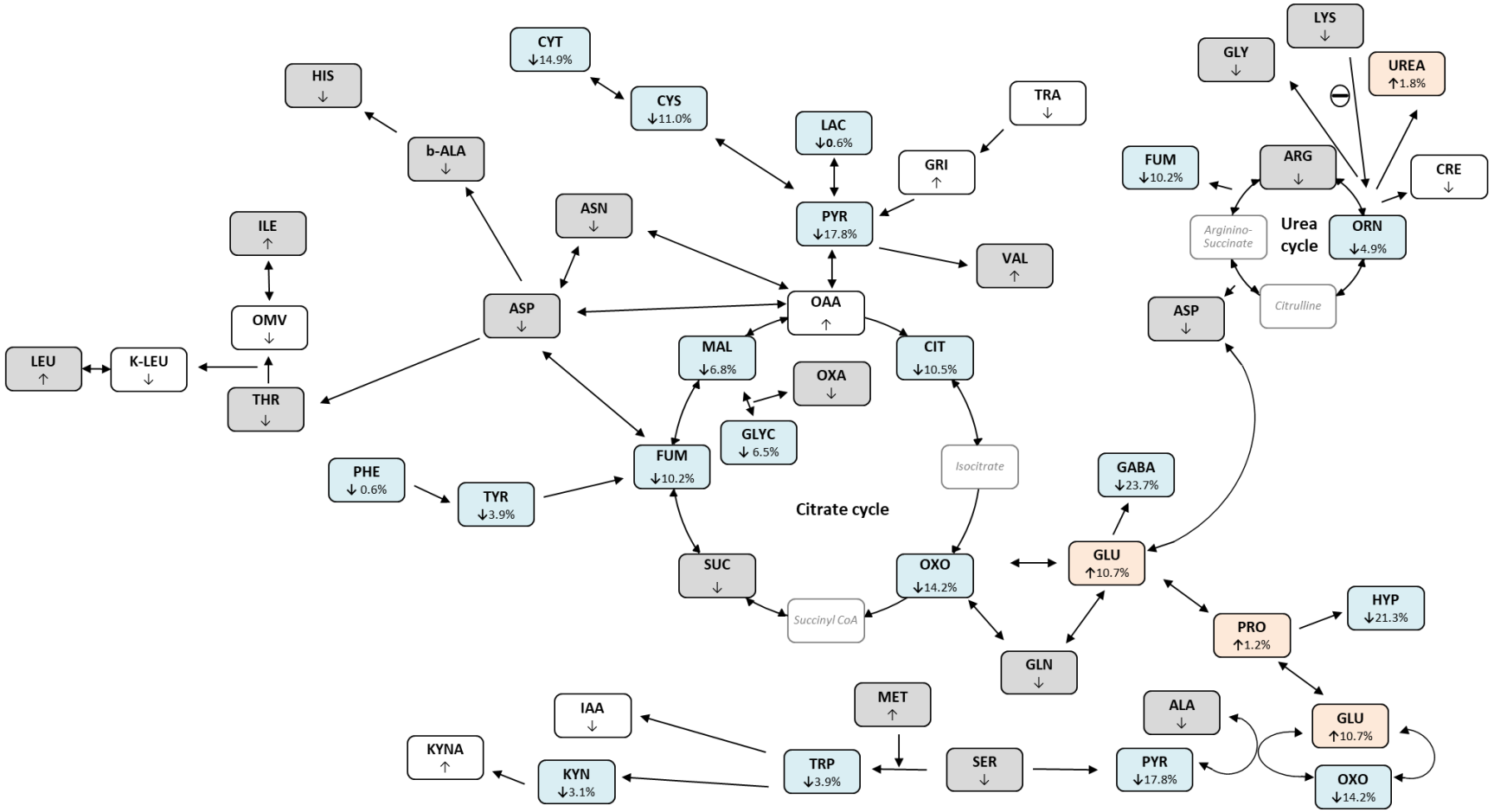

Figure 8 - Delta values and percentage deviation from week 8 (T2) to 17 weeks (T3) of intervention in the recommended protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

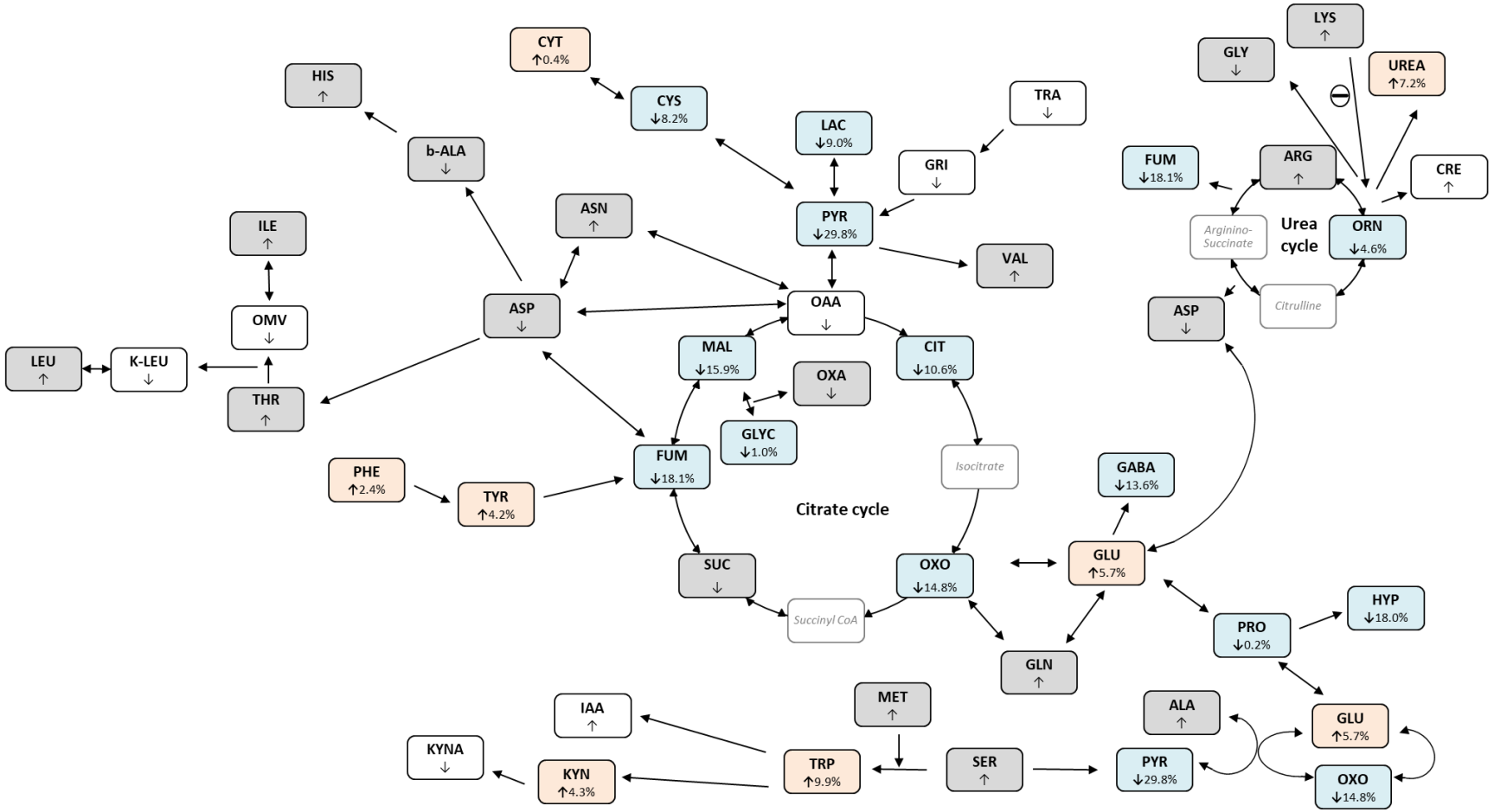

Figure 9 - Delta values and percentage deviation from Baseline (T1) to 17 weeks (T3) of intervention in the recommended protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

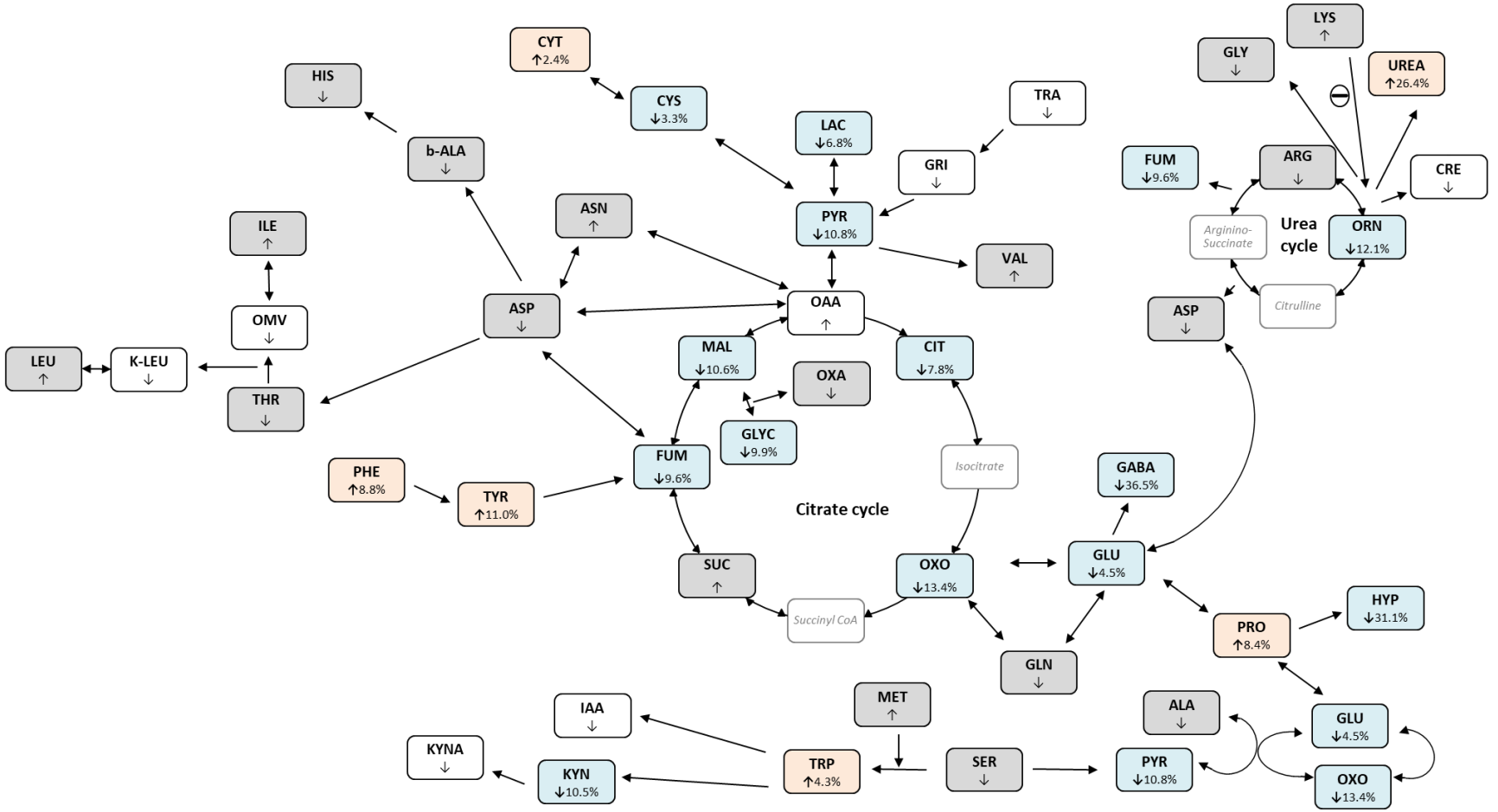

Figure 10 - Delta values and percentage deviation from Baseline (T1) to week 8 (T2) of intervention in the high protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

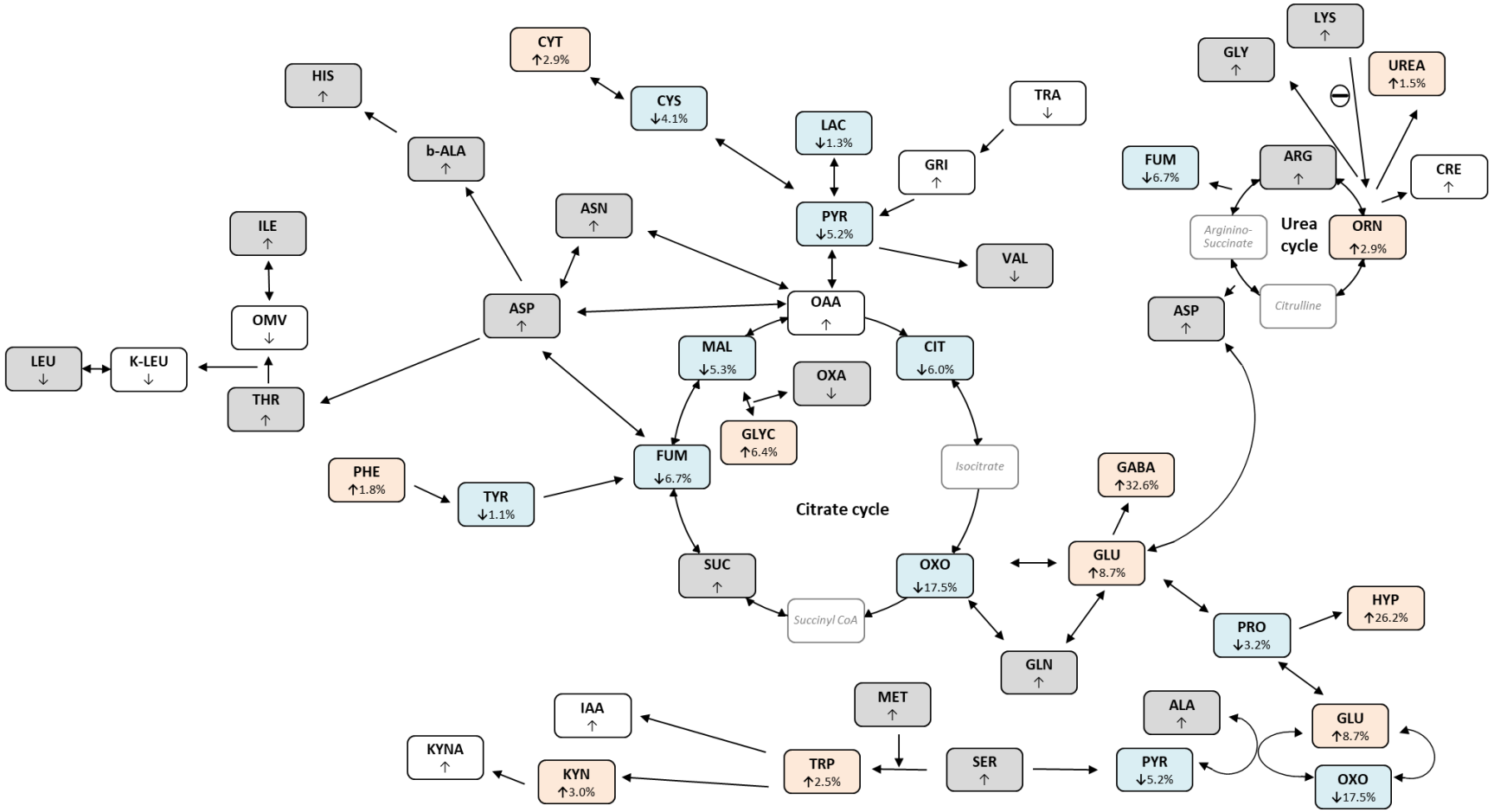

Figure 11 - Delta values and percentage deviation from week 8 (T2) to 17 weeks (T3) of intervention in the high protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

