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Changes in GSH and taurine levels in respose to different cage beddings and dietary patterns

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3. List of abbreviations

ACN	Acetonitrile
Ad lib	Ad libitum: mice with free access to food
Ad lib Antibiotics	Ad libitum fed mice, treated with antibiotics
Ad lib C	Ad lib mice kept on cellulose bedding
Ad lib CC	Ad lib mice kept on corncob bedding
Ad lib W	Ad lib mice kept on wooden bedding
CR	Mice restricted to 75% of normal food intake
CR Antibiotics	Mice restricted to 75% of normal food intake, treated with antibiotics
CR C	Mice restricted to 75% of normal food intake kept on cellulose bedding
CR CC	Mice restricted to 75% of normal food intake kept on cornb bedding
CR W	Mice restricted to 75% of normal food intake kept on wooden bedding
Ctrl Ad lib	Control group, ad libitum fed mice
Ctrl	Control group
FA	formic acid
FT Ad lib-R	Ad libitum fed mice, treated with antibiotics, received faecal transplant from ad libitum fed mice
FT CR-R	Ad libitum fed mice, treated with antibiotics, received faecal transplant from mice restricted to 75% of normal food intake
GSH	glutathione

GSTA3	Glutathione S-Transferase Alpha 3
H2O	water
HPLC-MS	High pressure liquid chromatography and mass spectrometry
LTD	Low taurine diet
MeOH	methanol
MGST1	Microsomal Glutathione S-Transferase 1
NTD	Normal taurine diet
ON	overnight fasted mice
ON C	overnight fasted mice kept on cellulose bedding
ON CC	overnight fasted mice kept on corncob bedding
ON E	overnight fasted mice kept without bedding
ON G	overnight fasted mice kept on grids (no access to the cage bottom)
ON W	overnight fasted mice kept on wooden bedding
qPCR	quantitative polymerasechainreaction
RT	retention time
SEM	standard error of the mean
St	standard
Tau	taurine
TauT	Taurine transporter

4. Abstract

Different diets, especially caloric restriction and fasting have shown positive influences on oxidative stress levels before. As taurine and GSH also have been shown to be involved in mechanisms of the redox-system, it was interesting to verify, if different dietary patterns might influence these levels as well. It has been shown before that caloric restriction increases taurine levels and decreases GSH levels in jejunum, whereas it shows the opposite effect in feces. Therefore, an experiment with antibiotic treated mice was performed, to see if there is any influence of microbiota on taurine and GSH-levels. To investigate into mechanisms, taurine transporter and glutathione transferases expression have been measured as well. We failed to prove any significant influences gene expressions of taurine transporters and glutathione transferases, however it was shown that microbiota has an influence on GSH and taurine levels. This proves that microbiota may have an influence on antioxidative responses.

5. Introduction

The experiments performend for this theses are considering many different aspects. The following sub-points aim to give an overview about the topics, that are relevant for the performed experiments, such as the different diets the mice were treated with, as well as the biomarkers and metabolites that are relevant for the outcomes of the experiments.

5.1.Diets

5.1.1. Caloric Restriction

Caloric restriction means in most cases, a reduction of 10-50% of normal food intake. It has shown positive impacts on healthy lifespan and aging in divers model organisms. (1) It has also been shown, that it increases glutathione reductase activity and GSH/GSSG ratios. (2) It was proven, that caloric restriction reduces antioxidative stress and it is suggested, that that this is the reason for the positive effects of caloric restriction. (2–4) It is proven, that there are positive effects of caloric restriction on diabetes, inflammatory gastrointestinal diseases and that intestinal mucosa and microbiota responses to caloric restriction. (2,5,6)

5.1.2. Fasting diets

Fasting is part of most religions in the world. As metabolic diseases dramatically increase, restrictive diets tend to get more popular again. (7) Obesity is one of the major risk factors for these diseases, especially for elderly persons. Obesity and aging in combination are risk factors for serious health problems, resulting in increasing the risk of disease and death. (8). Fasting comes in many different forms, such as intermitted fasting (for example: 12:12, 14:10, 18:6) were people only eat in a specific time frame of the day, or eat one day-fast the other, where people only consume water or non-caloric herbal teas every other day. As fasting has shown positive influence on obesity, cardiometabolic risks and inflammation, we wanted to know, if it also has an influence on taurine and GSH levels in the body. (8–10)

5.1.3. Fibre enriched diets

High fibre intake has shown a positive influence on diabetes management, coronary heart disease risk, overweight/obesity and overall chronic diseases. (11–14) Fibre supplementation also showed a positive influence on weight loss. (15) As fecal markers related to inflammation have shown to be improved by dietary fibre invention in obese

patients, we were if there is also an influence on taurine transport in the gastrointestinal tract, due to different fibre contents. (16)

5.1.4. Cage bedding

Mice generally should be housed on cage bedding, as it is important for maintaining their body temperature, as a nesting material, to absorb urine and for their general wellbeing. (17,18) However, as mice tend to eat their cage bedding, especially when on a caloric restricted diet, it has shown influences on metabolic outcomes.(17)

5.1.Metabolites of interest

5.1.1. Taurine

The main source of taurine (2-aminoethanesufonic acid) is dietary protein(19). It was described to be an essential amino acid for cats, and their bigger feline cospecies but it is also known to be a conditionally unalienable amino acid for humans. (20) Taurine is essentially involved in several biological processes as membrane stabilisation, immunity and reproduction. (20,21) It has been proven that high dietary taurine has antiapoptotic and antiatherogenic effects. (22) The beneficial effects of taurine are not tissue specific, however there are elevated levels in inflammatory cells and other tissues exposed to greater levels of oxidants, what suggests that taurine plays an role in inflammation in response to oxidative stress. (23) It is the most abundant amino acid in humans bodies. (23) Taurine has been proved to be effective to treat congestive heart failure and is approved as a therapy for this condition in Japan, however it also might be worth investigating into the possibilities of taurine in treatments of other diseases. (24) A previous study has shown, that the expression of antibacterial and inflammatory factors is regulated by restrictive diets what leads to the question, if taurine levels are influenced by restricted diets as well. (6) In mice (other than in human) 95% of bile acids are conjugated to taurine, whereas only 5% of bile acids are conjugated to glycine. (25,26)

5.1.2. GSH

GSH (Glutathione) is a compound of three peptides: glutamate, cysteine and glycine. (27) It is known as a major endogenous antioxidant and its functions are associated to many cell life activities that contribute to the development of many human diseases, such as cardiovascular-, immune- and aging diseases, as well as diabetes. (27,28) In mammalian cells it is the main endogenous nonprotein antioxidant, as well as the most abundant low-

molecular-weight thiol. (28,29) During aging, GSH levels progressively decline, however, it plays an important role in reducing oxidative damages, preventing apoptosis and maintaining the intracellular redox balance. (28–31)

5.1.Biomarkers of interest

5.1.1. TauT

Taurine transport is mainly controlled by ubiquitous expression of the taurine transporter (TauT), which is sodium/chlorine-dependent and located on the plasma membrane. (32) The taurine transporter is influenced by divers cellular stimuli, such as electrochemical charge, ionic environment and pH changes. (32) Studies with taurine-transporter-knockout mice have shown the importance of taurine and its membrane transport as an "anti-aging-protein" and for the development and maintenance of normal organ morphology and functions. (33,34) The absence of TauT and consequently taurine in the tissue leads to cardiac and skeletal muscle abnormality. (35)

5.1.2. MGST1 and GSTA3

MGST1 (Microsomal Glutathione S-Transferase 1) and GSTA3 (Glutathione S-Transferase Alpha 3) are part of the glutathione transferase family. They are involved in the protection against DNA damage, maintenance of cell integrity and oxidative stress by catalysing glutathione-conjugations to electrophilic substrates. (36) Besides being involved in the degradation of tyrosine and the biosynthesis of leukotrienes, prostaglandins, testosterone and progesterone, they also inactivate secondary metabolites released by oxidative stress. (37) Studies show, that MGST1 might be a promising target in the treatment and prevention of preeclampsia, which is a common pregnancy-specific syndrome, linked to the PI3K/AKT/mTOR pathway. (38) GSTA3 has also shown a significantly decreased expression in mice when put on a caloric restricted diet or an intermitted fasting diet. (39) GSTs in general are a group of enzymes, which are responsible for the catalysation of GSH to various electrophilic substrates, creating water-soluble complexes that can be excreted by urine and bile. (25,40)

6. Aim

Many studies have shown the importance of taurine in body tissue, especially in antioxidative processes. (6,23) Also, GSH has been proven to be important for many biological processes, including maintaining the intracellular redox-balance. (28) Dietary

restriction has shown antioxidant and anti-inflammatory effects and to improve life span and health span in several animal models. (3,4,6,25,41,42) As all aspects seem to have an influence on health span, healthy aging and antioxidative mechanisms it is time to find out, if there are any correlations between the different factors. In addition to previously mentioned points, fibre has shown to improve some inflammatory markers. (16) To investigate into the impact of different "inflammatory diets" on taurine and GSH, we measured contents of said metabolites in the intestine and some additional tissues. To get an idea about how the transport of GSH and taurine is influenced, we also attempted to measure some known transporters in the intestine.