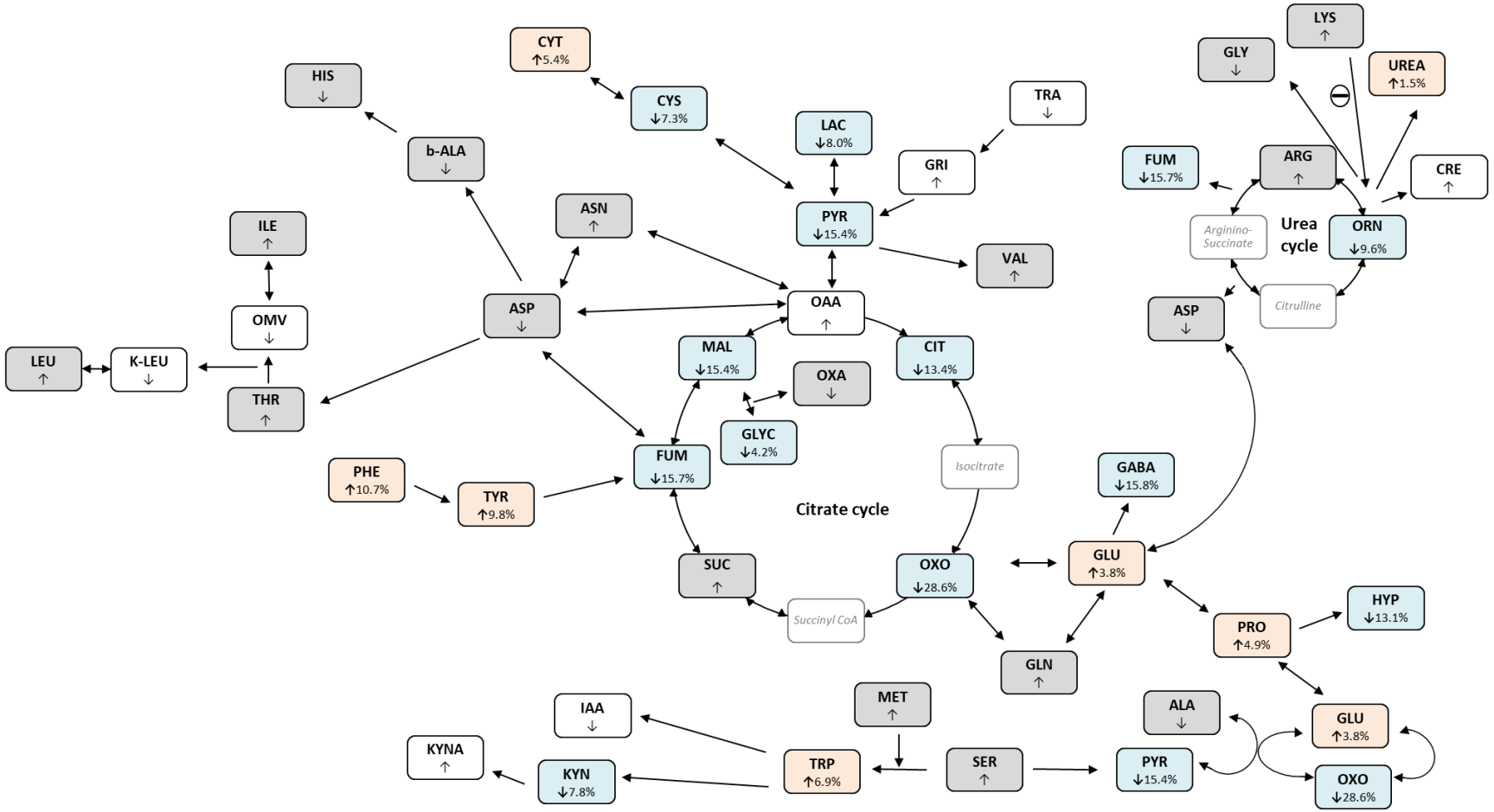

Figure 12 - Delta values and percentage deviation from Baseline (T1) to 17 weeks (T3) of intervention in the high protein group. Metabolites present in the QC are highlighted in grey, untargeted metabolites are white. Light tinted metabolites were not included into the analysis. Blue boxes show a decrease and orange boxes an increase of the 19 metabolites which were significantly different (Bonferroni post-hoc test).

When comparing the HP+T with the other two groups, it is noticeable that there were many more negative delta values in the HP+T between T1 and T2. This is especially remarkable for the non-significant metabolites (17 metabolites with negative delta values). Between T2 and T3, the delta values increased for more metabolites in comparison to the other two intervention groups (29 metabolites in HP+T, 10 in RP+T and 12 in CON). This suggests that increased protein intake may influenced some metabolites of energy metabolism.

Regarding the percentage differences, GABA/4-hydroxyproline showed the largest differences between the test phases, especially in the HP+T. The area of the metabolite decreased by 36.5 % from T1 to T2. From T2 to T3, the metabolite increased by 32.6 %. From T1 to T3 the area decreased by 15.8 %. For 4-hydroxyproline, the values were similar. From T1 to T2, the value decreased by 31.1 % and from T2 to T3, it increased by 26.2 %. From the beginning to the end of the intervention it was reduced by 13.1 %. Since values of the metabolites were quite close, we assume that the metabolite GABA/4-hydroxyproline was 4-hydroxyproline, not GABA. There was a group x time effect for both GABA/4-hydroxyproline and 4-hydroxyproline. Looking at the post-hoc results (**Table 8**), a significance could be seen only in the HP+T between T1 and T2 and within the group ($p = 0.006$). This indicates that the higher protein intake influenced the metabolite and that resistance training between T2 and T3 compensated this effect or that the metabolism had become accustomed to the increased protein intake. On the other hand, the significant effect may also have been due to older people being a very heterogeneous group (Kondoh, et al., 2020), as the values within the HP+T differed greatly.

There was an increase in the metabolites of the citrate cycle during physical activity, and the concentrations decreased again during exhaustion (Gibala, 2001). When looking at the delta values of the citrate cycle, no fundamental changes could be found between the different interventions. Malic acid, citric acid, fumaric acid and 2-oxoglutaric acid decreased similarly from baseline to the end of the intervention in all groups, only succinic acid and oxaloacetic acid differed.

According to the article by Maurer, et al. (2021), physiological changes by physical activity are best detectable in succinic acid. In the present thesis succinic acid did not change significantly. Oxaloacetic acid also did not change significantly,

but there was a decrease in metabolite from T1 to T2 in the RP+T, while the reverse was true for the other two groups.

2-oxoglutaric acid plays an essential role in the citrate cycle. The level of the metabolite depends on nutritional status, physical activity, and aging (Shahmirzadi, et al., 2020). This metabolite also showed a significant difference in our results. A time effect was found for 2-oxoglutartic acid (**Table 7**). The post-hoc tests showed that the metabolite changed significantly in the CON and HP+T between T1 and T3. The only group in which the metabolite did not change significantly was the RP+T. Significances could also be seen within the CON and within the HP+T. Thus, no intervention effects could be found for the metabolite. One possible explanation would be seasonal variations. Other metabolites of the citrate cycle, like citric acid, fumaric acid and malic acid also showed time effects. The effects on the metabolites of the citrate cycle may have been caused by seasonal fluctuations or other external influences.

Regarding the metabolites of the citrate cycle, as mentioned above, the four significant metabolites (malic acid, citric acid, fumaric acid, 2-oxoglutaric acid) decreased in all groups. Lactic acid and pyruvic acid also decreased in all groups and from the beginning to the end of the intervention. While these 6 metabolites showed time effects pyruvic acid also showed a group effect. Pyruvic acid differed significantly between RP+T and HP+T ($p = 0.028$). This difference is probably due to the increased protein intake in the HP+T. Similarly, to the metabolites of the citrate cycle, lactic acid and pyruvic acid also normally increase while exercising (Schranner, et al., 2020). During vigorous physical activity, blood glucose is required for energy production through glycolysis. Pyruvate and lactic acid are therefore elevated and decrease again within 60 minutes after physical activity (Schranner, et al., 2020). It is assumed that lactic acid and pyruvic acid may behave similarly to the metabolites of the citrate cycle.

As for glutamic acid, the MANOVA results showed a group effect. Glutamic acid was significantly different between the CON and the RP+T and decreased from T1 to T2 in all groups. In turn, between T2 and T3, the delta values were positive in all groups. Considering the whole intervention period, the delta values increased in the HP+T and in the RP+T from T1 to T3, while the delta value decreased in the

CON. The difference of the mean values of T3 to T2 of glutamic acid (**Table 14**) was 10.7 % in the RP+T, while it was only 3.2 % in the CON. Since the two groups differed between T2 and T3 mainly in resistance training, this could explain the significant effect. However, it is interesting to note that the HP+T was not significantly different from the CON.

Focusing on amino acids at the peptide level, dipeptides from glycine and leucine and dipeptides from glycine and proline appear to decrease in both plasma and serum after exercise (Schranner, et al., 2020). In the present study glycine and leucine were not significantly different. Proline showed a group effect between RP+T and HP+T. The delta values showed a decrease between T2 and T3 in the HP+T but an increase in the RP+T. The different protein intake might be a possible explanation.

After a high-protein diet, there are increased levels of BCAAs and urea in blood and muscle. Other amino acids of the consumed protein usually do not show a significant increase after increased protein intake (Holeček, 2018).

In muscle tissue, oxidation of leucine occurs during sustained physical activity (Gibala, 2001). In our case leucine increased between T2 and T3 in the RP+T, in the HP+T the delta value decreased. However, the metabolite did not change significantly during the intervention, it is possible that differences would have been detectable immediately after a training or during a training.

For urea a group and a time effect were found. In the HP+T there were some significances between T1 and T2 (positive delta value) and T2 and T3 (positive delta value), but also between the CON and the HP+T, and between the RP+T and the HP+T. Thus, there is evidence that increased protein intake may affect metabolism.

Urea increased by 26.4 % between T1 and T2, but only by 1.5 % between T2 and T3 in the HP+T. It is suspected that repletion occurred from time T2, or it is possible that the concentrations were too high, the peak was therefore overloaded, and this could no longer be detected by ChromaTOF during the measurement. Another possibility is that levelling off has occurred between T2 and T3.

Cysteine and cystine behaved similarly. The MANOVA showed time effects for the two amino acids. The areas of both metabolites differed significantly between T2 and T3 in the CON and generally within the CON. Cysteine was also significant

within the RP+T. Looking at the delta values, both metabolites increased between T1 and T2 and decreased after T2 until the end of the intervention. During the whole duration of the intervention (T1-T3), they decreased. No effect was found on cysteine and cystine by the interventions. This suggests that physical activity and increased protein intake did not affect the two metabolites.

Phenylalanine, tyrosine, and tryptophan increase in individuals who exercise (Ishikura, et al., 2013). Looking at the present results, the delta levels of phenylalanine and tryptophan increased in the HP+T, but they also increased when comparing T1 and T2, where no exercise was performed. Tyrosine showed the same pattern in all groups and there was only a significant effect in the HP+T. This effect could also be seen with phenylalanine, but phenylalanine also increased significantly between T1 and T3 in the HP+T. Tryptophan was significant within the RP+T, but also increased significantly from T1 to T2 in the RP+T. It is not possible to conclude clearly from the results whether the training showed an influence; this would require more detailed studies. Furthermore, the large variations within the groups were a problem, either due to the heterogeneity of the study population or caused by seasonal differences.

4. Conclusio

The aim of this master thesis was to investigate the effect of increased protein intake and resistance training on the metabolic profile in older adults. The citrate cycle and the urea cycle were examined in more detail, as well as metabolites closely related to these cycles.

The results show that 19 metabolites were significantly affected. Although metabolites of the citrate cycle tend to increase with physical activity in literature, they decreased in our studies. The results of this master thesis indicate significant differences in citrate cycle metabolites between time points and within groups, but no group effects could be found for citrate cycle metabolites. On the one hand, seasonal variations could be the reason, on the other hand, other external influences may have affected the values. Unfortunately, the results did not prove any effect of training on the chosen metabolites.