7. Methods

7.1. List of materials and equipment

Disposables:

- Eppendorf-tubes: Microcentrifuge tubes, 1.5 ml and 2ml; Eppendorf Austria GmbH (Vienna, Austria)
- Tips: 20µl, 100µl, 200µl, 1000µl epT.I.P.S.® 384; Eppendorf

Equipment

- Microsoft Excel
- Pipettes: 2µl, 0,5-10µl, 10-100µl, 20-200µl, 100-1000µl; Eppendorf Refercene® 2, Eppendorf
- Electronic Pipettes: 0,5 – 10 µL, 5-100µl, Eppendorf Xplorer®; EppendorfBlock heater:
    - Block Heater, 2 Block, Digital, SBH130D, SBH200D; Stuart® via BioCote
    - Liebisch Labtherm 2009 Digital Block Heater
    - Thermostat 5320 Dry Bath Heat Block; Eppendorf; Netheler-Hinz GmBH; Hamburg
- Concentrator: Concentrator plus/ Vacufuge® plus; Eppendorf
- High-precision scale: Sartorius ENTRIS 224I-1S
- NanoDrop: NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer; ThermoFisher Scientific

- Centrifuges.:
    - Minicentrifuge: Biozym
    - Thermo Scientific™ Megafuge™ 8; fisher scientific
    - Thermo Scientific™ Megafuge™ 8; fisher scientific
- Steady Shaker: Fisherbrand™ Sea Star orbital shaker package; fisher scientific
- Vortex: Heidolph Reax 2000
- Water bath: GFL 1002; Müller-Scherr; Linz
- Homogenizer: Precellys®24 Tissue Homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France
- qPCR-system: applied biosystems®, QuantStudio™ 6 Flex Real-Time PCR System, Life Technologies Holding (Singapore)
- HPLC-MS: LCMS-8040 Liquid Chromatograph Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan)
    - Liquid Chromatograph LC-20 AD
    - Mass-Spectrometer: Ultimate 3000 (Thermo Fischer Scientific, Waltham, MA, US) and a micrOTOF-Q II (Bruker Daltonics, Bremen, Germany)
    - Autosampler SIL-20AC HT
    - Liquid Chromatograph LC-20 AD
    - Column Oven CTO-20AC
    - High Pressure Switching Valve FCV-20AH2o UV/VIS Detector SPD-20A
    - Column: Atlantis T3 3 µm column (2.1x150 mm, Waters, Milford, MA, USA)
    - LabSolutions (Software)

RNA-Extraction and qPCR

- RNA-Extraction-Kit:
  peqGOLD HP Total RNA Kit; peQlab; VWR International GmBH (Erlangen)
    - peqGOLD Collection Tubes
    - peqGOLD Perfectbind DNA removing columns
    - peqGOLD PerfectBind® RNA Columns
    - peqGOLD RNA Lysis buffer T

    - peqGOLD RNA Wash buffer I
    - peqGOLD RNA Wash buffer II
    - peqGOLD RNA-free water
- PCR-plates: Frame Star® 384 Well Skirted PCR Plates, Roche Style; 4titude®; Brooks Life Sciences
- qPCR Seal: 4titude; Brooks Life Sciences
- Syringe needles: Braun Sterican Gr. 14
- Syringes: Braun Omnifix®-F 1ml
- SybrGreen-Mastermix: Applied Biosystems, Life Technologies, Carlsbad, CA, USA
- Primer: Roche, Mannheim, Germany
- Reverse transcription: SuperScript® II Reverse Transcriptase (Invitrogen™, Life Technologies, Carlsbad, CA, USA)

Taurine and GSH extraction and HPLC-MS

- Starlab Impact Restistant Screw Cap Tubes: 2.0ml
- HPLC-vials: 2mL; Sigma-Aldrich Chemie GmBH
- Glutathione: Sigma-Aldrich, St. Louis, MO, USA
- Taurine: Sigma-Aldrich, St. Louis, MO, USA
- Ethanol: EMPLURA® Ethanol absolute
- Methanol: ROTISOLV® HPLC Gradient Grade; ROTH
- Acetonitrile: ROTISOLV® HPLC Gradient Grade; ROTH
- Formic Acid: ROTIPURAN® ≥98 %, p.a., ACS; ROTH
- HPLC water: ROTISOLV® HPLC Gradient Grade, ROTH

Mice and treatment

- Male C57Bl/6 mice: Janvier Labs (Le Genest-Saint-Isle, France)
- Diets:
    - V153x R/M-H auto diet from SSNIFF-Spezialdiäten GmbH (Soest, Germany)
    - V1535 R/M-H Extrudate; SSNIFF-Spezialdiäten GmbH (Soest, Germany)

    - Arbocel Performance Small, pellets, cellulose; J. Rettenmaier & Söhne GmbH + Co KG (Vienna, Austria)
    - Lignocel select, poplar, cubic; J. Rettenmaier & Söhne GmbH + Co KG (Vienna, Austria)
    - RehoFix MK 3500, maize grains; J. Rettenmaier & Söhne GmbH + Co KG (Vienna, Austria)
- Antibiotics: Sigma-Aldrich, Vienna, Austria
- Beddings:
    - wooden (Lignocel select from J. Rettenmaier & Söhne GmbH + Co KG; Vienna, Austria)
    - corncob (RehoFix MK 3500 from J. Rettenmaier & Söhne GmbH + Co KG; Vienna, Austria)
    - cellulose (Arborcel Performance Small from J. Rettenmaier & Söhne GmbH + Co KG; Vienna, Austria)

7.2.Experimental setups

If not stated differently, the following parameter apply for each experiment. Male mice of the inbreed type C57Bl/6 from Janviere Ing Labs were purchased. They were housed on wooden bedding in a 12h-light/12h-dark cycle. Since the outcome of measured parameter can vary between different genders, the experiments were accomplished with male mice, to keep the quantity of needed animals small. For the success of the experiments it was important that mice were kept under standard specific-pathogenfree (SPF) conditions. If not stated differently, they were fed with V152x R/M-H auto diet from SSNIFF-Spezialdiaeten GmbH (Soest, Germany) and had free water access. When 12 weeks old, they were assigned to the different experimental groups. All groups had free water access. Ad libitum (Ctrl Ad lib) mice were housed with free access to food. CR mice received a diet which was reduced to 75% of normal food intake for 14 days.

7.2.1. Experiment 1: The influence of overnight fasting on GSH- and taurine levels

Control group (Ad lib)	Experimental Group (ON)
Wooden bedding (W)	Wooden bedding (W)
Cellulose bedding (C)	Cellulose bedding (C)
Corncob bedding (CC)	Corncob bedding (CC)
	No bedding – empty cages (E)
	On grids – no access to cage bottom (G)

Table 7-1: Different groups of experiment 1, two main diet groups housed on different beddings

Mice were divided into two main groups: ad libitum (Ad lib) and overnight fasted (ON) mice. Additionally, the groups were divided into three subgroups according to bedding type. There were three different beddings, wooden bedding (W), cellulose bedding (C) and corncob bedding (CC). For ON mice, there were two more subgroups. One subgroup was housed in an empty cage without any bedding (E), the second group was housed on grids, with no access to the cage bottom (G) to prevent coprophagy. Each group consisted of ten animals, due to loss in dissection, the end groups included between six and ten samples. Ad lib mice had unlimited access to food and water. They served as a control group in the study. ON mice were provides with an unlimited amount of food during the day. In the evening, the food was removed but there was still free access to water. They were housed without food for 16 hours, afterwards they regained free access to food. The process was repeated a week later. After the night fasting, dissection took place in the morning. The dissection process was the same for all mice groups. They were euthanized using an isoflurane overdose and the blood was drawn by cardiac puncture. To separate plasma from blood cells, 20 µl/ml aprotinin, 10 µl/ml EDTA and 10 µl/ml dipeptidyl peptidase were mixed with the blood and the mixture was centrifuged at 4°C for 10 minutes at 3.600xG. Tissues (liver, brain, muscle, kidney, heart) were extracted. Scrapings of small intestine mucosa as well as the content of cecum were collected. Liquid nitrogen was used to snap freeze the samples, before they were stored at -80°C for further analysis. (17)

7.2.2. Experiment 2: The influence of caloric restriction on GSH- and taurine levels

Control Group (Ad lib)	Experimental Group (CR)
Wooden bedding (W)	Wooden bedding (W)
Cellulose bedding (C)	Cellulose bedding (C)
Corncob bedding (CC)	Corncob bedding (CC)

Table 7-2: Different groups Experiment 2, two diet groups housed on different beddings

There were two main groups: Ad libitum (Ad lib) feed and mice fed a caloric restricted diet (CR). As an additional factor, the groups were divided into three groups each, separated by the cage bedding, they were housed on. The beddings used and the treatment of Ad lib mice went according to experiment 1. For the CR groups, calorie intake was limited to 75% of normal food intake for 14 days. After the experimental time, mice were euthanized using an isoflurane overdose. Samples were collected and stored as descripted in section 7.2.1. (17)

7.2.3. Experiment 3: The influence of microbiota on GSH- and taurine levels

Normal Microbiota	No Microbiota	Transplanted Microbiota
Ad libitum (Ctrl Ad lib)	Ad libitum mice treated with antibiotics (Ad lib Antibiotics)	Faecal transplant from Ctrl Ad lib mice (FT Ad lib-R)
Caloric Restriction (CR)	Caloric restricted mice treated with antibiotics (CR Antibiotics)	Faecal transplant from CR mice (FT CR-R)

Table 7-3: Different Groups Experiment 3, three different microbiota schemes, two different diet groups

These two groups served as control groups for the further experiments. They also provided the feces for the microbiota transplant. To eliminate microbiota, four groups of mice where treated with antibiotics. A mix of neomycin 1g/l, metronidazole 1g/l, ampicillin 1g/l and vancomycin 0,5g/l was used. The mice were repeatedly gavaged 200µl of the cocktail. The groups that maintained without microbiota were gavaged previously mentioned cocktail 3 times in 14 days. Mice that received a microbiota transplant were gavaged said antibiotic cocktail five and three days before the microbiota transplant took place. Microbiota transplant was performed twice in a two-day-interval. For the transplant, fresh feces of Ctrl Ad lib or CR mice was vortexed with sterile PBS,

centrifuged at 1000 x g for three minutes. The supernatant was gavaged to the mice immediately. Antibiotics treated mice without microbiota transplant were divided in two groups, one of the groups had free access to food (Ad lib Antibiotics), the other one was limited to 75% of usual food intake for 14 days (CR Antibiotics). Mice were euthanized seven days after the first gavage by an isoflurane overdose. Samples were taken and stored as descripted in section 7.2.1. (43)

7.2.4. Experiment 4: The influence of fibre content on GSH- and taurine levels

Control Group	Fibre Groups
Standard chow (std. chow)	5% fibre
	20% sol. Fibre (20% soluble fibre)
	20% cellulose

Table 7-4: Different Groups experiment 4, four groups, different fibre content in each diet

The mice were divided into four groups. All groups were fed ad libitium. The control group (std. chow) received standard chow, which contains 5% of fibre. The other groups received chow with other fibre sources and contents. The 5% fibre group was fed a chow, that also contains 5% of fibre but the fibre source is pectin and cellulose. The other groups received either chow with 20% soluble fibre or 20% cellulose. After 14 days of treatment, mice were euthanized by an isoflurane overdose. Samples were taken and stored as descripted in section 7.2.1.