Pyruvic acid and lactic acid showed a similar pattern as the metabolites of the citrate cycle. The delta values decreased in all groups and at all time points. It is assumed that the results were close to those of the metabolites of the citrate cycle because pyruvic acid and lactic acid are tightly related in the metabolic pathway.

As for the urea cycle, a group and time effect could be found for urea, with significances between T1 and T2 as well as T1 and T3. There was also a significant difference between the CON and HP+T, and between the RP+T and HP+T. Urea increased significantly more in HP+T compared to RP+T and CON (lowest values in CON). Urea increased by 26.4 % between T1 and T2, but only by 1.5 % between T2 and T3 in HP+T. It is suggested that saturation occurred from T2, that concentrations were too high, or that a levelling off occurred. It can still be assumed that an increased protein intake may had an influence.

Since older adults are a rather heterogeneous group reasons for the observed effects can be diverse and may also be related to seasonal effects or other influences such as gender (see the master's thesis of Sabine Trettenhahn), lifestyle, diet, health conditions, medication, and others.

Metabolomics is still a fairly young discipline and requires further studies to better understand the more precise mechanisms. It would be beneficial if more information can be provided on the metabolism of citrate cycle, urea cycle and metabolites closely related to the two metabolic pathways, as well as studies regarding metabolomics and aging, exercise, and high-protein diets.

According to the conclusion of the master thesis of Sabine Trettenhahn with the title "Gender-specific differences in the metabolome of older adults" the metabolome differed significantly between males and females. Higher values could be seen in metabolites of men and the body composition showed a significant difference between men and women. Protein intake in contrast showed no significant difference between males and females.

Sabine Trettenhahn did not find any strong associations between metabolites and body composition parameters, but there were trends showing that body composition affects the metabolome differently depending on gender.

In her master thesis Sabine Trettenhahn suggests looking more closely on energy consumption, intracellular and extracellular mass, physical fitness parameters and serological parameters such as inflammation status or blood lipid levels to explain the observed differences in the metabolome. Furthermore, fat, carbohydrate and energy intake could be considered as they are important for metabolism. This would also be an interesting option for the topic of the present master thesis.

In conclusion and in summary, there are still some unanswered questions about the issues addressed in both master theses and these could be used as the basis for further research.

5. Fazit

Ziel dieser Masterarbeit war es, die Auswirkungen einer erhöhten Proteinzufuhr und eines Krafttrainings auf das Stoffwechselprofil älterer Menschen zu untersuchen. Der Zitratzyklus und der Harnstoffzyklus wurden genauer untersucht, ebenso wie Metaboliten, die eng mit diesen Zyklen zusammenhängen.

Die Ergebnisse zeigen, dass 19 Metaboliten signifikant waren. Obwohl die Metaboliten des Zitratzyklus in der Literatur bei körperlicher Aktivität tendenziell ansteigen, nahmen sie in unseren Studien ab. Die Ergebnisse dieser Masterarbeit deuten auf signifikante Unterschiede bei den Metaboliten des Zitratzyklus zwischen den Zeitpunkten und innerhalb der Gruppen hin, aber es konnten keine Gruppeneffekte für die Metaboliten des Zitratzyklus gefunden werden. Einerseits könnten jahreszeitliche Schwankungen der Grund dafür sein, andererseits könnten auch andere äußere Einflüsse die Werte beeinflusst haben. Leider konnten die Ergebnisse keinen Einfluss des Trainings auf die ausgewählten Metaboliten nachweisen.

Pyruvat und Laktat zeigten ein ähnliches Muster wie die Metaboliten des Zitratzyklus. Die Delta-Werte nahmen in allen Gruppen und zu allen Zeitpunkten ab. Es wird angenommen, dass die Ergebnisse, denen der Metaboliten des Citratzyklus nahekamen, da Pyruvat und Laktat im Stoffwechselweg eng miteinander verbunden sind.

Was den Harnstoffzyklus anbelangt, so konnte für Harnstoff ein Gruppen- und Zeiteffekt festgestellt werden, mit Signifikanzen zwischen T1 und T2 sowie T1 und T3. Es gab auch einen signifikanten Unterschied zwischen CON und HP+T sowie zwischen RP+T und HP+T. Der Harnstoff stieg bei HP+T im Vergleich zu RP+T und CON signifikant stärker an (niedrigste Werte bei CON). Der Harnstoff stieg zwischen T1 und T2 um 26,4 %, aber nur um 1,5 % zwischen T2 und T3 in HP+T. Es wird vermutet, dass ab T2 eine Sättigung eintrat, dass die Konzentrationen zu hoch waren oder dass ein Abflachen stattfand. Es kann weiterhin angenommen werden, dass eine erhöhte Proteinzufuhr einen Einfluss gehabt haben könnte.

Da ältere Erwachsene eine recht heterogene Gruppe sind, können die Gründe für die beobachteten Effekte vielfältig sein und auch mit jahreszeitlichen Effekten oder anderen Einflüssen wie Geschlecht (siehe die Masterarbeit von Sabine Tretten-

hahn), Lebensstil, Ernährung, Gesundheitszustand, Medikamenteneinnahme und anderen zusammenhängen.

Die Metabolomik ist noch eine recht junge Disziplin und erfordert weitere Studien, um die genaueren Mechanismen besser zu verstehen. Es wäre von Vorteil, wenn mehr Informationen über den Stoffwechsel des Citratzyklus, des Harnstoffzyklus und der mit den beiden Stoffwechselwegen eng verbundenen Metaboliten zur Verfügung gestellt werden könnten, ebenso wie Studien über Metabolomik und Altern, Bewegung und proteinreiche Ernährung.

Nach dem Fazit der Masterarbeit von Sabine Trettenhahn mit dem Titel "Gender-specific differences in the metabolome of older adults" unterschied sich das Metabolom signifikant zwischen Männern und Frauen. Es zeigten sich höhere Werte bei den Metaboliten der Männer und die Körperzusammensetzung zeigte einen signifikanten Unterschied zwischen Männern und Frauen. Die Proteinzufuhr hingegen zeigte keinen signifikanten Unterschied zwischen Männern und Frauen.

Sabine Trettenhahn fand keine starken Assoziationen zwischen Metaboliten und Parametern der Körperzusammensetzung, aber es gab Trends, die zeigen, dass die Körperzusammensetzung das Metabolom je nach Geschlecht unterschiedlich beeinflusst.

Sabine Trettenhahn schlägt in ihrer Masterarbeit vor, den Energieverbrauch, die intrazelluläre und extrazelluläre Masse, körperliche Fitnessparameter und serologische Parameter wie den Entzündungsstatus oder die Blutfettwerte genauer zu betrachten, um die beobachteten Unterschiede im Metabolom zu erklären. Darüber hinaus könnte die Fett-, Kohlenhydrat- und Energieaufnahme berücksichtigt werden, da sie für den Stoffwechsel wichtig sind. Auch dies wäre eine interessante Option für das Thema der vorliegenden Masterarbeit.

Abschließend und zusammenfassend lässt sich sagen, dass es noch einige offene Fragen zu den in beiden Masterarbeiten behandelten Themen gibt, die als Grundlage für weitere Forschungen genutzt werden könnten.

6. Summary

There is not much literature in the field of metabolomics in the older population related to high protein intake and physical activity. The research question addressed in this master's thesis was whether physical activity and higher protein intake in the aging population impacts metabolism and what this means for metabolomics research.

Plasma samples from the NutriAging study were measured using GC-MS (Gas Chromatography-Mass Spectrometry) and analyzed using ChromaTOF and MS-Dial programs. The participates were 155 healthy adults aged 65 to 84 years.

To answer the research question, the differences between intervention groups (high protein plus resistance training group, recommended protein plus resistance training group, and control group) and between test phases (Baseline, week 8 after intervention start and 17 weeks after intervention start) in metabolites of the citrate cycle, urea cycle and related metabolites were investigated.

The results of this master's thesis showed that there were some significant differences in metabolites between groups and time points. Regarding the citrate cycle, citric acid and 2-oxoglutaric acid decreased significantly in the HP+T from T1 to T3. Fumaric acid and lactic acid decreased in all groups. Pyruvic acid decreased from T1 to T3, but the significant differences were also present in the CON and RP+T (lactic acid additionally in the HP+T). Urea, tryptophan (from T1 to T2 in the RP+T), and phenylalanine (from T1 to T3 in the HP+T) increased during the intervention. Urea increased significantly from T1 to T3 in the HP+T and indicates that protein intake had an impact on the metabolite. 4-hydroxyproline, glycolic acid and ornithine decreased significantly from T1 to T2 in the HP+T. A total of 19 metabolites showed significant differences, providing evidence that exercise and high protein intake may had an impact on metabolites, but there were also significances within groups that could be due to seasonal differences or heterogeneity of the group.

7. Zusammenfassung

Es gibt nicht viel Literatur auf dem Gebiet der Metabolomik in der älteren Bevölkerung im Zusammenhang mit einer hohen Proteinzufuhr und körperlicher Aktivität. Die Forschungsfrage, die in dieser Masterarbeit behandelt wurde, war, ob sich körperliche Aktivität und eine höhere Proteinzufuhr in der alternden Bevölkerung auf den Stoffwechsel auswirkt und was dies für die Metabolomikforschung bedeutet.

Plasmaproben aus der NutriAging-Studie wurden mit GC-MS (Gaschromatographie-Massenspektrometrie) gemessen und mit den Programmen ChromaTOF und MS-Dial analysiert. Bei den Teilnehmern handelte es sich um 155 gesunde Erwachsene im Alter von 65 bis 84 Jahren.

Zur Beantwortung der Forschungsfrage wurden die Unterschiede zwischen den Interventionsgruppen (Gruppe mit hohem Proteingehalt und Krafttraining, Gruppe mit empfohlenem Proteingehalt und erneutem Krafttraining, sowie Kontrollgruppe) und zwischen den Testphasen (Baseline, Woche 8 nach Interventionsbeginn und 17 Wochen nach Interventionsbeginn) bei den Metaboliten des Zitratzyklus, des Harnstoffzyklus und damit zusammenhängenden Metaboliten untersucht.

Die Ergebnisse dieser Masterarbeit zeigten, dass es einige signifikante Unterschiede in den Metaboliten zwischen den Gruppen und Zeitpunkten gab. Was den Zitratzyklus betrifft, so nahmen Citrat und 2-Oxoglutarsäure in der HP+T von T1 bis T3 signifikant ab. Fumarsäure und Laktat nahmen in allen Gruppen ab. Pyruvat nahm von T1 bis T3 ab, aber die signifikanten Unterschiede waren auch in der CON und RP+T vorhanden (Laktat zusätzlich in der HP+T). Harnstoff, Tryptophan (von T1 auf T2 in der RP+T) und Phenylalanin (von T1 auf T3 in der HP+T) nahmen während der Intervention zu. Harnstoff stieg von T1 bis T3 in der HP+T signifikant an, was darauf hindeutet, dass die Eiweißzufuhr einen Einfluss auf diesen Metaboliten hatte. 4-Hydroxyprolin, Glykolsäure und Ornithin nahmen bei der HP+T von T1 auf T2 signifikant ab. Insgesamt 19 Metaboliten wiesen signifikante Unterschiede auf, was darauf hindeutet, dass Bewegung und eine hohe Proteinzufuhr einen Einfluss auf die Metaboliten hatten, aber es gab auch Signifikanzen innerhalb der Gruppen, die auf saisonale Unterschiede oder die Heterogenität der Gruppe zurückzuführen sein könnten.

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9. Appendix

Table 10 - Delta values between measurement time points of significant metabolites in the different intervention groups 1