7.2.5. Experiment 5: The effect of dietary taurine on GSH- and taurine levels in mice

Control groups	Normal taurine diet (NTD)	Low taurine diet (LTD)
Ad libitum (Ad lib)	Ad libitium (Ctrl NTD)	Ad libitum (Ctrl LTD)
Caloric restricted (CR)	Caloric restricted (CR NTD)	Caloric restricted (CR LTD)

Table 7-5: Different groups of experiment 5, three different contents of taurine in food, each feed either ad libitum or restricted to 75% of normal food intake

Mice were divided into 6 groups. There were three ad libitum groups, which had unlimited access to food and water and three groups that received a caloric restricted diet, that was limited to 75% of normal food intake. One Ad lib group and one CR group

received standard chow, these groups served as control groups for further analysis. There were two further diets, normal taurine diet and low taurine diet. Table 7-6 shows the differences between the diets.

	Low taurine diet (LTD)	Normal taurine diet (NTD)
Casein	10	10
Cornstarch	52,3	51,8
Sucrose	10	10
Maltodextrin	12,5	12,5
Soybean oil	5	5
Fibre mix	3	3
Vitamin mix w/o choline	1	1
Mineral mix	6	6
Choline	0,2	0,2
Taurine	**0**	**0,5**

Table 7-6: The difference between low taurine and normal taurine diet, ingrediencies g/100g; Low taurine diet without any extra taurine

7.2.6. Experiment 6: Human Data: The Effect of fasting on GSH- and taurine levels in human feces

In this study, 55 participants took part. All of them had to provide written consent. There were 16 males and 35 females, the age range was 23-75 years (mean 45,25 years). Before the study, weight (75,9 ± 12,85 kg) and BMI (25,93 ± 3,93 kg/m²) were documented. To the fasting group, 24 participants were assigned, 31 to the non-fasting group. Dropouts only occurred in the fasting group, which narrowed the group down to 20 people. The non-fasting group did not have any lifestyle changes, the fasting group was supported by a fasting coach. They were required to follow Buchinger fasting and advised to drink 2-3L of non-energy liquids, like water or herbal tea, daily. At noon, they got a fruit juice, which was organic and freshly squeezed. In the evening, they ate vegetable soup (only the liquid). Participants were asked to provide stool samples before the start of the study, as well as directly after the fasting break. The samples were saved at -80°C until further analysis. (7)

7.3.Measurements HPLC-MS

7.3.1. Sample preparation

An adapted protocol form the method of Budinska et al. and Ito et al was used. (44,45)

7.3.1.1. Plasma samples

For the extraction of GSH and taurine out of plasma samples, 50 µl of plasma were taken. 150 µl of methanol was added, then the mixture was vortexed and placed in ice. The ice box with the samples were put on the steady shaker, which was set to 150 RPM for 10 minutes. Afterwards the diluted plasma samples were centrifuged at four degrees and 12.000 g for 10 minutes. 100µl of the supernatant was transferred to a new 1,5ml column. The supernatant was evaporated in the concentrator for 18 minutes at 60°C until completely dry. The sample was dissolved in 50µl methanol, vortexed very thoroughly and the mixture was transferred to the vials for measurement with HPLC-MS.

7.3.1.2. Intestinal samples

For the extraction of GSH and taurine out of intestinal samples, the mucosa was cut into pieces of 6 to 10mg and put into 1,5ml Eppendorf-tubes. Weights were protocolled and multiplied by 9, to get the amount of methanol in µl that has to be added later. For the following process, it was important, that the sample was at the bottom of the Eppendorf-tube to achieve equal treatment for all samples. If that was not the case, samples were pushed down to the bottom of the tubes using a needle. To disrupt the tissue, a freeze-dry-method was used. A water bath was set to 37°C, and a box of dry ice was prepared. 70% ethanol was added to the dry ice. The samples were placed alternately in the water bath and the dry ice for 10 seconds. This process was repeated five times. Subsequently, the calculated amount of methanol (-20°C) was added to each sample. A screwdriver was used to disrupt the tissue. Therefore, it was snipped against the Eppendorf-tubes, until the sample was completely dissolved. Afterwards, the samples were vortexed very well. The mixture was centrifuged at 4°C and 12.000g for 10 minutes, then the supernatant was transferred to new 1,5ml Eppendorf-tubes. In order not to have any tissue residues, the supernatant was centrifuged again at 4°C and 12.000g for 10 minutes. The supernatant was transferred to vials for measurement.

7.3.1.3. Liver samples

For the extraction of GSH and taurine out of liver samples, the liver was cut into pieces of 6-10mg. The weight was protocolled and the pieces were put into precellies. The measured weight was multiplied by 9, to get the amount of methanol in µl. The required quantity of methanol was added to the precellies and the precellies were placed in the precelly shaker, which was set to 5000 2x30-15. Afterwards, the precellies were vortexed and centrifuged at 4000g and 4°C for 5 minutes. The supernatant was transferred to 1,5ml Eppendorf-tubes and these were centrifuged at 12.000g and 4°C for 10 minutes. The supernatant was transferred to vials for measurement.

7.3.2. Preparation of standard series

To calculate the concentrations of taurine and GSH in the tissues, taurine- and GSH-standards were prepared. Therefore, 2 mg of taurine were weighed into an 2ml Eppendorf. Because neither GSH, nor taurine are dissolvable in methanol, 500µl H2O were added to dissolve the taurine. Then 500µl methanol were added, to create a standard A with a concentration of 2mg/ml. The mixture was vortexed properly. Standard series was prepared according to Table 7-7.

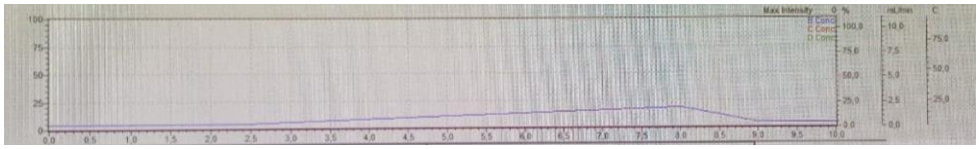
Standard Nr	Methanol µl	Adjunct	Concentration mg/ml	Concentration µg/ml
Standard A	500 (+500µl H2O)	2mg taurine/GSH	2	2000,0
Standard 1	900	100µl St A	0,2	200,0
Standard 2	900	100µl St 1	0,02	20,0
Standard 3	500	500µl St 2	0,01	10,0
Standard 4	500	500µl St 3	0,005	5,0
Standard 5	500	500µl St 4	0,0025	2,5
Standard 6	800	200µl St 4	0,001	1,0
Standard 7	500	500µl St 6	0,0005	0,5
Standard 8	500	500µl St 7	0,00025	0,25
Standard 9	500	500µl St 8	0,000125	0,125
Standard 10	500	500µl St 9	0,0000625	0,0625
Standard 11	500	500µl St 10	0,00003125	0,0313
Standard 12	500	500µl St 11	0,000015625	0,0156
Standard 13	500	500µl St 12	7,8125E-06	0,0078
Standard 14	500	500µl St 13	3,90625E-06	0,0039

Table 7-7: standard preparation for HPLC-MS measurements, depending on the measured tissue, the according standard-concentrations were measured

7.3.3. Measurement

To measure the levels of taurine, GSH and different conjugates, a LCMS-8040 from Shimadzu was used with an Atlantis® T3 3µm column.
For the measurements, two different solutions were prepared: solvent A and solvent B. For solvent A, H2O (1L) was mixed with 0,1% (1ml) of formic acid. For solvent B, Acetonitrile (1L) was mixed with 0,1% (1ml) of formic acid.

The measurement started with 100% of solvent A. The concentration of solvent B was increased up to 20% at minute 8. Afterwards, the concentration of solvent B dropped to 0% at minute 10. After 10 minutes, the measurement was complete, the next sample was ready for measurement.



Picture 7-1: Ratio of solvent A to solvent B in course of the measurement, starting with 100% of solvent A, concentration of solvent B increasing up tp 20% at minute 8, dropping back again to 0% until the end of the measurement

At the beginning of each measurement, GSH and taurine standards were measured, starting with the lowest concentration. The measurement of the standards was followed by a washing step. Afterwards, samples were measured. Here, a washing step was conducted every 3 samples. Because taurine and GSH are not stable forever, once the measurement of the samples was finished, the standards were measured again, to create valuable standard curves for calculation.

After measurement, analyzation and integration of relevant peaks was conducted. With the applied method, GSH, taurine and conjugates of both are expected to show at RT of 2.5 minutes. Values were documented for further calculations in Microsoft Excel.

7.4.Measurements qPCR

7.4.1. Sample preparation

7.4.1.1. RNA-extraction

RNA was extracted out of jejunum or rather ileum samples. Therefore, PEQGOLD Total RNA Kit (S-Line) was used. The samples were cut into the required pieces and placed in 1.5 ml Eppendorf-tubes. To disrupt the sample, 200 µl of Lysis Buffer were added and the disruption was achieved by repeatedly drawing up with the pipette. Following, a syringe was used to disrupted the tissue further. The mixture was drawn up several times, until the tissue was completely lysed. The syringe was used to transfer the lysate to the green DNA-removing columns. These were placed into 2ml collection tubes, included in the kit. The DNA-removing columns with the lysate were centrifuged at 10.000g for one minute at room temperature. Afterwards DNA-removing columns were discarded. To the lysate 200µl of 70% ethanol were added. Both were mixed using the pipette and drawing up the mixture several times. Then the mixture was transferred directly to the membrane of the orange RNA-binding columns. These were placed into included 2ml collection tubes and centrifuged at 10.000g at room temperature for one minute. The RNA has now

bound to the membrane, the flow-through and collection tubes were discarded. RNA-binding columns were now placed onto new 2ml collection tubes and 500µl of Wash Buffer I were added to the RNA-binding columns. The columns were centrifuged again at 10.000g for one minute at room temperature. The flow-through was discarded, the 2ml collection tubes were reused. 500µl of Wash Buffer II was added to the RNA-binding columns. These were centrifuged at 10.000g for one minute at room temperature. After the flow-through was disposed the washing step with Wash Buffer II was repeated. Afterwards, the flow-through was discarded again and the RNA-binding columns were dried by centrifuging them for 2 minutes at 10.000g and room temperature. RNA-binding columns were placed into 1,5ml Eppendorf-tubes and 50ul of RNAse-free Water was applied directly to the membrane. The tubes were centrifuged at 6.000g and room temperature for one minute, to elute the RNA from the membrane. Eppendorf-tubes were placed on ice, RNA-binding columns were discarded. The concentration of RNA was measured using Nano Drop and the samples were stored at -80°C until further use.