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	ΔT1, T2	ΔT2, T3	ΔT1, T3
2-Oxoglutaric acid	CON	0.021 ± 0.010	0.019 ± 0.010	0.016 ± 0.006	0.018 ± 0.009	-0.002	-0.003	-0.005
	RP+T	0.019 ± 0.008	0.019 ± 0.008	0.017 ± 0.007	0.018 ± 0.008	0.000	-0.003	-0.003
	HP+T	0.020 ± 0.009	0.018 ± 0.009	0.014 ± 0.006	0.017 ± 0.009	-0.003	-0.003	-0.006
	Total	0.020 ± 0.009	0.019 ± 0.009	0.015 ± 0.006	0.018 ± 0.008	-0.002	-0.003	-0.005
4-Hydroxyproline	CON	0.035 ± 0.019	0.036 ± 0.016	0.035 ± 0.017	0.035 ± 0.017	0.001	-0.001	0.000
	RP+T	0.041 ± 0.019	0.043 ± 0.030	0.034 ± 0.017	0.039 ± 0.023	0.002	-0.009	-0.007
	HP+T	0.042 ± 0.018	0.029 ± 0.009	0.036 ± 0.020	0.036 ± 0.017	-0.013	0.008	-0.005
	Total	0.039 ± 0.019	0.036 ± 0.020	0.035 ± 0.018	0.037 ± 0.019	-0.003	-0.001	-0.004
alpha-Tocopherol	CON	0.298 ± 0.078	0.294 ± 0.065	0.299 ± 0.061	0.297 ± 0.068	-0.003	0.004	0.001
	RP+T	0.274 ± 0.051	0.315 ± 0.072	0.285 ± 0.077	0.291 ± 0.069	0.041	-0.030	0.012
	HP+T	0.295 ± 0.063	0.269 ± 0.061	0.268 ± 0.055	0.278 ± 0.061	-0.026	-0.001	-0.027
	Total	0.290 ± 0.066	0.292 ± 0.068	0.285 ± 0.065	0.289 ± 0.066	0.002	-0.007	-0.005
Citric acid	CON	0.549 ± 0.160	0.533 ± 0.149	0.502 ± 0.114	0.528 ± 0.142	-0.016	-0.031	-0.047
	RP+T	0.550 ± 0.139	0.549 ± 0.167	0.492 ± 0.129	0.530 ± 0.147	0.000	-0.058	-0.058
	HP+T	0.531 ± 0.138	0.489 ± 0.117	0.460 ± 0.100	0.493 ± 0.122	-0.042	-0.029	-0.071
	Total	0.543 ± 0.146	0.523 ± 0.146	0.485 ± 0.115	0.517 ± 0.138	-0.020	-0.038	-0.058
Cysteine	CON	0.137 ± 0.018	0.143 ± 0.028	0.124 ± 0.025	0.135 ± 0.025	0.006	-0.019	-0.013
	RP+T	0.133 ± 0.024	0.138 ± 0.016	0.123 ± 0.026	0.131 ± 0.023	0.004	-0.015	-0.011
	HP+T	0.143 ± 0.024	0.139 ± 0.024	0.133 ± 0.029	0.138 ± 0.026	-0.005	-0.006	-0.010
	Total	0.138 ± 0.022	0.140 ± 0.024	0.127 ± 0.027	0.135 ± 0.025	0.002	-0.013	-0.012
Cystine	CON	0.416 ± 0.142	0.448 ± 0.127	0.351 ± 0.182	0.405 ± 0.156	0.033	-0.097	-0.065
	RP+T	0.379 ± 0.134	0.448 ± 0.099	0.381 ± 0.151	0.403 ± 0.132	0.069	-0.067	0.002
	HP+T	0.410 ± 0.114	0.420 ± 0.113	0.432 ± 0.131	0.421 ± 0.119	0.010	0.012	0.022
	Total	0.403 ± 0.131	0.439 ± 0.115	0.387 ± 0.159	0.410 ± 0.137	0.035	-0.052	-0.016
Fumaric acid	CON	0.018 ± 0.004	0.017 ± 0.004	0.015 ± 0.003	0.017 ± 0.004	-0.001	-0.002	-0.003
	RP+T	0.018 ± 0.003	0.016 ± 0.003	0.015 ± 0.003	0.016 ± 0.003	-0.002	-0.002	-0.003
	HP+T	0.017 ± 0.003	0.016 ± 0.004	0.015 ± 0.003	0.016 ± 0.003	-0.002	-0.001	-0.003
	Total	0.018 ± 0.004	0.016 ± 0.004	0.015 ± 0.003	0.016 ± 0.004	-0.001	-0.001	-0.003
GABA/ 4-Hydroxyproline	CON	0.003 ± 0.002	0.004 ± 0.002	0.004 ± 0.003	0.004 ± 0.002	0.000	0.001	0.001
	RP+T	0.004 ± 0.002	0.005 ± 0.004	0.004 ± 0.002	0.004 ± 0.003	0.001	-0.001	-0.001
	HP+T	0.005 ± 0.003	0.003 ± 0.001	0.004 ± 0.003	0.004 ± 0.002	-0.002	0.001	-0.001
	Total	0.004 ± 0.002	0.004 ± 0.003	0.004 ± 0.003	0.004 ± 0.002	0.000	0.000	0.000
Glutamic acid	CON	0.430 ± 0.144	0.395 ± 0.157	0.407 ± 0.143	0.411 ± 0.148	-0.035	0.013	-0.022
	RP+T	0.358 ± 0.117	0.342 ± 0.118	0.378 ± 0.117	0.359 ± 0.117	-0.016	0.036	0.020
	HP+T	0.395 ± 0.103	0.377 ± 0.136	0.410 ± 0.165	0.394 ± 0.136	-0.018	0.033	0.015
	Total	0.397 ± 0.126	0.373 ± 0.140	0.400 ± 0.144	0.390 ± 0.137	-0.024	0.026	0.003
Glycolic acid	CON	0.018 ± 0.004	0.019 ± 0.004	0.019 ± 0.005	0.019 ± 0.004	0.001	0.000	0.001
	RP+T	0.019 ± 0.005	0.020 ± 0.006	0.019 ± 0.003	0.019 ± 0.005	0.001	-0.001	0.000
	HP+T	0.019 ± 0.003	0.017 ± 0.003	0.019 ± 0.003	0.018 ± 0.003	-0.002	0.001	-0.001
	Total	0.019 ± 0.004	0.019 ± 0.004	0.019 ± 0.004	0.019 ± 0.004	0.000	0.000	0.000

Values are shown as mean±stdv and delta values are added (ΔT1, T2; ΔT2, T3 and ΔT1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 11 - Delta values between measurement time points of significant metabolites in the different intervention groups 2

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	ΔT1, T2	ΔT2, T3	ΔT1, T3
Kynurenine	CON	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
	RP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
	HP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.000	0.002 ± 0.001	0.000	0.000	0.000
	Total	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
Lactic acid	CON	16.923 ± 2.117	16.396 ± 2.271	15.701 ± 1.798	16.340 ± 2.114	-0.527	-0.695	-1.222
	RP+T	17.261 ± 2.327	15.811 ± 1.689	15.714 ± 2.181	16.262 ± 2.180	-1.450	-0.096	-1.546
	HP+T	17.328 ± 2.697	16.158 ± 2.055	15.950 ± 2.144	16.479 ± 2.374	-1.170	-0.208	-1.378
	Total	17.157 ± 2.370	16.146 ± 2.039	15.788 ± 2.017	16.364 ± 2.218	-1.011	-0.358	-1.369
Malic acid	CON	0.010 ± 0.003	0.009 ± 0.003	0.008 ± 0.002	0.009 ± 0.003	-0.001	-0.001	-0.002
	RP+T	0.009 ± 0.003	0.009 ± 0.002	0.008 ± 0.003	0.009 ± 0.003	-0.001	-0.001	-0.001
	HP+T	0.009 ± 0.003	0.008 ± 0.003	0.007 ± 0.002	0.008 ± 0.003	-0.001	0.000	-0.001
	Total	0.009 ± 0.003	0.008 ± 0.003	0.008 ± 0.002	0.009 ± 0.003	-0.001	-0.001	-0.002
Ornithine	CON	1.382 ± 0.418	1.329 ± 0.347	1.329 ± 0.319	1.347 ± 0.362	-0.053	-0.001	-0.054
	RP+T	1.290 ± 0.341	1.295 ± 0.309	1.231 ± 0.283	1.272 ± 0.310	0.004	-0.063	-0.059
	HP+T	1.378 ± 0.275	1.211 ± 0.224	1.246 ± 0.311	1.278 ± 0.279	-0.167	0.035	-0.132
	Total	1.354 ± 0.352	1.279 ± 0.301	1.273 ± 0.307	1.302 ± 0.322	-0.075	-0.007	-0.082
Phenylalanine	CON	0.691 ± 0.128	0.712 ± 0.146	0.685 ± 0.111	0.696 ± 0.129	0.020	-0.027	-0.006
	RP+T	0.686 ± 0.112	0.707 ± 0.118	0.703 ± 0.128	0.699 ± 0.119	0.020	-0.004	0.017
	HP+T	0.660 ± 0.100	0.718 ± 0.118	0.731 ± 0.107	0.703 ± 0.112	0.058	0.013	0.071
	Total	0.679 ± 0.114	0.712 ± 0.128	0.706 ± 0.116	0.699 ± 0.120	0.033	-0.007	0.026
Proline	CON	6.380 ± 2.677	7.125 ± 3.130	5.974 ± 1.918	6.493 ± 2.645	0.744	-1.150	-0.406
	RP+T	6.212 ± 2.667	6.128 ± 1.885	6.201 ± 2.164	6.180 ± 2.237	-0.084	0.073	-0.011
	HP+T	6.858 ± 2.184	7.433 ± 2.249	7.196 ± 2.527	7.162 ± 2.315	0.575	-0.237	0.338
	Total	6.492 ± 2.509	6.939 ± 2.563	6.451 ± 2.254	6.628 ± 2.448	0.447	-0.488	-0.041
Pyruvic acid	CON	0.608 ± 0.214	0.520 ± 0.186	0.451 ± 0.177	0.526 ± 0.202	-0.089	-0.069	-0.157
	RP+T	0.567 ± 0.205	0.484 ± 0.211	0.398 ± 0.192	0.483 ± 0.212	-0.083	-0.086	-0.169
	HP+T	0.611 ± 0.270	0.546 ± 0.170	0.517 ± 0.188	0.558 ± 0.215	-0.066	-0.028	-0.094
	Total	0.597 ± 0.230	0.518 ± 0.188	0.458 ± 0.190	0.524 ± 0.211	-0.079	-0.060	-0.139
Tryptophan	CON	2.209 ± 0.557	2.402 ± 0.418	2.306 ± 0.374	2.306 ± 0.459	0.193	-0.096	0.097
	RP+T	2.080 ± 0.501	2.378 ± 0.391	2.285 ± 0.525	2.248 ± 0.487	0.298	-0.093	0.205
	HP+T	2.220 ± 0.401	2.315 ± 0.442	2.373 ± 0.429	2.303 ± 0.425	0.095	0.059	0.154
	Total	2.175 ± 0.492	2.366 ± 0.417	2.323 ± 0.437	2.288 ± 0.456	0.190	-0.043	0.147
Tyrosine	CON	2.595 ± 0.469	2.706 ± 0.506	2.685 ± 0.458	2.662 ± 0.477	0.111	-0.020	0.090
	RP+T	2.535 ± 0.394	2.750 ± 0.538	2.642 ± 0.431	2.642 ± 0.462	0.215	-0.108	0.107
	HP+T	2.482 ± 0.488	2.755 ± 0.533	2.725 ± 0.455	2.654 ± 0.504	0.273	-0.030	0.243
	Total	2.539 ± 0.454	2.735 ± 0.520	2.686 ± 0.446	2.653 ± 0.480	0.195	-0.049	0.147
Urea	CON	23.163 ± 7.442	24.103 ± 6.347	24.478 ± 7.940	23.915 ± 7.234	0.940	0.374	1.314
	RP+T	23.166 ± 5.489	24.400 ± 5.558	24.829 ± 6.530	24.132 ± 5.857	1.234	0.430	1.663
	HP+T	23.722 ± 6.945	29.991 ± 7.564	30.446 ± 6.678	28.053 ± 7.654	6.269	0.456	6.724
	Total	23.352 ± 6.701	26.170 ± 7.067	26.588 ± 7.591	25.370 ± 7.251	2.818	0.418	3.236

Values are shown as mean±stdv and delta values are added (ΔT1, T2; ΔT2, T3 and ΔT1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

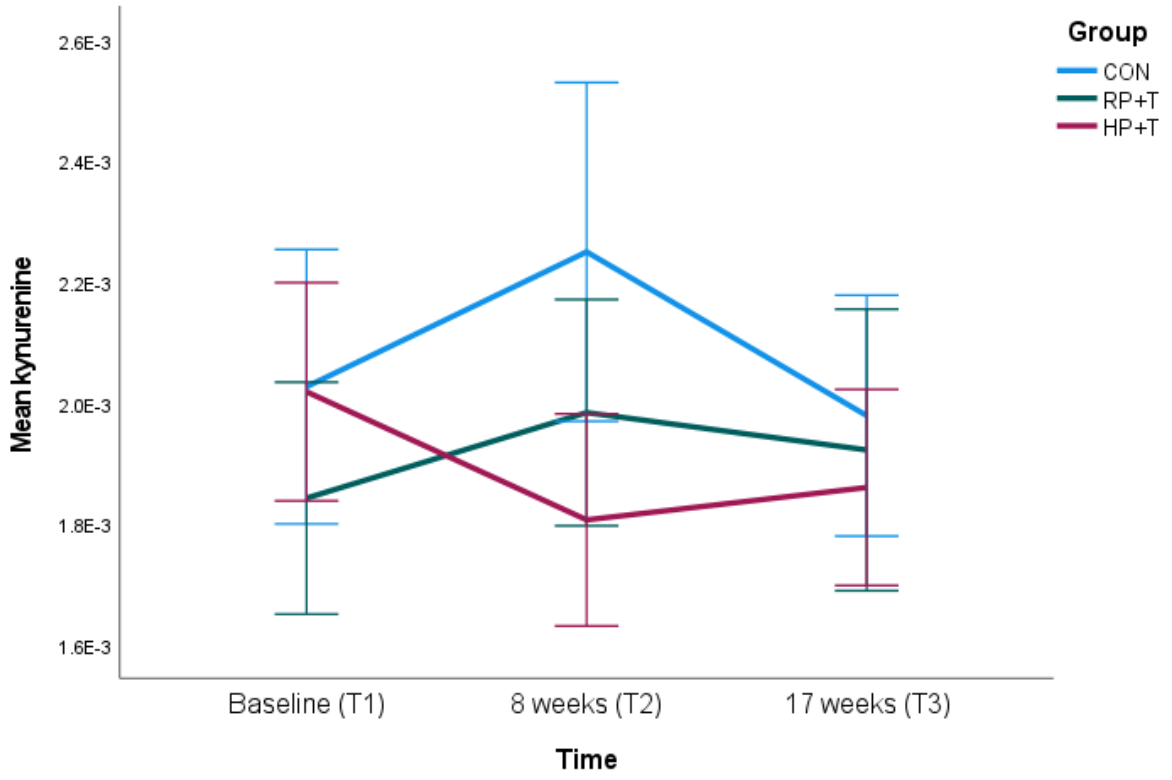

Figure 13 - Development of Kynurenine during intervention in the different groups (95% confidence interval)

The line chart above (**Figure 13**) shows the significant effect of Kynurenine between the three interventions in more detail. For the delta values, it was not possible to tell from the numbers alone whether the value decreased or increased because the areas of the metabolite were so small (0.02).

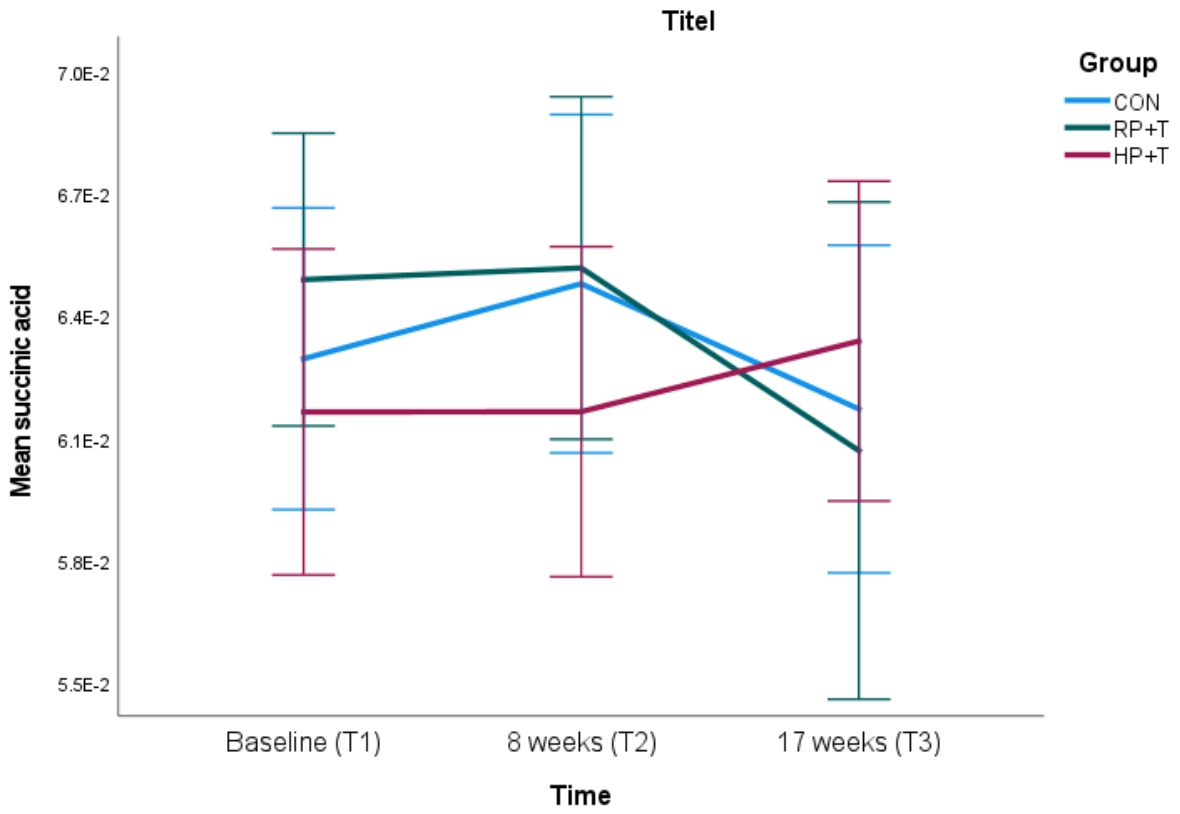

Figure 14 – Development of succinic acid during intervention in the different groups (95% confidence interval)

Succinic acid was not significant but showed a slight tendency to decrease in the CON and in the RP+T from baseline to week 8. In the HP+T, the values remained quite constant. From T2 to the end of the intervention, the values in the CON and in the RP+T showed a slight tendency to decrease, while in the HP+T tendency to increase.

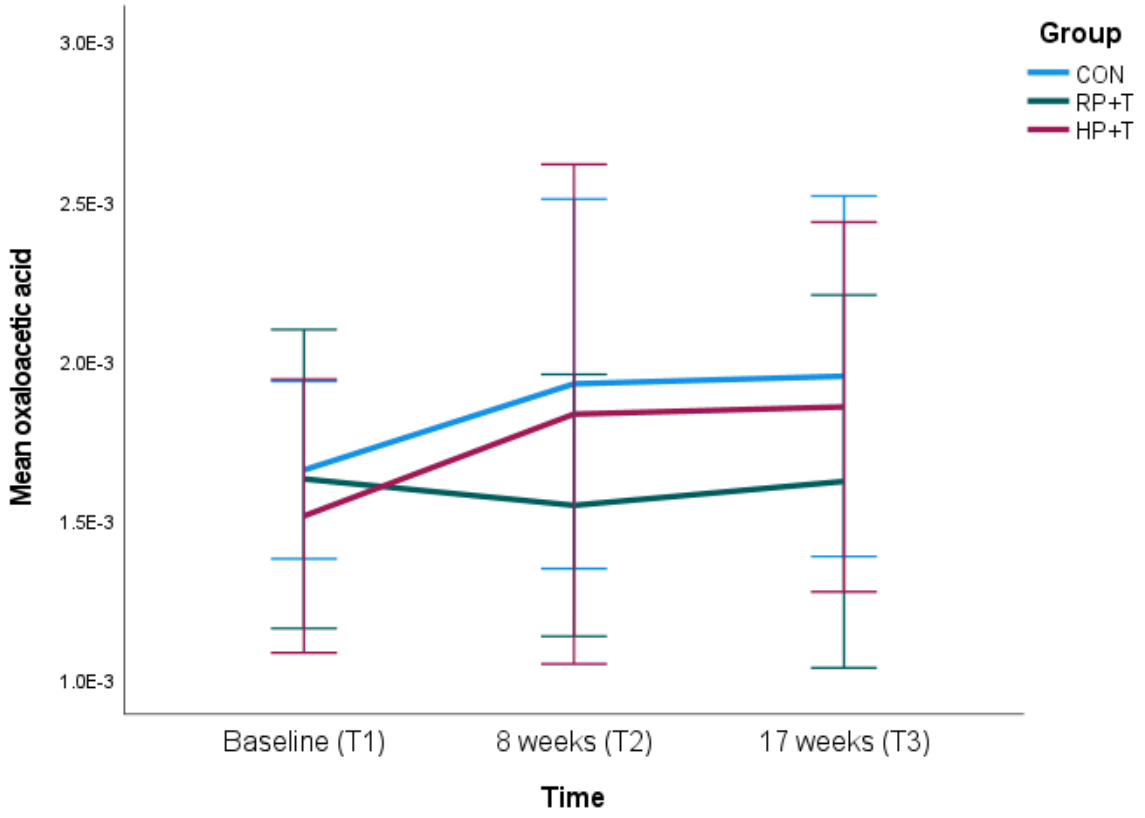

Figure 15 - Development of oxaloacetic acid during intervention in the different groups (95% confidence interval)

Oxaloacetic acid, like succinic acid, was not significantly different, but there was a slight tendency for the metabolite to decrease from T1 to T2 in the RP+T, while the reverse was true for the other two groups

Table 12 - Delta values between measurement time points of not significant metabolites in the different intervention groups 1

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	ΔT1, T2	ΔT2, T3	ΔT1, T3
3-Methyl-2-oxopentanoic acid	CON	0.218 ± 0.052	0.214 ± 0.044	0.199 ± 0.038	0.210 ± 0.046	-0.004	-0.015	-0.020
	RP+T	0.207 ± 0.045	0.207 ± 0.042	0.194 ± 0.050	0.203 ± 0.046	0.000	-0.013	-0.013
	HP+T	0.209 ± 0.057	0.201 ± 0.042	0.198 ± 0.035	0.203 ± 0.046	-0.007	-0.003	-0.011
	Gesamt	0.212 ± 0.052	0.208 ± 0.043	0.197 ± 0.040	0.206 ± 0.046	-0.004	-0.011	-0.015
Alanine	CON	8.859 ± 1.960	9.046 ± 2.024	8.681 ± 1.879	8.862 ± 1.944	0.187	-0.364	-0.177
	RP+T	8.450 ± 1.717	8.967 ± 1.419	8.813 ± 2.147	8.743 ± 1.780	0.517	-0.154	0.363
	HP+T	8.759 ± 1.947	8.690 ± 1.784	8.696 ± 1.738	8.715 ± 1.808	-0.069	0.006	-0.063
	Gesamt	8.707 ± 1.878	8.903 ± 1.775	8.724 ± 1.899	8.778 ± 1.848	0.196	-0.179	0.018
Arginine	CON	0.045 ± 0.010	0.046 ± 0.014	0.047 ± 0.009	0.046 ± 0.011	0.002	0.001	0.003
	RP+T	0.042 ± 0.009	0.047 ± 0.009	0.045 ± 0.009	0.045 ± 0.009	0.005	-0.002	0.003
	HP+T	0.045 ± 0.007	0.045 ± 0.007	0.047 ± 0.007	0.045 ± 0.007	0.000	0.002	0.002
	Gesamt	0.044 ± 0.008	0.046 ± 0.011	0.046 ± 0.008	0.045 ± 0.009	0.002	0.000	0.002
Asparagine	CON	0.161 ± 0.033	0.165 ± 0.040	0.159 ± 0.023	0.162 ± 0.033	0.003	-0.006	-0.003
	RP+T	0.166 ± 0.035	0.175 ± 0.029	0.167 ± 0.029	0.169 ± 0.031	0.009	-0.008	0.001
	HP+T	0.166 ± 0.027	0.172 ± 0.032	0.176 ± 0.033	0.171 ± 0.031	0.006	0.004	0.010
	Gesamt	0.164 ± 0.031	0.170 ± 0.035	0.167 ± 0.029	0.167 ± 0.032	0.006	-0.003	0.003
Aspartic acid	CON	0.003 ± 0.001	0.004 ± 0.004	0.003 ± 0.001	0.004 ± 0.002	0.000	-0.001	0.000
	RP+T	0.003 ± 0.001	0.004 ± 0.005	0.003 ± 0.001	0.003 ± 0.003	0.000	-0.001	0.000
	HP+T	0.004 ± 0.005	0.003 ± 0.001	0.004 ± 0.005	0.004 ± 0.004	-0.001	0.001	0.000
	Gesamt	0.004 ± 0.003	0.004 ± 0.004	0.003 ± 0.003	0.004 ± 0.003	0.000	0.000	0.000
beta-Alanine	CON	0.023 ± 0.011	0.022 ± 0.010	0.022 ± 0.009	0.022 ± 0.010	-0.002	0.000	-0.001
	RP+T	0.023 ± 0.008	0.021 ± 0.006	0.020 ± 0.008	0.021 ± 0.008	-0.002	-0.001	-0.003
	HP+T	0.022 ± 0.012	0.019 ± 0.009	0.020 ± 0.012	0.021 ± 0.011	-0.003	0.001	-0.002
	Gesamt	0.023 ± 0.011	0.021 ± 0.009	0.021 ± 0.010	0.021 ± 0.010	-0.002	0.000	-0.002
Creatinine	CON	0.308 ± 0.071	0.314 ± 0.077	0.315 ± 0.079	0.313 ± 0.075	0.006	0.001	0.007
	RP+T	0.316 ± 0.073	0.316 ± 0.063	0.316 ± 0.080	0.316 ± 0.071	0.001	-0.001	0.000
	HP+T	0.328 ± 0.075	0.323 ± 0.071	0.334 ± 0.081	0.328 ± 0.075	-0.005	0.011	0.006
	Gesamt	0.317 ± 0.073	0.318 ± 0.071	0.322 ± 0.080	0.319 ± 0.074	0.001	0.004	0.005
Glutamine	CON	2.852 ± 0.465	2.987 ± 0.564	2.910 ± 0.410	2.916 ± 0.483	0.135	-0.077	0.058
	RP+T	2.827 ± 0.489	3.053 ± 0.453	2.910 ± 0.495	2.930 ± 0.484	0.226	-0.143	0.083
	HP+T	2.898 ± 0.396	2.837 ± 0.326	2.941 ± 0.308	2.892 ± 0.345	-0.061	0.104	0.043
	Gesamt	2.860 ± 0.447	2.956 ± 0.467	2.921 ± 0.403	2.912 ± 0.441	0.095	-0.035	0.061
Glyceric acid	CON	0.030 ± 0.006	0.030 ± 0.008	0.036 ± 0.024	0.032 ± 0.015	-0.001	0.006	0.005
	RP+T	0.032 ± 0.006	0.029 ± 0.005	0.031 ± 0.019	0.031 ± 0.012	-0.003	0.002	-0.001
	HP+T	0.031 ± 0.006	0.029 ± 0.004	0.032 ± 0.016	0.031 ± 0.010	-0.002	0.003	0.001
	Gesamt	0.031 ± 0.006	0.029 ± 0.006	0.033 ± 0.020	0.031 ± 0.013	-0.002	0.004	0.002
Glycine	CON	5.084 ± 0.983	5.312 ± 1.190	5.066 ± 1.144	5.154 ± 1.105	0.227	-0.245	-0.018
	RP+T	4.969 ± 1.192	5.126 ± 1.406	4.940 ± 1.172	5.012 ± 1.250	0.157	-0.185	-0.028
	HP+T	5.332 ± 1.036	5.000 ± 1.015	5.223 ± 1.099	5.185 ± 1.050	-0.332	0.223	-0.110
	Gesamt	5.134 ± 1.065	5.153 ± 1.199	5.082 ± 1.132	5.123 ± 1.130	0.019	-0.071	-0.052
Histidine	CON	0.726 ± 0.142	0.697 ± 0.159	0.694 ± 0.120	0.705 ± 0.141	-0.029	-0.003	-0.032
	RP+T	0.688 ± 0.148	0.727 ± 0.145	0.709 ± 0.123	0.708 ± 0.138	0.040	-0.019	0.021
	HP+T	0.719 ± 0.152	0.684 ± 0.128	0.717 ± 0.127	0.707 ± 0.136	-0.036	0.034	-0.002
	Gesamt	0.713 ± 0.147	0.701 ± 0.145	0.706 ± 0.123	0.707 ± 0.138	-0.011	0.005	-0.007
Indole 3-acetate	CON	0.083 ± 0.059	0.084 ± 0.078	0.079 ± 0.071	0.082 ± 0.069	0.000	-0.005	-0.005
	RP+T	0.071 ± 0.042	0.073 ± 0.031	0.073 ± 0.039	0.072 ± 0.037	0.002	0.000	0.002
	HP+T	0.076 ± 0.050	0.070 ± 0.044	0.073 ± 0.052	0.073 ± 0.048	-0.005	0.003	-0.003
	Gesamt	0.077 ± 0.051	0.076 ± 0.056	0.075 ± 0.057	0.076 ± 0.055	-0.001	-0.001	-0.002
Isoleucine	CON	2.044 ± 0.642	2.065 ± 0.503	1.940 ± 0.508	2.016 ± 0.553	0.021	-0.125	-0.103
	RP+T	1.941 ± 0.495	1.928 ± 0.396	1.978 ± 0.479	1.949 ± 0.454	-0.013	0.050	0.038
	HP+T	1.893 ± 0.433	1.977 ± 0.463	1.979 ± 0.415	1.949 ± 0.435	0.084	0.003	0.087
	Gesamt	1.963 ± 0.536	1.995 ± 0.460	1.964 ± 0.466	1.974 ± 0.487	0.033	-0.031	0.001