7.4.1.2. Precipitation

Based on the measurement, it was decided whether the RNA samples were pure enough for reverse transcription. If this was not the case, precipitation was performed to purify the 50µl of extracted RNA-sample. For precipitation, 5µl of sodium acetate and 150 µl of 75% ethanol (4°C) were added. The samples were vortexed and stored at -20°C for at least two hours. The samples were centrifuged at 4°C and 12.000g for 15 minutes. The supernatant was discarded and the Eppendorf-tubes were dried by carefully removing ethanol with caution to not interrupt the loose RNA pellet at the bottom of the tube. The pellet was subsequently reconstituted in 50µl RNAse free water and concentration was conducted. Samples were then stored at -80°C until further use.

7.4.1.3. Reverse transcription

Reverse transcription was performed using qScript Reverse Transcriptase. RNA concentrations were harmonized for all reactions by diluting samples of higher RNA concentrations with nuclease free water. First of all, the required amount of water was pipetted to new 1,5 ml Eppendorf-tubes. Secondly, the pure RNA sample was pipetted to the wall of the Eppendorf. 5µl of MasterMix were pipetted to the other side of the

Eppendorf. When all samples were prepared, samples were centrifuged shortly to combine all the reagents and start the reaction. To mix the ingredients properly, several flicks against the Eppendorf-tubes were performed. The samples were put to block heaters of different heat stages. Five minutes at 22°C, followed by 30 minutes at 42°C. To stop the reaction, samples were placed at 85°C for 5 minutes. Temperatures were controlled using a mercury thermometer. After the last heating step, the samples were put on racks to cool down for 5 minutes, afterwards samples were placed on ice. Subsequently 380µl of RNAse free water were added. Samples were stored at -20°C until further use.

7.4.1. Measurement

For the detection of gene transcription levels, qPCR was performed. The following three genes were investigated: MGST1, GSTA3 and TauT. Furthermore, Eef1α1 was detected as a housekeeping gene for latter calculation of 2^ddCTs. Therefore, 384-well-plates were prepared. cDNA samples were pipetted in triplicates for each gene to measure. Plates were sealed and stored at -20°C until detection. On the day of measurement, MasterMixes for each gene were prepared according to Table 7-8. 8µl of MasterMix were pipetted on each well. Plates were sealed and put into qPCR-machine for measurement. CT values were documented into a Microsoft Excel sheet for further analysis.

MasterMix preparation	µl/well
SybrGreen	5
Primer forward	0,3
Primer reverse	0,3
RNAse free water	2,4
total	8

Table 7-8: MasterMix preparation, 8 µl per well of MasterMix, 2µl of sample, 10µl of measuring volume

7.5.Analysis

7.5.1. Analysis HPLC-MS results

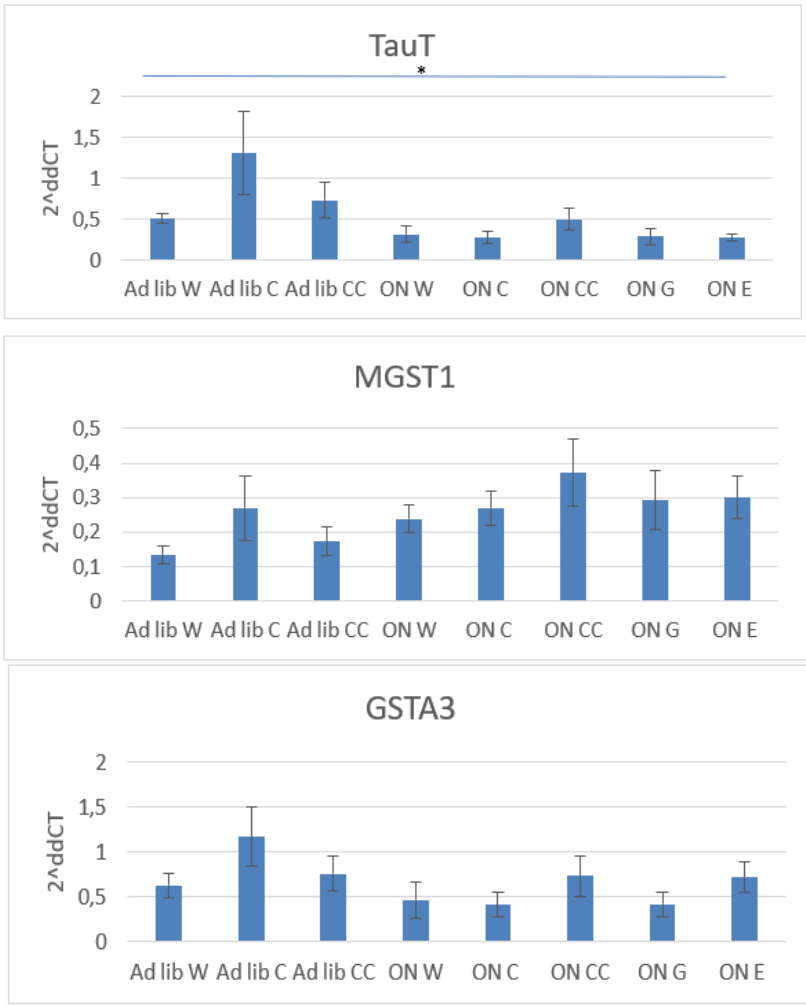
The AUCs calculated were organized and standard curves were drawn, based on the means of the standard measurements before and after sample measurements. Final concentrations of taurine and GSH in the tissues were calculated using the standard curves. AUCs and concentrations were analysed by two-tailed Student's t-tests and Bonferroni corrected based on the number of groups, α-level was defined as 5%.

7.5.2. Analysis qPCR results

CT values were organized and assigned to the matching samples. Samples were organized according to their groups. All CT levels for the measured genes were normalized based on the CT-values of Eef1α1. ΔΔCTs were calculated. Final ΔΔCTs were analysed using two-tailed Student's t-tests and Bonferroni corrected based on the number of groups, α-level was defined as 5%.

8. Results

8.1. Overnight fasting



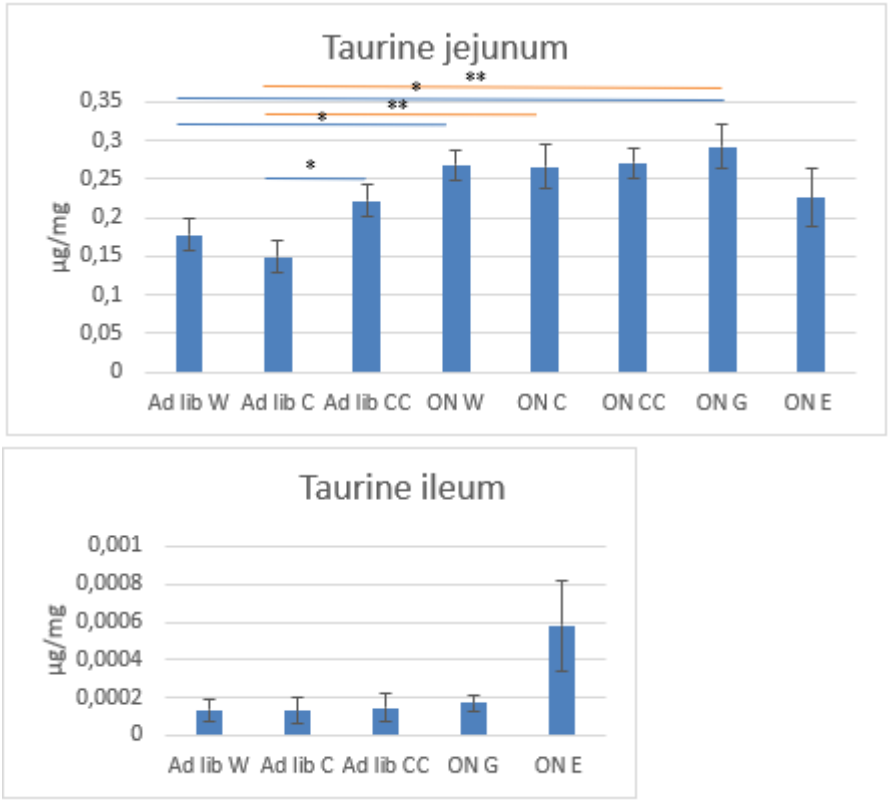
* non significant difference after Bonferroni correction

** significant difference after bonferroni correction

Graph 8-1: Relative mRNA expression of TauT, MGST1 and GSTA3 measured in ileum of ON mice, there is no statistically significant influence of ON on gene expression

In case of the ON experiment, RNA for the measurement of gene expression was extracted out of jejunum of mice. The outcomes are presented in Graph 8-1. In the expression of TauT, there was a difference between Ad lib W (0.51±0.06) and ON E (0.28±0.05) based on a p-level of 0.05, however, the difference did not remain significant

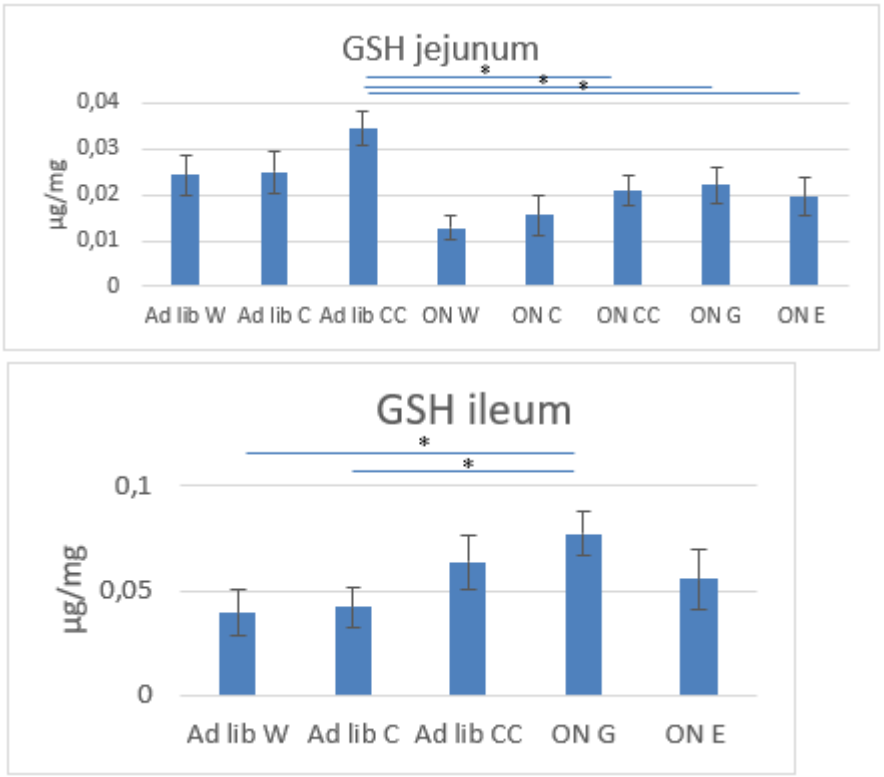
after Bonferroni correction. MGST1 and GSTA3 did not show any statistically significant differences in gene expression between the groups. We were not able to prove that ON has a statistically significant influence on the expression of taurine and glutathione transporters. Nonetheless, there seems to be a strong trend in lower expression of TauT and GSTA3, and higher expression of MGST1 in ON groups.



Graph 8-2: Taurine levels in jejunum and ileum after ON compared to ad libitum group, ON statistically significant increases taurine levels in jejunum

For further investigation taurine levels in jejunum and ileum were measured. The results are presented in Graph 8-2. Taurine contents in ileum did not show any statistically significant differences between the groups. However, in jejunum there were statistically significant higher taurine contents in the jejunum of mice of the ON G (0.29±0.02µg/mg) group compared to Ad lib C (0.15±0.02µg/mg). The p-value after Bonferroni correction was defined as $p<0{,}00625$. There was also a statistically significant higher amount of taurine in the jejunum of ON C mice (0.27±0.03µg/mg), compared to the group of mice which was fed ad libitium and housed on the same bedding (Ad lib C) (0.15±0.02µg/mg). Other differences did not remain statistically significant after Bonferroni correction. These results indicate, that even if the expression of taurine transporters is not statistically

significant affected by ON, there are significant higher taurine levels in jejunum while fasting. The intake of cage bedding also seems to have an influence on taurine levels.

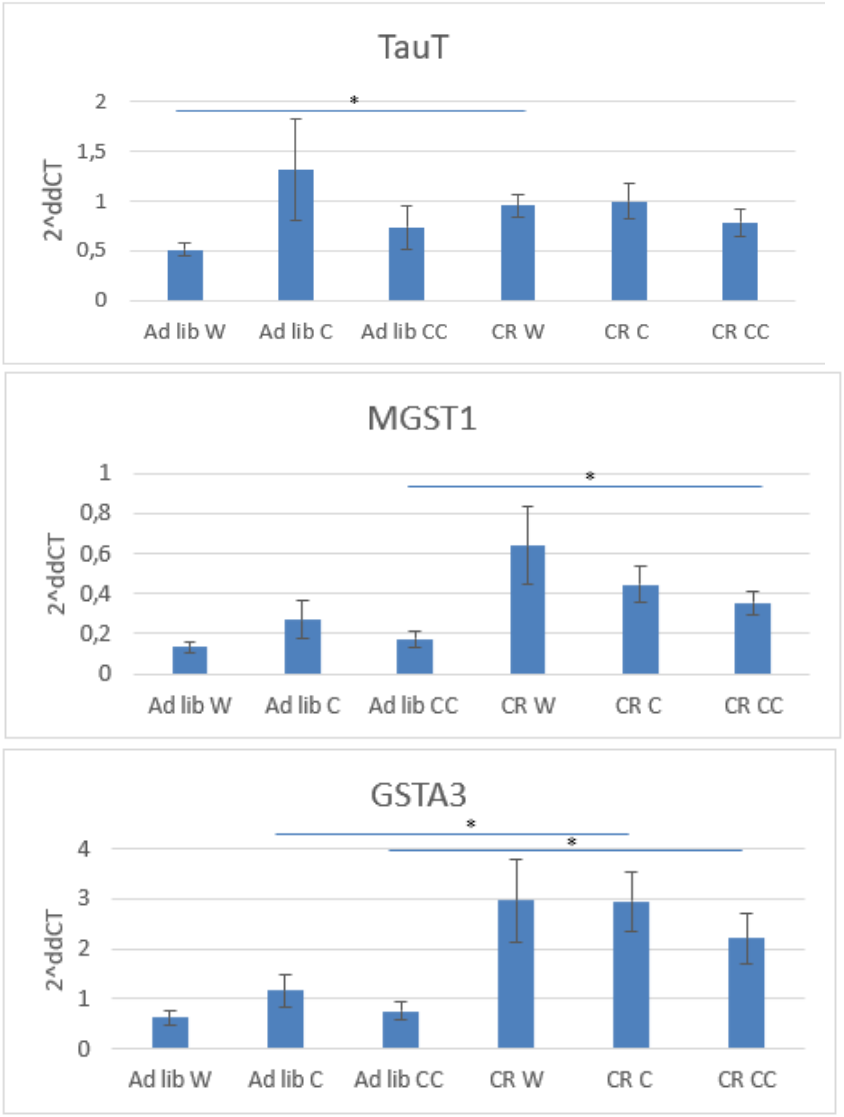


Graph 8-3: GSH-levels in ileum and jejunum after ON, ON has no statistically significant influence on GSH levels in jejunum and ileum

* *

To compare with the influence on taurine levels, GSH levels were measured in jejunum and ileum. The outcomes are shown in Graph 8-3. In jejunum, there was a slightly lower content of GSH in ON G (0.022±0.004µg/mg), ON E (0.020±0.004µg/mg) and ON CC (0.021±0.003µg/mg) mice, compared to Ad lib CC mice (0.034±0.004µg/mg). However, after Bonferroni correction, the differences did not remain significant. In ileum, a similar effect was shown. GSH content was slightly higher in ON G mice (0.037±0.010µg/mg) compared to Ad lib W group (0.039±0.011µg/mg) and Ad lib C group (0.042±0.010µg/mg). Unfortunately, these differences did not remain statistically significant after Bonferroni correction (p-value < 0,01). These results indicate, that GSH levels show the opposite reaction to ON than taurine.

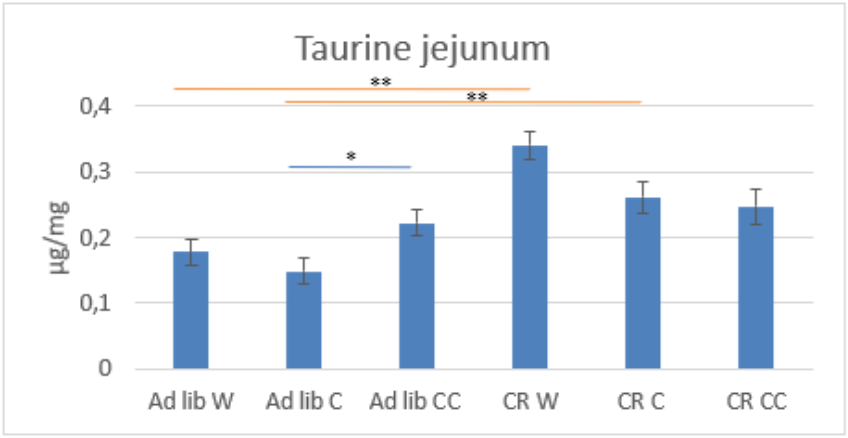
8.2.Caloric restriction

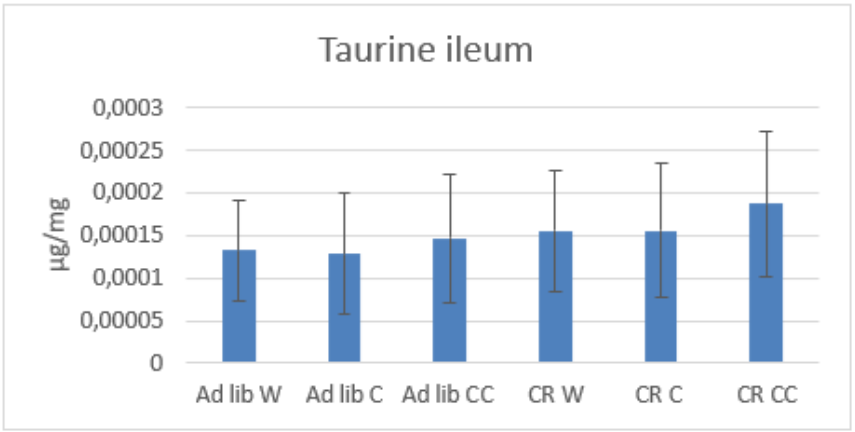


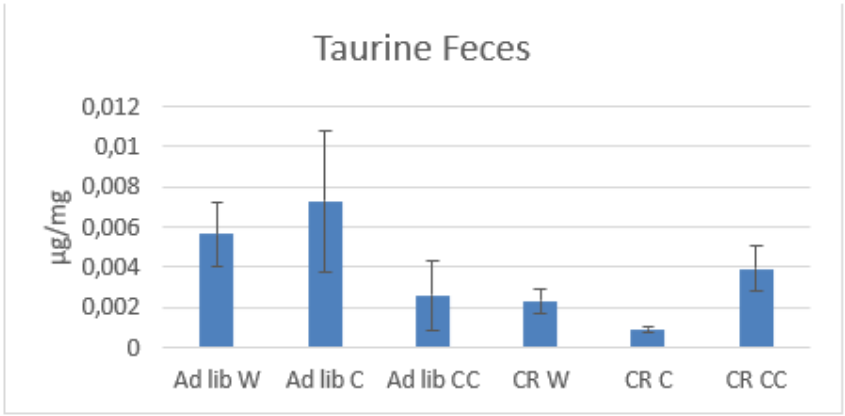
Graph 8-4: Relative mRNA expression of TauT, MGST1 and GSTA3 measured in ileum after CR, there is no statistically significant influence of CR on gene expression

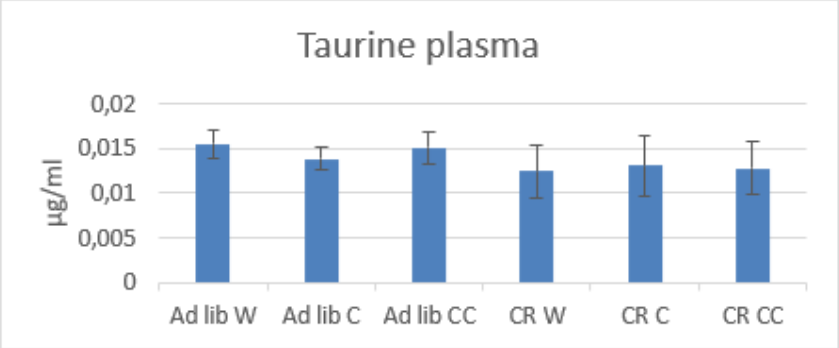
For the CR experiment, RNA was extracted out of jejunum of the mice. Results of the measurements of gene expression are presented in Graph 8-4. There was a slightly higher expression of TauT in Ad lib W group (0.51±0.06) than in CR W group (0.95±0.11). Also, MGST1 seemed to be higher expressed in CR CC group (0.35± 0.06) compared to Ad lib CC (0.17±0.04) to some extent. There were also higher 2^ddCTs of GSTA3 in CR C (2.95±0.59) and CR CC mice (2.21±0.50), compared to the Ad lib groups housed on the same bedding (Ad lib C: 1.17±0.33; Ad lib CC: 0.76±0.19). Unfortunately, after Bonferroni correction, none of the differences remained statistically significant (p-value

< 0,0083). However, the data shows that CR might lead to higher expressions of taurine transporters and glutathione transferases. Maybe bigger groups would have shown statistically significant differences.