Values are shown as mean±stdv and delta values are added (ΔT1, T2; ΔT2, T3 and ΔT1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 13 - Delta values between measurement time points of not significant metabolites in the different intervention groups 2

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	ΔT1, T2	ΔT2, T3	ΔT1, T3
Ketoleucine	CON	0.095 ± 0.025	0.093 ± 0.021	0.085 ± 0.018	0.091 ± 0.022	-0.002	-0.008	-0.010
	RP+T	0.090 ± 0.022	0.091 ± 0.022	0.085 ± 0.024	0.089 ± 0.022	0.000	-0.006	-0.005
	HP+T	0.093 ± 0.031	0.092 ± 0.024	0.088 ± 0.018	0.091 ± 0.025	-0.001	-0.004	-0.005
	Total	0.093 ± 0.026	0.092 ± 0.022	0.086 ± 0.020	0.090 ± 0.023	-0.001	-0.006	-0.007
Kynurenic acid	CON	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
	RP+T	0.002 ± 0.002	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
	HP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
	Gesamt	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000	0.000	0.000
Leucine	CON	3.572 ± 0.959	3.640 ± 0.796	3.446 ± 0.755	3.553 ± 0.838	0.069	-0.194	-0.126
	RP+T	3.494 ± 0.688	3.501 ± 0.702	3.603 ± 0.767	3.533 ± 0.714	0.008	0.102	0.110
	HP+T	3.445 ± 0.735	3.736 ± 0.858	3.669 ± 0.787	3.617 ± 0.797	0.291	-0.068	0.223
	Gesamt	3.507 ± 0.809	3.632 ± 0.790	3.566 ± 0.768	3.568 ± 0.788	0.126	-0.066	0.060
Lysine	CON	1.204 ± 0.167	1.236 ± 0.169	1.251 ± 0.168	1.230 ± 0.168	0.032	0.016	0.048
	RP+T	1.231 ± 0.175	1.265 ± 0.171	1.238 ± 0.186	1.244 ± 0.176	0.034	-0.027	0.007
	HP+T	1.246 ± 0.176	1.272 ± 0.182	1.278 ± 0.149	1.265 ± 0.168	0.027	0.006	0.033
	Gesamt	1.226 ± 0.172	1.256 ± 0.173	1.256 ± 0.167	1.246 ± 0.171	0.031	0.000	0.031
Methionine	CON	0.219 ± 0.043	0.229 ± 0.044	0.220 ± 0.032	0.223 ± 0.040	0.010	-0.009	0.001
	RP+T	0.216 ± 0.046	0.222 ± 0.043	0.225 ± 0.049	0.221 ± 0.046	0.006	0.003	0.008
	HP+T	0.215 ± 0.041	0.227 ± 0.048	0.231 ± 0.039	0.224 ± 0.043	0.012	0.004	0.016
	Gesamt	0.217 ± 0.043	0.226 ± 0.045	0.225 ± 0.040	0.223 ± 0.043	0.009	-0.001	0.008
Oxalic acid	CON	0.071 ± 0.036	0.073 ± 0.052	0.059 ± 0.017	0.068 ± 0.038	0.002	-0.014	-0.012
	RP+T	0.065 ± 0.016	0.068 ± 0.037	0.061 ± 0.015	0.064 ± 0.025	0.003	-0.006	-0.003
	HP+T	0.067 ± 0.024	0.066 ± 0.013	0.063 ± 0.018	0.065 ± 0.019	-0.001	-0.003	-0.004
	Gesamt	0.068 ± 0.027	0.069 ± 0.038	0.061 ± 0.017	0.066 ± 0.029	0.001	-0.008	-0.007
Oxaloacetate	CON	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	0.000	0.000	0.000
	RP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.001	0.000	0.000	0.000
	HP+T	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	0.000	0.000	0.000
	Gesamt	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	0.000	0.000	0.000
Serine	CON	0.763 ± 0.180	0.763 ± 0.214	0.753 ± 0.171	0.760 ± 0.188	0.001	-0.011	-0.010
	RP+T	0.771 ± 0.207	0.841 ± 0.243	0.807 ± 0.240	0.807 ± 0.230	0.070	-0.034	0.036
	HP+T	0.791 ± 0.240	0.750 ± 0.199	0.799 ± 0.192	0.780 ± 0.210	-0.041	0.049	0.008
	Gesamt	0.775 ± 0.208	0.781 ± 0.219	0.784 ± 0.200	0.780 ± 0.208	0.007	0.003	0.009
Succinic acid	CON	0.063 ± 0.012	0.065 ± 0.013	0.062 ± 0.013	0.063 ± 0.012	0.002	-0.003	-0.001
	RP+T	0.065 ± 0.010	0.065 ± 0.011	0.061 ± 0.017	0.064 ± 0.013	0.000	-0.004	-0.004
	HP+T	0.062 ± 0.012	0.062 ± 0.012	0.063 ± 0.012	0.062 ± 0.012	0.000	0.002	0.002
	Gesamt	0.063 ± 0.011	0.064 ± 0.012	0.062 ± 0.013	0.063 ± 0.012	0.001	-0.002	-0.001
Threonic acid	CON	0.210 ± 0.075	0.204 ± 0.073	0.181 ± 0.080	0.198 ± 0.077	-0.006	-0.023	-0.029
	RP+T	0.203 ± 0.076	0.213 ± 0.062	0.191 ± 0.068	0.202 ± 0.069	0.010	-0.023	-0.013
	HP+T	0.202 ± 0.074	0.194 ± 0.068	0.180 ± 0.084	0.192 ± 0.076	-0.008	-0.014	-0.022
	Gesamt	0.205 ± 0.075	0.203 ± 0.068	0.183 ± 0.078	0.197 ± 0.074	-0.002	-0.020	-0.022
Threonine	CON	0.559 ± 0.140	0.584 ± 0.134	0.562 ± 0.128	0.568 ± 0.133	0.026	-0.022	0.004
	RP+T	0.565 ± 0.100	0.591 ± 0.110	0.570 ± 0.106	0.575 ± 0.105	0.026	-0.021	0.005
	HP+T	0.574 ± 0.102	0.573 ± 0.117	0.593 ± 0.104	0.580 ± 0.107	-0.001	0.020	0.019
	Gesamt	0.566 ± 0.116	0.582 ± 0.121	0.575 ± 0.114	0.574 ± 0.117	0.017	-0.007	0.009
Valine	CON	8.196 ± 1.818	8.367 ± 1.513	8.002 ± 1.574	8.188 ± 1.633	0.170	-0.364	-0.194
	RP+T	7.945 ± 1.312	8.085 ± 1.332	8.192 ± 1.431	8.074 ± 1.349	0.140	0.107	0.246
	HP+T	8.129 ± 1.328	8.898 ± 1.661	8.596 ± 1.425	8.541 ± 1.498	0.768	-0.302	0.467
	Gesamt	8.101 ± 1.515	8.464 ± 1.538	8.257 ± 1.492	8.274 ± 1.518	0.363	-0.207	0.156

Values are shown as mean±stdv and delta values are added (ΔT1, T2; ΔT2, T3 and ΔT1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 14 - Deviation in percent between measurement time points of significant metabolites in the different intervention groups 1

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	% T1, T2	% T2, T3	% T1, T3
2-Oxoglutaric acid	CON	0.021 ± 0.010	0.019 ± 0.010	0.016 ± 0.006	0.018 ± 0.009	-9.8	-17.7	-25.7
	RP+T	0.019 ± 0.008	0.019 ± 0.008	0.017 ± 0.007	0.018 ± 0.008	-0.7	-14.2	-14.8
	HP+T	0.020 ± 0.009	0.018 ± 0.009	0.014 ± 0.006	0.017 ± 0.009	-13.4	-17.5	-28.6
	Total	0.020 ± 0.009	0.019 ± 0.009	0.015 ± 0.006	0.018 ± 0.008	-8.5	-16.6	-23.7
4-Hydroxyproline	CON	0.035 ± 0.019	0.036 ± 0.016	0.035 ± 0.017	0.035 ± 0.017	3.8	-3.2	0.4
	RP+T	0.041 ± 0.019	0.043 ± 0.030	0.034 ± 0.017	0.039 ± 0.023	4.3	-21.3	-18.0
	HP+T	0.042 ± 0.018	0.029 ± 0.009	0.036 ± 0.020	0.036 ± 0.017	-31.1	26.2	-13.1
	Total	0.039 ± 0.019	0.036 ± 0.020	0.035 ± 0.018	0.037 ± 0.019	-8.7	-1.5	-10.1
alpha-Tocopherol	CON	0.298 ± 0.078	0.294 ± 0.065	0.299 ± 0.061	0.297 ± 0.068	-1.1	1.4	0.3
	RP+T	0.274 ± 0.051	0.315 ± 0.072	0.285 ± 0.077	0.291 ± 0.069	15.1	-9.4	4.3
	HP+T	0.295 ± 0.063	0.269 ± 0.061	0.268 ± 0.055	0.278 ± 0.061	-8.9	-0.3	-9.2
	Total	0.290 ± 0.066	0.292 ± 0.068	0.285 ± 0.065	0.289 ± 0.066	0.6	-2.5	-1.9
Citric acid	CON	0.549 ± 0.160	0.533 ± 0.149	0.502 ± 0.114	0.528 ± 0.142	-2.9	-5.9	-8.6
	RP+T	0.550 ± 0.139	0.549 ± 0.167	0.492 ± 0.129	0.530 ± 0.147	-0.1	-10.5	-10.6
	HP+T	0.531 ± 0.138	0.489 ± 0.117	0.460 ± 0.100	0.493 ± 0.122	-7.8	-6.0	-13.4
	Total	0.543 ± 0.146	0.523 ± 0.146	0.485 ± 0.115	0.517 ± 0.138	-3.7	-7.3	-10.8
Cysteine	CON	0.137 ± 0.018	0.143 ± 0.028	0.124 ± 0.025	0.135 ± 0.025	4.4	-13.3	-9.5
	RP+T	0.133 ± 0.024	0.138 ± 0.016	0.123 ± 0.026	0.131 ± 0.023	3.2	-11.0	-8.2
	HP+T	0.143 ± 0.024	0.139 ± 0.024	0.133 ± 0.029	0.138 ± 0.026	-3.3	-4.1	-7.3
	Total	0.138 ± 0.022	0.140 ± 0.024	0.127 ± 0.027	0.135 ± 0.025	1.4	-9.6	-8.4
Cystine	CON	0.416 ± 0.142	0.448 ± 0.127	0.351 ± 0.182	0.405 ± 0.156	7.9	-21.7	-15.5
	RP+T	0.379 ± 0.134	0.448 ± 0.099	0.381 ± 0.151	0.403 ± 0.132	18.1	-14.9	0.4
	HP+T	0.410 ± 0.114	0.420 ± 0.113	0.432 ± 0.131	0.421 ± 0.119	2.4	2.9	5.4
	Total	0.403 ± 0.131	0.439 ± 0.115	0.387 ± 0.159	0.410 ± 0.137	8.8	-11.8	-4.0
Fumaric acid	CON	0.018 ± 0.004	0.017 ± 0.004	0.015 ± 0.003	0.017 ± 0.004	-6.9	-9.9	-16.1
	RP+T	0.018 ± 0.003	0.016 ± 0.003	0.015 ± 0.003	0.016 ± 0.003	-8.8	-10.2	-18.1
	HP+T	0.017 ± 0.003	0.016 ± 0.004	0.015 ± 0.003	0.016 ± 0.003	-9.6	-6.7	-15.7
	Total	0.018 ± 0.004	0.016 ± 0.004	0.015 ± 0.003	0.016 ± 0.004	-8.4	-8.9	-16.6
GABA/ 4-Hydroxyproline	CON	0.003 ± 0.002	0.004 ± 0.002	0.004 ± 0.003	0.004 ± 0.002	4.7	17.2	22.8
	RP+T	0.004 ± 0.002	0.005 ± 0.004	0.004 ± 0.002	0.004 ± 0.003	13.3	-23.7	-13.6
	HP+T	0.005 ± 0.003	0.003 ± 0.001	0.004 ± 0.003	0.004 ± 0.002	-36.5	32.6	-15.8
	Total	0.004 ± 0.002	0.004 ± 0.003	0.004 ± 0.003	0.004 ± 0.002	-8.5	6.2	-2.9
Glutamic acid	CON	0.430 ± 0.144	0.395 ± 0.157	0.407 ± 0.143	0.411 ± 0.148	-8.1	3.2	-5.2
	RP+T	0.358 ± 0.117	0.342 ± 0.118	0.378 ± 0.117	0.359 ± 0.117	-4.5	10.7	5.7
	HP+T	0.395 ± 0.103	0.377 ± 0.136	0.410 ± 0.165	0.394 ± 0.136	-4.5	8.7	3.8
	Total	0.397 ± 0.126	0.373 ± 0.140	0.400 ± 0.144	0.390 ± 0.137	-6.0	7.0	0.7
Glycolic acid	CON	0.018 ± 0.004	0.019 ± 0.004	0.019 ± 0.005	0.019 ± 0.004	2.8	1.8	4.7
	RP+T	0.019 ± 0.005	0.020 ± 0.006	0.019 ± 0.003	0.019 ± 0.005	5.9	-6.5	-1.0
	HP+T	0.019 ± 0.003	0.017 ± 0.003	0.019 ± 0.003	0.018 ± 0.003	-9.9	6.4	-4.2
	Total	0.019 ± 0.004	0.019 ± 0.004	0.019 ± 0.004	0.019 ± 0.004	-0.7	0.7	0.0