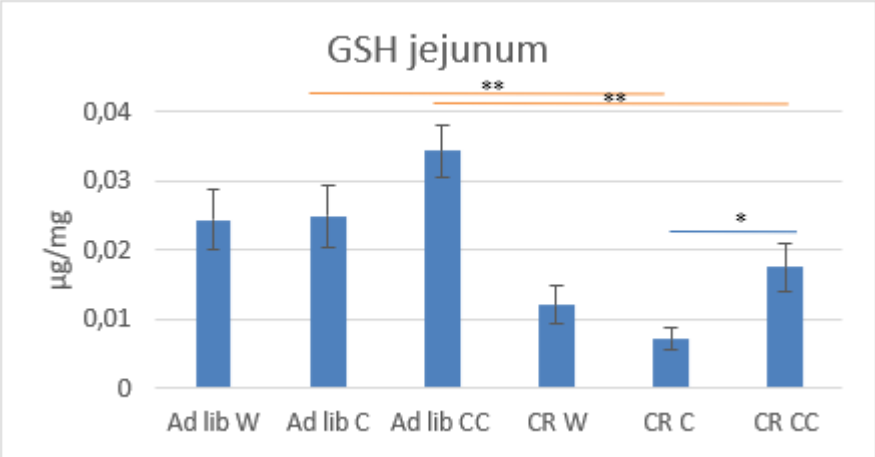


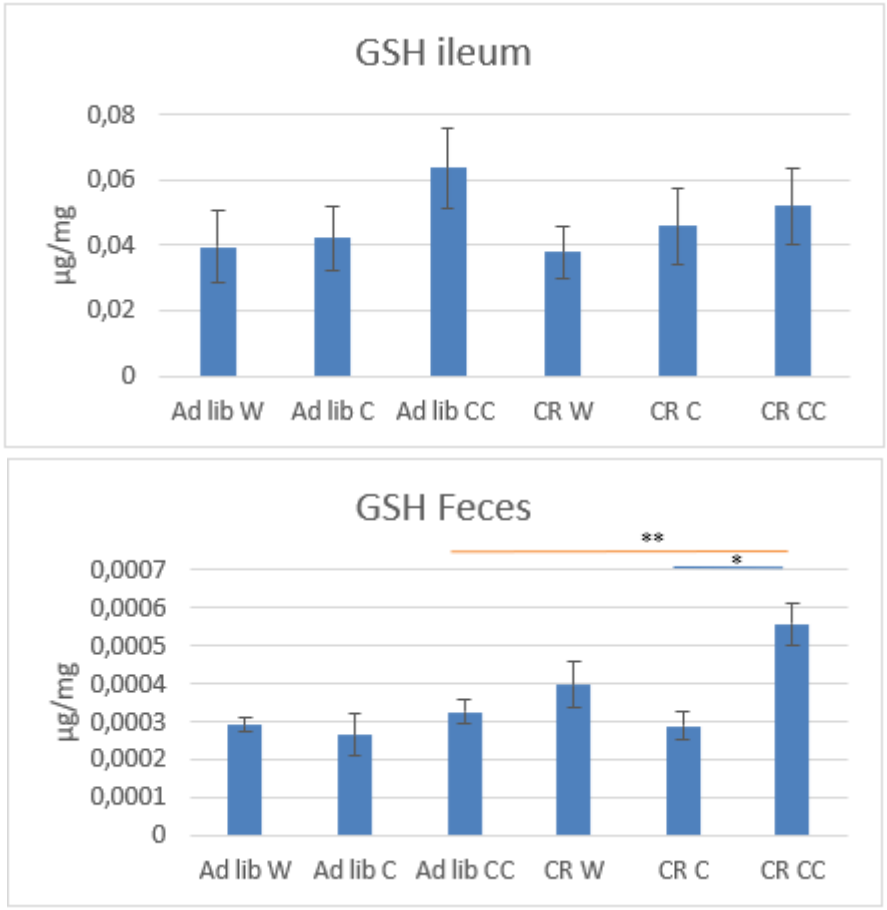




Graph 8-5: Taurine in different tissues after CR, CR statistically significant increases taurine contents in the mucosa of jejunum, but not in other tissues

For further investigation, taurine content of different tissue was measured. The results are presented in Graph 8-5. Taurine levels in plasma stayed almost constant. In ileum and feces no statistically significant changes could be proven. In jejunum, there was a slightly different content of taurine between the ad libitum groups housed on cellulose bedding (0.15±0.02µg/mg), versus the ad libitum group housed on corncob bedding (0.22±0.02µg/mg). However, this change did not remain significant after Bonferroni correction (p-value < 0.0083). Nonetheless, there were statistically significant higher taurine contents in jejunum of CR W (0.34±0.02µg/mg) and CR C mice (0.26±0.02µg/mg) compared to their conspecifics housed on the same bedding. Again, it was shown, that CR leads to higher taurine contents in jejunum. The results also indicate, that there might be an influence of bedding on taurine levels in the jejunum.



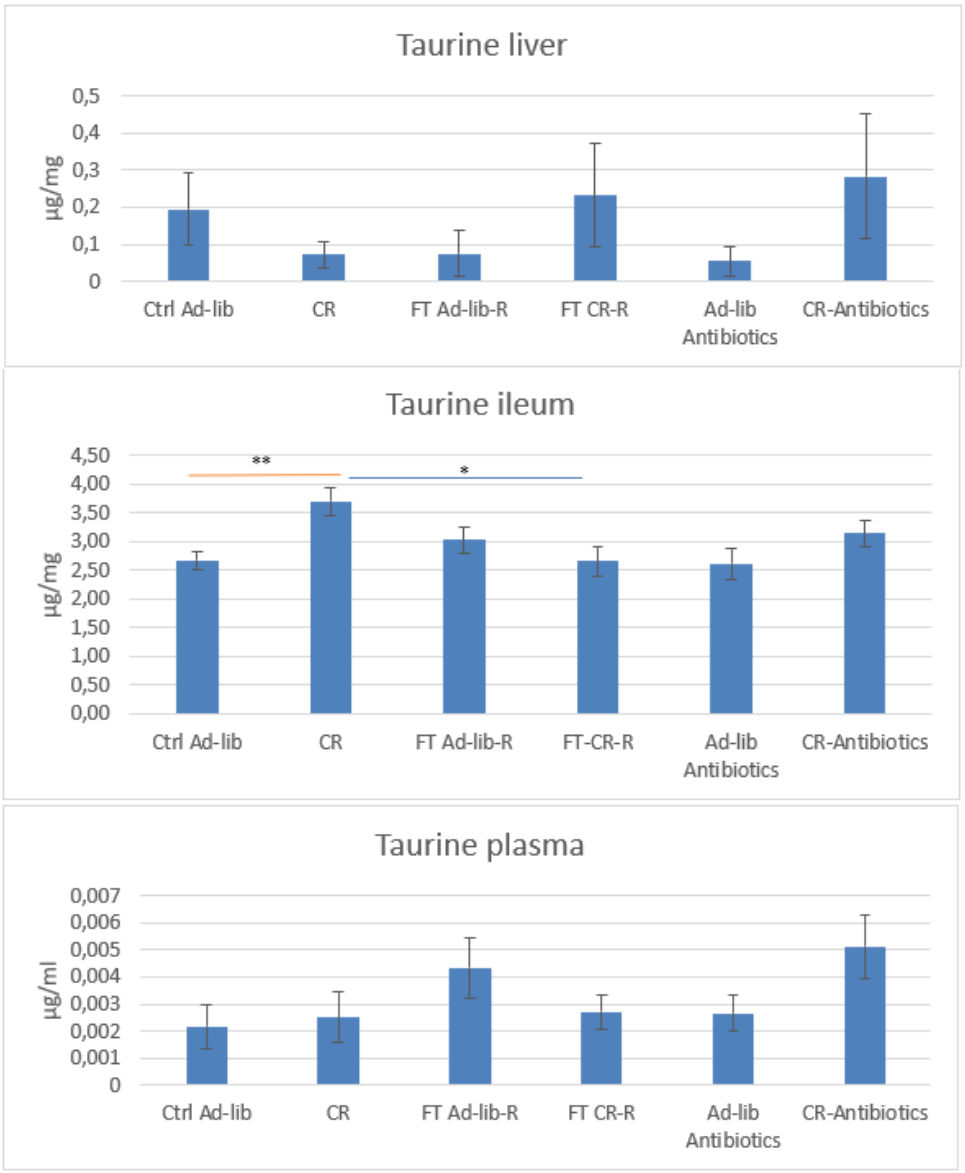


Graph 8-6: GSH levels in different tissues after CR, CR statistically significant lowers GSH contents in the mucosa of the jejunum and increases GSH levels in feces

To compare taurine contents and GSH contents, GSH was measured in the same tissues as taurine. The results are presented in Graph 8-6. It was not possible to measure GSH in plasma with applied method. In jejunum, GSH contents showed a similar response to the ON experiment. There was a statistically significant lower GSH content in CR C (0.007±0.002µg/mg) and CR CC (0.018±0.003µg/mg) compared to the ad libitium mouse groups housed on the same bedding (Ad lib C = 0.025±0.004µg/mg; Ad lib CC = 0.034±0.004µg/mg). This result was confirmed after Bonferroni correction (p-value < 0,0083). There was also a slightly higher GSH content in CR CC (0.018±0.003µg/mg) mice's jejunum compared to CR C mice (0.007±0.002µg/mg) but these changes were not proven as statistically significant after Bonferroni correction. Changes in GSH content in ileum could not be proven statistically, however, in feces, a statistically significant higher GSH content was shown in CR CC (0.56±0.05ng/mg) compared to Ad lib CC (0.32±0.03ng/mg). There was also a slightly lower GSH content in feces from mice of

the CR C group (0.29±0.04ng/mg) than in the CR CC group (0.56±0.05ng/mg), yet the difference did not stay statistically significant after Bonferroni correction. (Concentrations in feces presented as ng/mg due to very low concentrations.) These results indicate again, that taurine and GSH are opponents. It is an interesting finding, that even if GSH content in ad libitum fed groups is higher in jejunum, the excretion in feces is higher in CR groups.

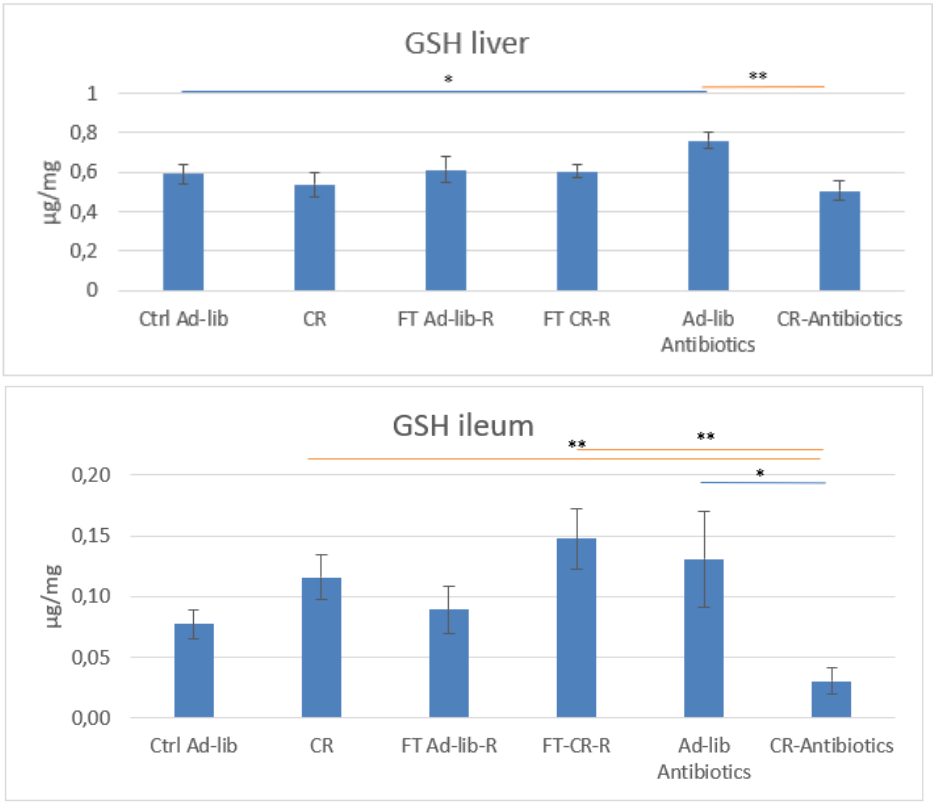
8.3.Microbiota



Graph 8-7: Taurine in different tissue in response to different microbiota, CR increases the taurine content of the mucosa in ileum statistically significant, microbiota does not seem to have a statistically significant impact

To investigate the influence of microbiota an experiment with antibiotics treated mice was performed and the taurine content of different tissue was measured. The results are

shown in Graph 8-7. There were not found any statistical differences between the taurine levels in liver and plasma, however, in ileum there was a slightly lower content in CR group (3.69±0.24µg/mg) versus FT-CR-R (2.66±0.26µg/mg), nonetheless this difference did not stay statistically significant after Bonferroni correction ($p<0,0083$). There was a statistically significant difference in ileum taurine content between the Ctrl Ad-lib group (2.67±0.16µg/mg) and the CR group (3.69±0.24µg/mg), the taurine content was higher in the CR mice.

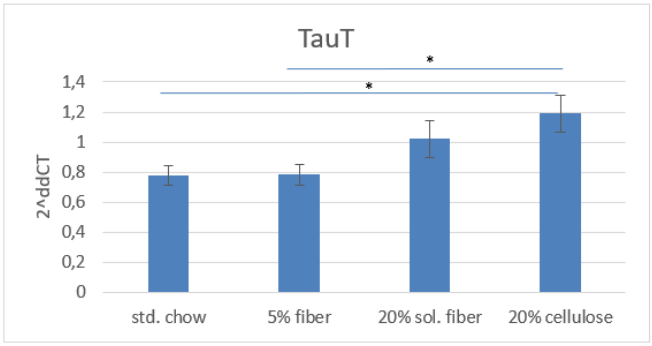


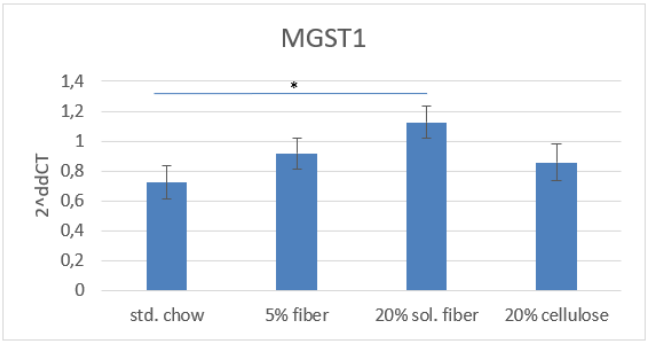
Graph 8-8: GSH content in different tissue in response to different microbiota, microbiota shows a statistically significant influence on GSH levels in the mucosa of ileum

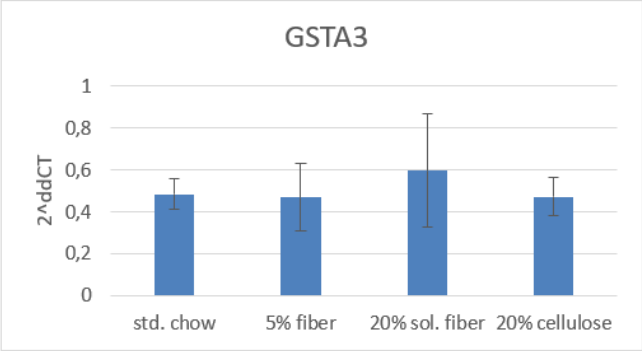
To compare changes in taurine content, GSH contents were also measured. Results of the measurements are presented in

Graph 8-8. Again, it was not possible to measure content of GSH in plasma with applied method. GSH content in liver shows to be slightly higher in Ad-lib Antibiotics group (0.76±0.04µg/mg), than in Ctrl Ad lib (0.59±0.05µg/mg), however this difference does not remain significant after Bonferroni correction ($p<0,0083$). Nonetheless there is a significant lower GSH content in liver of mice in the CR-Antibiotics group (0.51±0.05µg/mg) than in the Ad-lib Antibiotics group (0.76±0.04µg/mg). This change seems to be connected to CR. In ileum, the same effect is shown in antibiotics group, although it does not remain significant after Bonferroni correction. However, there are statistically significant higher GSH contents in ileum of CR (0.116±0.018µg/mg) and FT-CR-R mice (0.148±0.025µg/mg), than in CR antibiotics mice (0.031±0.011µg/mg). This effect remains statistically significant ($p<0,0083$) and seems to be a direct effect of microbiota. The results indicate, that microbiota could have at least some influence on taurine and GSH metabolism, however, there might be more crucial effects of CR.

8.4.Fibre



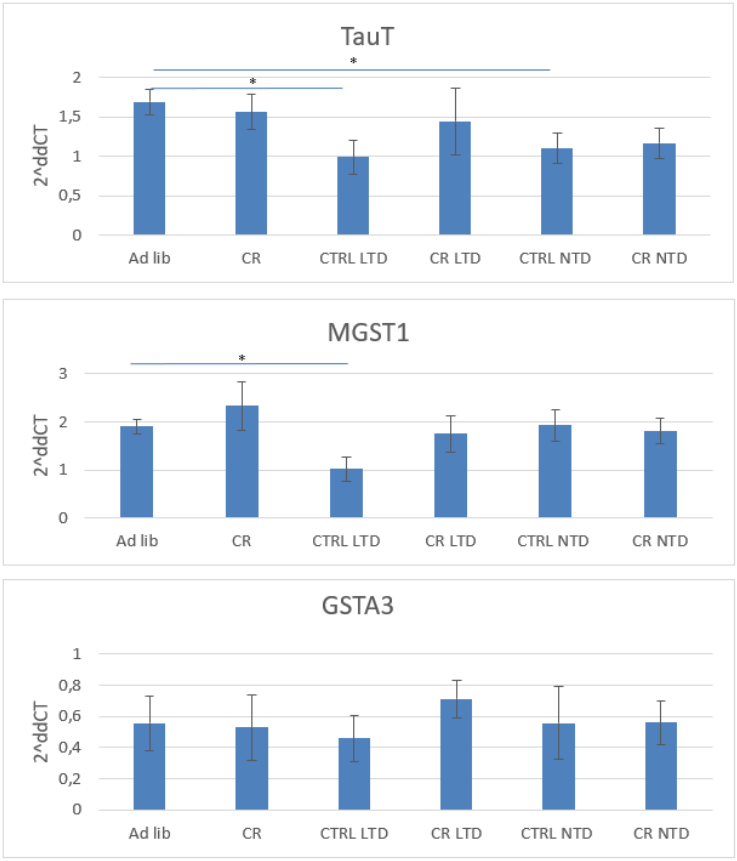




Graph 8-9: Relative mRNA expression of TauT, MGST1 und GSTA3 in the mucosa of jejunum in response to different fibre contents, the consumption of different (amounts of) fibre has no statistically significant impact on the expression of taurine transporters and glutathione transferases