Values are shown as mean±stdv and percentage deviations are added (%T1, T2; %T2, T3 and %T1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 15 - Deviation in percent between measurement time points of significant metabolites in the different intervention groups 2

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	% T1, T2	% T2, T3	% T1, T3
Kynurenine	CON	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	11.0	-12.0	-2.4
	RP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	7.7	-3.1	4.3
	HP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.000	0.002 ± 0.001	-10.5	3.0	-7.8
	Total	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	2.7	-5.0	-2.4
Lactic acid	CON	16.923 ± 2.117	16.396 ± 2.271	15.701 ± 1.798	16.340 ± 2.114	-3.1	-4.2	-7.2
	RP+T	17.261 ± 2.327	15.811 ± 1.689	15.714 ± 2.181	16.262 ± 2.180	-8.4	-0.6	-9.0
	HP+T	17.328 ± 2.697	16.158 ± 2.055	15.950 ± 2.144	16.479 ± 2.374	-6.8	-1.3	-8.0
	Total	17.157 ± 2.370	16.146 ± 2.039	15.788 ± 2.017	16.364 ± 2.218	-5.9	-2.2	-8.0
Malic acid	CON	0.010 ± 0.003	0.009 ± 0.003	0.008 ± 0.002	0.009 ± 0.003	-7.9	-10.8	-17.8
	RP+T	0.009 ± 0.003	0.009 ± 0.002	0.008 ± 0.003	0.009 ± 0.003	-9.7	-6.8	-15.9
	HP+T	0.009 ± 0.003	0.008 ± 0.003	0.007 ± 0.002	0.008 ± 0.003	-10.6	-5.3	-15.4
	Total	0.009 ± 0.003	0.008 ± 0.003	0.008 ± 0.002	0.009 ± 0.003	-9.3	-7.9	-16.5
Ornithine	CON	1.382 ± 0.418	1.329 ± 0.347	1.329 ± 0.319	1.347 ± 0.362	-3.8	0.0	-3.9
	RP+T	1.290 ± 0.341	1.295 ± 0.309	1.231 ± 0.283	1.272 ± 0.310	0.3	-4.9	-4.6
	HP+T	1.378 ± 0.275	1.211 ± 0.224	1.246 ± 0.311	1.278 ± 0.279	-12.1	2.9	-9.6
	Total	1.354 ± 0.352	1.279 ± 0.301	1.273 ± 0.307	1.302 ± 0.322	-5.5	-0.5	-6.0
Phenylalanine	CON	0.691 ± 0.128	0.712 ± 0.146	0.685 ± 0.111	0.696 ± 0.129	2.9	-3.8	-0.9
	RP+T	0.686 ± 0.112	0.707 ± 0.118	0.703 ± 0.128	0.699 ± 0.119	3.0	-0.6	2.4
	HP+T	0.660 ± 0.100	0.718 ± 0.118	0.731 ± 0.107	0.703 ± 0.112	8.8	1.8	10.7
	Total	0.679 ± 0.114	0.712 ± 0.128	0.706 ± 0.116	0.699 ± 0.120	4.9	-1.0	3.9
Proline	CON	6.380 ± 2.677	7.125 ± 3.130	5.974 ± 1.918	6.493 ± 2.645	11.7	-16.1	-6.4
	RP+T	6.212 ± 2.667	6.128 ± 1.885	6.201 ± 2.164	6.180 ± 2.237	-1.4	1.2	-0.2
	HP+T	6.858 ± 2.184	7.433 ± 2.249	7.196 ± 2.527	7.162 ± 2.315	8.4	-3.2	4.9
	Total	6.492 ± 2.509	6.939 ± 2.563	6.451 ± 2.254	6.628 ± 2.448	6.9	-7.0	-0.6
Pyruvic acid	CON	0.608 ± 0.214	0.520 ± 0.186	0.451 ± 0.177	0.526 ± 0.202	-14.6	-13.2	-25.9
	RP+T	0.567 ± 0.205	0.484 ± 0.211	0.398 ± 0.192	0.483 ± 0.212	-14.6	-17.8	-29.8
	HP+T	0.611 ± 0.270	0.546 ± 0.170	0.517 ± 0.188	0.558 ± 0.215	-10.8	-5.2	-15.4
	Total	0.597 ± 0.230	0.518 ± 0.188	0.458 ± 0.190	0.524 ± 0.211	-13.3	-11.6	-23.3
Tryptophan	CON	2.209 ± 0.557	2.402 ± 0.418	2.306 ± 0.374	2.306 ± 0.459	8.7	-4.0	4.4
	RP+T	2.080 ± 0.501	2.378 ± 0.391	2.285 ± 0.525	2.248 ± 0.487	14.3	-3.9	9.9
	HP+T	2.220 ± 0.401	2.315 ± 0.442	2.373 ± 0.429	2.303 ± 0.425	4.3	2.5	6.9
	Total	2.175 ± 0.492	2.366 ± 0.417	2.323 ± 0.437	2.288 ± 0.456	8.8	-1.8	6.8
Tyrosine	CON	2.595 ± 0.469	2.706 ± 0.506	2.685 ± 0.458	2.662 ± 0.477	4.3	-0.8	3.5
	RP+T	2.535 ± 0.394	2.750 ± 0.538	2.642 ± 0.431	2.642 ± 0.462	8.5	-3.9	4.2
	HP+T	2.482 ± 0.488	2.755 ± 0.533	2.725 ± 0.455	2.654 ± 0.504	11.0	-1.1	9.8
	Total	2.539 ± 0.454	2.735 ± 0.520	2.686 ± 0.446	2.653 ± 0.480	7.7	-1.8	5.8
Urea	CON	23.163 ± 7.442	24.103 ± 6.347	24.478 ± 7.940	23.915 ± 7.234	4.1	1.6	5.7
	RP+T	23.166 ± 5.489	24.400 ± 5.558	24.829 ± 6.530	24.132 ± 5.857	5.3	1.8	7.2
	HP+T	23.722 ± 6.945	29.991 ± 7.564	30.446 ± 6.678	28.053 ± 7.654	26.4	1.5	28.3
	Total	23.352 ± 6.701	26.170 ± 7.067	26.588 ± 7.591	25.370 ± 7.251	12.1	1.6	13.9

Values are shown as mean±stdv and percentage deviations are added (%T1, T2; %T2, T3 and %T1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 16 - Deviation in percent between measurement time points of not significant metabolites in the different intervention groups 1

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	% T1, T2	% T2, T3	% T1, T3
3-Methyl-2-oxopentanoic acid	CON	0.218 ± 0.052	0.214 ± 0.044	0.199 ± 0.038	0.210 ± 0.046	-1.9	-7.2	-9.0
	RP+T	0.207 ± 0.045	0.207 ± 0.042	0.194 ± 0.050	0.203 ± 0.046	0.0	-6.1	-6.1
	HP+T	0.209 ± 0.057	0.201 ± 0.042	0.198 ± 0.035	0.203 ± 0.046	-3.5	-1.7	-5.2
	Gesamt	0.212 ± 0.052	0.208 ± 0.043	0.197 ± 0.040	0.206 ± 0.046	-1.9	-5.1	-6.9
Alanine	CON	8.859 ± 1.960	9.046 ± 2.024	8.681 ± 1.879	8.862 ± 1.944	2.1	-4.0	-2.0
	RP+T	8.450 ± 1.717	8.967 ± 1.419	8.813 ± 2.147	8.743 ± 1.780	6.1	-1.7	4.3
	HP+T	8.759 ± 1.947	8.690 ± 1.784	8.696 ± 1.738	8.715 ± 1.808	-0.8	0.1	-0.7
	Gesamt	8.707 ± 1.878	8.903 ± 1.775	8.724 ± 1.899	8.778 ± 1.848	2.3	-2.0	0.2
Arginine	CON	0.045 ± 0.010	0.046 ± 0.014	0.047 ± 0.009	0.046 ± 0.011	3.6	2.3	5.9
	RP+T	0.042 ± 0.009	0.047 ± 0.009	0.045 ± 0.009	0.045 ± 0.009	11.6	-4.9	6.2
	HP+T	0.045 ± 0.007	0.045 ± 0.007	0.047 ± 0.007	0.045 ± 0.007	-0.8	4.4	3.6
	Gesamt	0.044 ± 0.008	0.046 ± 0.011	0.046 ± 0.008	0.045 ± 0.009	4.3	0.8	5.2
Asparagine	CON	0.161 ± 0.033	0.165 ± 0.040	0.159 ± 0.023	0.162 ± 0.033	2.1	-3.6	-1.6
	RP+T	0.166 ± 0.035	0.175 ± 0.029	0.167 ± 0.029	0.169 ± 0.031	5.2	-4.7	0.3
	HP+T	0.166 ± 0.027	0.172 ± 0.032	0.176 ± 0.033	0.171 ± 0.031	3.7	2.4	6.1
	Gesamt	0.164 ± 0.031	0.170 ± 0.035	0.167 ± 0.029	0.167 ± 0.032	3.5	-1.9	1.6
Aspartic acid	CON	0.003 ± 0.001	0.004 ± 0.004	0.003 ± 0.001	0.004 ± 0.002	13.7	-20.2	-9.3
	RP+T	0.003 ± 0.001	0.004 ± 0.005	0.003 ± 0.001	0.003 ± 0.003	12.7	-15.6	-4.9
	HP+T	0.004 ± 0.005	0.003 ± 0.001	0.004 ± 0.005	0.004 ± 0.004	-23.3	24.5	-4.5
	Gesamt	0.004 ± 0.003	0.004 ± 0.004	0.003 ± 0.003	0.004 ± 0.003	-0.9	-5.4	-6.3
beta-Alanine	CON	0.023 ± 0.011	0.022 ± 0.010	0.022 ± 0.009	0.022 ± 0.010	-6.7	1.3	-5.4
	RP+T	0.023 ± 0.008	0.021 ± 0.006	0.020 ± 0.008	0.021 ± 0.008	-7.8	-5.3	-12.7
	HP+T	0.022 ± 0.012	0.019 ± 0.009	0.020 ± 0.012	0.021 ± 0.011	-12.4	5.6	-7.5
	Gesamt	0.023 ± 0.011	0.021 ± 0.009	0.021 ± 0.010	0.021 ± 0.010	-8.9	0.7	-8.2
Creatinine	CON	0.308 ± 0.071	0.314 ± 0.077	0.315 ± 0.079	0.313 ± 0.075	1.8	0.3	2.1
	RP+T	0.316 ± 0.073	0.316 ± 0.063	0.316 ± 0.080	0.316 ± 0.071	0.3	-0.3	0.0
	HP+T	0.328 ± 0.075	0.323 ± 0.071	0.334 ± 0.081	0.328 ± 0.075	-1.5	3.4	1.9
	Gesamt	0.317 ± 0.073	0.318 ± 0.071	0.322 ± 0.080	0.319 ± 0.074	0.2	1.2	1.4
Glutamine	CON	2.852 ± 0.465	2.987 ± 0.564	2.910 ± 0.410	2.916 ± 0.483	4.7	-2.6	2.0
	RP+T	2.827 ± 0.489	3.053 ± 0.453	2.910 ± 0.495	2.930 ± 0.484	8.0	-4.7	3.0
	HP+T	2.898 ± 0.396	2.837 ± 0.326	2.941 ± 0.308	2.892 ± 0.345	-2.1	3.7	1.5
	Gesamt	2.860 ± 0.447	2.956 ± 0.467	2.921 ± 0.403	2.912 ± 0.441	3.3	-1.2	2.1
Glyceric acid	CON	0.030 ± 0.006	0.030 ± 0.008	0.036 ± 0.024	0.032 ± 0.015	-2.7	21.0	17.8
	RP+T	0.032 ± 0.006	0.029 ± 0.005	0.031 ± 0.019	0.031 ± 0.012	-8.8	7.3	-2.1
	HP+T	0.031 ± 0.006	0.029 ± 0.004	0.032 ± 0.016	0.031 ± 0.010	-7.9	12.2	3.3
	Gesamt	0.031 ± 0.006	0.029 ± 0.006	0.033 ± 0.020	0.031 ± 0.013	-6.3	14.1	7.0
Glycine	CON	5.084 ± 0.983	5.312 ± 1.190	5.066 ± 1.144	5.154 ± 1.105	4.5	-4.6	-0.4
	RP+T	4.969 ± 1.192	5.126 ± 1.406	4.940 ± 1.172	5.012 ± 1.250	3.2	-3.6	-0.6
	HP+T	5.332 ± 1.036	5.000 ± 1.015	5.223 ± 1.099	5.185 ± 1.050	-6.2	4.5	-2.1
	Gesamt	5.134 ± 1.065	5.153 ± 1.199	5.082 ± 1.132	5.123 ± 1.130	0.4	-1.4	-1.0
Histidine	CON	0.726 ± 0.142	0.697 ± 0.159	0.694 ± 0.120	0.705 ± 0.141	-4.0	-0.5	-4.4
	RP+T	0.688 ± 0.148	0.727 ± 0.145	0.709 ± 0.123	0.708 ± 0.138	5.8	-2.6	3.0
	HP+T	0.719 ± 0.152	0.684 ± 0.128	0.717 ± 0.127	0.707 ± 0.136	-5.0	4.9	-0.3
	Gesamt	0.713 ± 0.147	0.701 ± 0.145	0.706 ± 0.123	0.707 ± 0.138	-1.6	0.7	-0.9
Indole 3-acetate	CON	0.083 ± 0.059	0.084 ± 0.078	0.079 ± 0.071	0.082 ± 0.069	0.4	-5.8	-5.5
	RP+T	0.071 ± 0.042	0.073 ± 0.031	0.073 ± 0.039	0.072 ± 0.037	3.5	-0.6	2.9
	HP+T	0.076 ± 0.050	0.070 ± 0.044	0.073 ± 0.052	0.073 ± 0.048	-7.2	3.6	-3.9
	Gesamt	0.077 ± 0.051	0.076 ± 0.056	0.075 ± 0.057	0.076 ± 0.055	-1.3	-1.4	-2.7
Isoleucine	CON	2.044 ± 0.642	2.065 ± 0.503	1.940 ± 0.508	2.016 ± 0.553	1.0	-6.0	-5.1
	RP+T	1.941 ± 0.495	1.928 ± 0.396	1.978 ± 0.479	1.949 ± 0.454	-0.7	2.6	1.9
	HP+T	1.893 ± 0.433	1.977 ± 0.463	1.979 ± 0.415	1.949 ± 0.435	4.4	0.1	4.6
	Gesamt	1.963 ± 0.536	1.995 ± 0.460	1.964 ± 0.466	1.974 ± 0.487	1.7	-1.6	0.1

Values are shown as mean±stdv and percentage deviations are added (%T1, T2; %T2, T3 and %T1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 17 - Deviation in percent between measurement time points of not significant metabolites in the different intervention groups 2

Metabolites	Group	Baseline (T1)	8 weeks (T2)	17 weeks (T3)	Total	% T1, T2	% T2, T3	% T1, T3
Ketoleucine	CON	0.095 ± 0.025	0.093 ± 0.021	0.085 ± 0.018	0.091 ± 0.022	-1.9	-8.8	-10.5
	RP+T	0.090 ± 0.022	0.091 ± 0.022	0.085 ± 0.024	0.089 ± 0.022	0.3	-6.1	-5.8
	HP+T	0.093 ± 0.031	0.092 ± 0.024	0.088 ± 0.018	0.091 ± 0.025	-0.9	-4.8	-5.6
	Total	0.093 ± 0.026	0.092 ± 0.022	0.086 ± 0.020	0.090 ± 0.023	-0.9	-6.7	-7.5
Kynurenic acid	CON	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	-12.9	20.0	4.5
	RP+T	0.002 ± 0.002	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	-14.0	2.9	-11.5
	HP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	-4.4	7.4	2.7
	Gesamt	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	-10.6	10.8	-0.9
Leucine	CON	3.572 ± 0.959	3.640 ± 0.796	3.446 ± 0.755	3.553 ± 0.838	1.9	-5.3	-3.5
	RP+T	3.494 ± 0.688	3.501 ± 0.702	3.603 ± 0.767	3.533 ± 0.714	0.2	2.9	3.1
	HP+T	3.445 ± 0.735	3.736 ± 0.858	3.669 ± 0.787	3.617 ± 0.797	8.4	-1.8	6.5
	Gesamt	3.507 ± 0.809	3.632 ± 0.790	3.566 ± 0.768	3.568 ± 0.788	3.6	-1.8	1.7
Lysine	CON	1.204 ± 0.167	1.236 ± 0.169	1.251 ± 0.168	1.230 ± 0.168	2.7	1.3	4.0
	RP+T	1.231 ± 0.175	1.265 ± 0.171	1.238 ± 0.186	1.244 ± 0.176	2.7	-2.2	0.5
	HP+T	1.246 ± 0.176	1.272 ± 0.182	1.278 ± 0.149	1.265 ± 0.168	2.1	0.5	2.6
	Gesamt	1.226 ± 0.172	1.256 ± 0.173	1.256 ± 0.167	1.246 ± 0.171	2.5	0.0	2.5
Methionine	CON	0.219 ± 0.043	0.229 ± 0.044	0.220 ± 0.032	0.223 ± 0.040	4.7	-4.0	0.5
	RP+T	0.216 ± 0.046	0.222 ± 0.043	0.225 ± 0.049	0.221 ± 0.046	2.7	1.2	3.9
	HP+T	0.215 ± 0.041	0.227 ± 0.048	0.231 ± 0.039	0.224 ± 0.043	5.4	1.9	7.5
	Gesamt	0.217 ± 0.043	0.226 ± 0.045	0.225 ± 0.040	0.223 ± 0.043	4.4	-0.5	3.8
Oxalic acid	CON	0.071 ± 0.036	0.073 ± 0.052	0.059 ± 0.017	0.068 ± 0.038	2.3	-19.2	-17.3
	RP+T	0.065 ± 0.016	0.068 ± 0.037	0.061 ± 0.015	0.064 ± 0.025	4.7	-9.5	-5.2
	HP+T	0.067 ± 0.024	0.066 ± 0.013	0.063 ± 0.018	0.065 ± 0.019	-1.5	-4.0	-5.4
	Gesamt	0.068 ± 0.027	0.069 ± 0.038	0.061 ± 0.017	0.066 ± 0.029	1.7	-11.5	-10.0
Oxaloacetate	CON	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	16.3	1.2	17.8
	RP+T	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.001	-5.1	4.9	-0.5
	HP+T	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	21.1	1.2	22.6
	Gesamt	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	11.5	2.1	13.9
Serine	CON	0.763 ± 0.180	0.763 ± 0.214	0.753 ± 0.171	0.760 ± 0.188	0.1	-1.4	-1.3
	RP+T	0.771 ± 0.207	0.841 ± 0.243	0.807 ± 0.240	0.807 ± 0.230	9.1	-4.0	4.7
	HP+T	0.791 ± 0.240	0.750 ± 0.199	0.799 ± 0.192	0.780 ± 0.210	-5.2	6.5	1.0
	Gesamt	0.775 ± 0.208	0.781 ± 0.219	0.784 ± 0.200	0.780 ± 0.208	0.9	0.3	1.2
Succinic acid	CON	0.063 ± 0.012	0.065 ± 0.013	0.062 ± 0.013	0.063 ± 0.012	2.9	-4.7	-2.0
	RP+T	0.065 ± 0.010	0.065 ± 0.011	0.061 ± 0.017	0.064 ± 0.013	0.4	-6.9	-6.5
	HP+T	0.062 ± 0.012	0.062 ± 0.012	0.063 ± 0.012	0.062 ± 0.012	0.0	2.8	2.8
	Gesamt	0.063 ± 0.011	0.064 ± 0.012	0.062 ± 0.013	0.063 ± 0.012	1.2	-2.9	-1.7
Threonic acid	CON	0.210 ± 0.075	0.204 ± 0.073	0.181 ± 0.080	0.198 ± 0.077	-2.8	-11.4	-13.8
	RP+T	0.203 ± 0.076	0.213 ± 0.062	0.191 ± 0.068	0.202 ± 0.069	5.0	-10.6	-6.2
	HP+T	0.202 ± 0.074	0.194 ± 0.068	0.180 ± 0.084	0.192 ± 0.076	-4.1	-7.0	-10.8
	Gesamt	0.205 ± 0.075	0.203 ± 0.068	0.183 ± 0.078	0.197 ± 0.074	-1.0	-9.7	-10.6
Threonine	CON	0.559 ± 0.140	0.584 ± 0.134	0.562 ± 0.128	0.568 ± 0.133	4.6	-3.7	0.7
	RP+T	0.565 ± 0.100	0.591 ± 0.110	0.570 ± 0.106	0.575 ± 0.105	4.6	-3.5	0.9
	HP+T	0.574 ± 0.102	0.573 ± 0.117	0.593 ± 0.104	0.580 ± 0.107	-0.2	3.6	3.4
	Gesamt	0.566 ± 0.116	0.582 ± 0.121	0.575 ± 0.114	0.574 ± 0.117	2.9	-1.3	1.6
Valine	CON	8.196 ± 1.818	8.367 ± 1.513	8.002 ± 1.574	8.188 ± 1.633	2.1	-4.4	-2.4
	RP+T	7.945 ± 1.312	8.085 ± 1.332	8.192 ± 1.431	8.074 ± 1.349	1.8	1.3	3.1
	HP+T	8.129 ± 1.328	8.898 ± 1.661	8.596 ± 1.425	8.541 ± 1.498	9.5	-3.4	5.7
	Gesamt	8.101 ± 1.515	8.464 ± 1.538	8.257 ± 1.492	8.274 ± 1.518	4.5	-2.4	1.9

Values are shown as mean±stdv and percentage deviations are added (%T1, T2; %T2, T3 and %T1, T3); Missing values were excluded from the analysis. CON (control group); RP+T (recommended protein group + resistance training); HP+T (high protein group + resistance training). T1 (Baseline; start of intervention); T2 (8 weeks after start of intervention); T3 (17 weeks after start of intervention); Blue cells show a decrease of the metabolite, orange cells an increase.

Table 18 - Correlations between protein intake and all selected metabolites

Correlation					
Protein_gKGd_Ph1		Protein_gKGd_Ph2		Protein_gKGd_Ph3	
Metabolite	r	Metabolite	r	Metabolite	r
T1_Phenylalanine	0.022	T2_Phenylalanine	0.065	T3_Phenylalanine	0.056
T1_Threonicacid	0.086	T2_Threonicacid	0.096	T3_Threonicacid	0.089
T1_Tyrosine	0.015	T2_Tyrosine	0.160	T3_Tyrosine	-0.017
T1_GABA/4Hydroxyproline	0.089	T2_GABA/4Hydroxyproline	-0.149	T3_GABA/4Hydroxyproline	-0.081
T1_2Oxoglutaricacid	0.118	T2_2Oxoglutaricacid	-0.014	T3_2Oxoglutaricacid	0.040
T1_Alanine	-0.101	T2_Alanine	-0.095	T3_Alanine	-0.059
T1_Arginine	0.047	T2_Arginine	0.070	T3_Arginine	0.005
T1_Asparagine	0.156	T2_Asparagine	0.210	T3_Asparagine	0.220
T1_Asparticacid	-0.087	T2_Asparticacid	-0.080	T3_Asparticacid	0.127
T1_betaAlanine	0.049	T2_betaAlanine	-0.089	T3_betaAlanine	-0.112
T1_Citricacid	-0.116	T2_Citricacid	-0.242	T3_Citricacid	-0.165
T1_Cystine	-0.071	T2_Cystine	-0.155	T3_Cystine	0.152
T1_Fumaricacid	-0.040	T2_Fumaricacid	-0.113	T3_Fumaricacid	-0.135
T1_Glutamicacid	-0.100	T2_Glutamicacid	-0.105	T3_Glutamicacid	-0.034
T1_Glutamine	0.000	T2_Glutamine	-0.058	T3_Glutamine	0.060
T1_Glycine	-0.022	T2_Glycine	-0.071	T3_Glycine	-0.008
T1_Glycolicacid	0.040	T2_Glycolicacid	-0.131	T3_Glycolicacid	-0.095
T1_Histidine	0.094	T2_Histidine	0.045	T3_Histidine	0.138
T1_Isoleucine	0.003	T2_Isoleucine	-0.063	T3_Isoleucine	0.024
T1_Lacticacid	-0.033	T2_Lacticacid	-0.027	T3_Lacticacid	-0.026
T1_Leucine	0.028	T2_Leucine	0.049	T3_Leucine	0.082
T1_Lysine	0.094	T2_Lysine	0.140	T3_Lysine	0.033
T1_Malicacid	-0.148	T2_Malicacid	-0.185	T3_Malicacid	-0.111
T1_Methionine	0.133	T2_Methionine	0.061	T3_Methionine	0.126
T1_Ornithine	0.420	T2_Ornithine	-0.075	T3_Ornithine	-0.061
T1_Oxaloacetate	0.062	T2_Oxaloacetate	0.052	T3_Oxaloacetate	-0.084
T1_Proline	-0.041	T2_Proline	-0.027	T3_Proline	0.156
T1_Pyruvicacid	-0.173	T2_Pyruvicacid	-0.013	T3_Pyruvicacid	0.053
T1_Serine	0.155	T2_Serine	-0.028	T3_Serine	0.124
T1_Succinicacid	0.028	T2_Succinicacid	-0.003	T3_Succinicacid	0.006
T1_Threonine	0.173	T2_Threonine	-0.007	T3_Threonine	0.080
T1_Tryptophan	-0.017	T2_Tryptophan	0.079	T3_Tryptophan	0.076
T1_Urea	0.103	T2_Urea	0.303	T3_Urea	0.244
T1_Valine	-0.052	T2_Valine	0.096	T3_Valine	0.122
T1_3Methyl2oxopentanoicacid	-0.117	T2_3Methyl2oxopentanoicacid	-0.080	T3_3Methyl2oxopentanoicacid	0.011
T1_4Hydroxyproline	0.147	T2_4Hydroxyproline	-0.194	T3_4Hydroxyproline	-0.023
T1_Cysteine	-0.161	T2_Cysteine	-0.133	T3_Cysteine	0.138
T1_Glycericacid	0.185	T2_Glycericacid	0.014	T3_Glycericacid	-0.163
T1_Oxalicacid	0.047	T2_Oxalicacid	-0.020	T3_Oxalicacid	0.087
T1_alphaTocopherol	0.091	T2_alphaTocopherol	-0.060	T3_alphaTocopherol	-0.220
T1_Indole3acetate	-0.082	T2_Indole3acetate	-0.032	T3_Indole3acetate	-0.108
T1_Kynurenicacid	-0.073	T2_Kynurenicacid	-0.042	T3_Kynurenicacid	-0.099
T1_Kynurenine	-0.071	T2_Kynurenine	-0.201	T3_Kynurenine	-0.172
T1_Creatinine	-0.062	T2_Creatinine	0.128	T3_Creatinine	-0.014
T1_Ketoleucine	-0.089	T2_Ketoleucine	-0.011	T3_Ketoleucine	0.093

Ph = phases 1-3 (Ph1 = Baseline, start of intervention (T1); Ph2 = after 8 weeks of intervention (T2) and Ph3 = after 17 weeks of intervention (T3). Colored cells signal a weak to moderate linear relationship (0.2 < r ≤ 0.5 or -0.2 < r ≤ -0.5). r = Correlation coefficient. gKGd = g per kg body weigth per day. Missing value handling: PAIRWISE, EXCLUDE