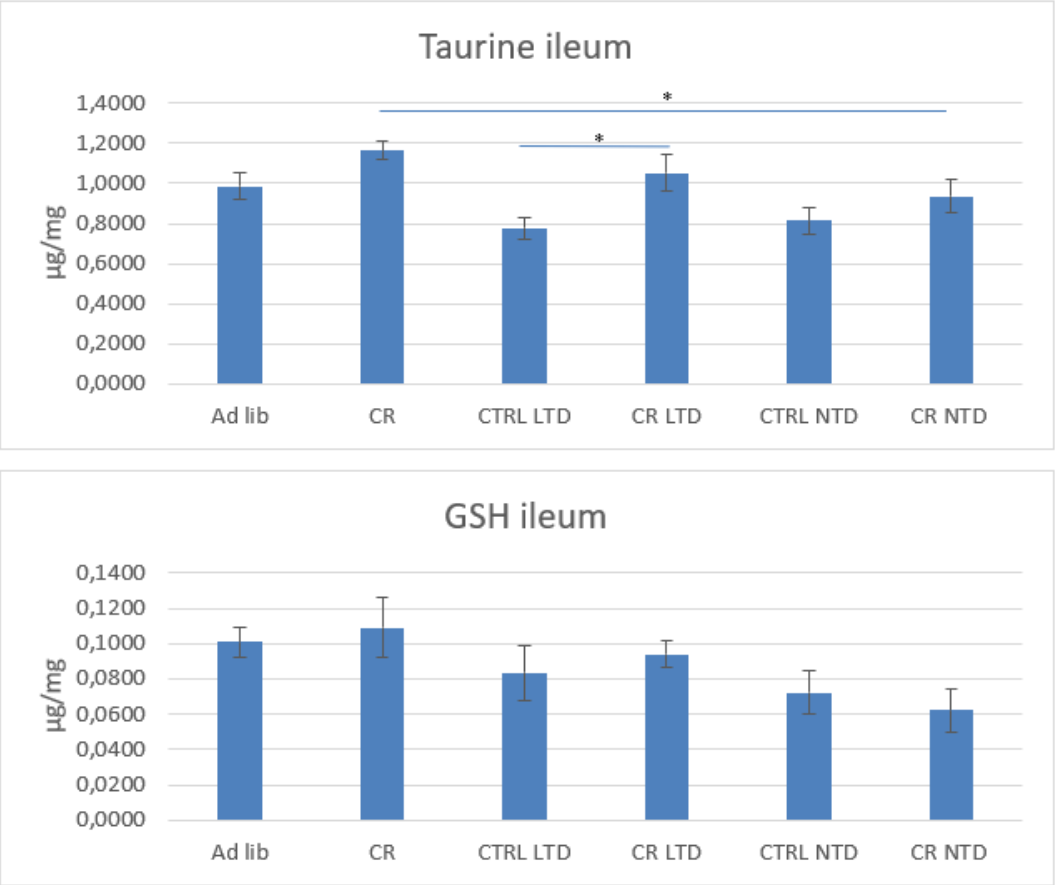
To investigate, if different fibre contents and compositions have an influence on the expression of taurine transporter and glutathione transferases, experiment 4 was performed. RNA for the measurements was extracted out of jejunum of the mice. The results are presented in Graph 8-4. A statistically significant influence of different fibre contents on GSTA3 could not be proven. There was a slightly higher expression of TauT in the 20% soluble fibre (1.02±0.12) and the 20% cellulose group (1.19±1.13) than in the std. chow group (0.78±0.06). However, the difference did not remain statistically significant after Bonferroni correction. (p-value < 0,0125). There was also a slightly higher expression of MGST1 in the 20% sol. fibre group (1.13±0.11) than in the std. chow group (0.72±0.11). Unfortunately, the differences did not remain statistically significant. Bigger group sizes might have led to better results.

8.5.Impact of taurine in food



Graph 8-10: Relative mRNA expression of TauT, MGST1 and GSTA3 in the mucosa of ileum, taurine in food does not influence the expression of taurine transporters and glutathione transferases

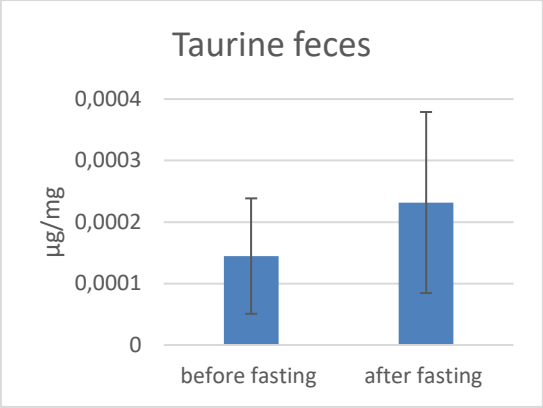
To investigate into gene expressions RNA was extracted out of ileum of the mice. Results are presented in Graph 8-10. TauT shows slightly lower expression in Ctrl LTD (0.99±0.22) and Ctrl NTD (1.10±0.19) groups, than in Ad lib group(1.69±0.16). However, after Bonferroni correction, these differences do not remain statistically significant (p-value<0,0083). In addition, MGST1 shows a slightly lower expression in Ctrl LTD group (1.02±0.25) than in Ad lib group (1.90±0.15), nonetheless, the difference is not statistically significant after Bonferroni correction. There are no statistically significant differences between the groups in expression of GSTA3. Taurine content of food might have an influence on the expression of taurine transporters and glutathione transferases, bigger group sizes might have shown significant differences between the treatments.

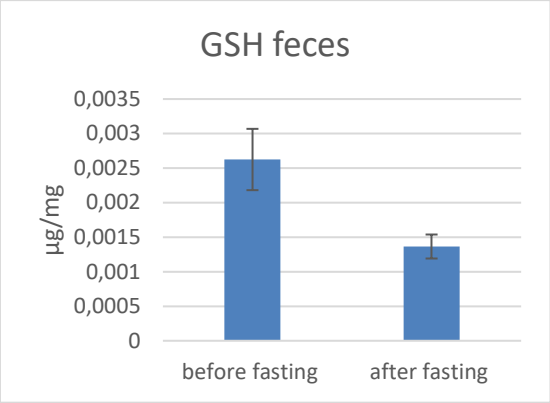


Graph 8-11: The impact of taurine content in food on taurine and GSH levels in ileum, taurine in food has no statistically significant impact on taurine and GSH levels in ileum

To investigate, whether taurine consumption has an impact on taurine and GSH contents in tissue, the concentration of the components in ileum was measured. Taurine contents were slightly lower in CR NTD group (0.94±0.08µg/mg) than in CR group (1.16±0.05µg/mg), whereas they were slightly higher in CR LTD group (1.05±0.09µg/mg) versus CTRL LTD group (0.78±0.06µg/mg). However, none of the differences remained statistically significant after Bonferroni correction (p-value < 0,0083). There were also no differences between the groups in taurine content in ileum. Taurine consumption might have an influence on taurine and GSH content in tissue, nonetheless further investigation is needed. Also, the contents in different kinds of tissue needs to be tested.

8.6.Human Study





Graph 8-12: Taurine and GSH levels in human feces before and after fasting, there is no statistically significant influence of fasting on taurine and GSH levels in human feces

To investigate into the effects of fasting on taurine and GSH levels in Human, the stool samples of the fasting group were analysed. The results are presented in Graph 8-12. Even if the means of taurine excretion (before: 0.14±0.09ng/mg; after: 0.23±0.15 ng/mg) were higher and the means of the GSH excretion (before 2.62±0.44ng/mg; after: 1.36±0.17ng/mg) were lower after fasting, none of the findings was statistically significant (p-value<0.05). (Excretions described as ng/mg due to very low concentrations.) A bigger group size and a longer fasting period might have found significant differences.

9. Discussion

Caloric restricted diets, as well as fasting have proven to be significantly beneficial for health, as well as for healthy life span. Studies have shown, that CR modulate the levels of GSH and taurine, as well as the expression of GSTs and conjugates of taurine and GSH. (5,25,43) It has also been shown, that microbiota plays a role in this regulation, as it influences the effects of CR on levels of insulin/leptin and on weight loss. (5,46) The outcomes of theses experiments are a fitting piece into the previous findings. The different possibilities of cage bedding also have already shown to have an influence on the outcomes of metabolic studies in mice due to different fibre and energy content. (17) This experiment has shown a similar effect on taurine and GSH levels. These findings suggest, that the content of fibre in nutrition has an effect on many health parameters and should not be underestimated. In humans, there was shown that fasting has a significant influence on the expression of genes related to the regulation of triglyceride levels, ketone body

production, insulin secretion and multiple regulatory metabolic processes, such as adipogenesis and fat metabolism. (7) Although the outcomes could not be statistically proven, there is a strong beneficial trend shown in the levels of GSH and taurine after fasting.

The presented experiments contained many different aspects of dietary patterns, such as caloric restriction, fibre intake, cage bedding, taurine intake, overnight fasting in mice, microbiota in mice and Buchinger fasting in Humans. Therefore, this thesis shows an overview on taurine and GSH responses to many different dietary patterns. However, there could be data on more tissues, especially for mucosa overall the intestine. There is also a good overview on taurine transporter and glutathione transferase expression in jejunum or ileum to different diets. Nonetheless it would be interesting to know, how these genes respond in course of the intestine. There also have been some difficulties in the laboratory work. Whereas it is easy to calculate the amount of methanol needed for the extraction out of the tissues, it is more than important, that the tissue is frozen while cutting and weighting. Otherwise it is nearly impossible to transfer the cut tissue into the Eppendorf-tubes and to get exact weights for further calculations. Also, methanol is known to be a little difficult to pipette, as it is pretty runny. It is easier, if used ice cold (as described in 7.3.1) and the pipette tip is wetted with methanol in advance. This is necessary, to not lose tiny drops of methanol and to get exact results in the end. It is also important that measurements are performed timely to the preparation, as levels of taurine and GSH may vary otherwise. However, the measurement of one sample in HPLC-MS takes ten minutes, and there are only 70 places in the machine. Including standard measurements, it was sometimes difficult, to time when to put the next samples into the machine. The interpretation of the peaks was pretty clear for taurine, however, GSH sometimes did not show exact peaks, resulting in difficulties with interpretation.

10. Conclusion

We were not able to find any significant influences of dietary inventions on gene expressions. However, es there are some insignificant differences between the groups, further research with bigger mouse groups and maybe less different diet groups should be performed, to investigate further into that topic. Nonetheless, it was shown once again, that caloric restricted diets have an influence on antioxidative compounds, as GSH and taurine. It is shown, that caloric restriction significantly increases levels of taurine in

jejunum, whereas it lowers levels of GSH. In feces, the effect is significantly opposed for GSH levels. These effects have been shown before by Gregor et. al. (25) Unfortunately, we have not been able to prove the same effects of fasting in humans, however, there is a slight difference in the means. This topic should be investigated further with a higher number of subjects and better unified groups. It would be possible to start a study with subjects in a consistent age and BMI group. Nonetheless, it is known that it is generally difficult to recruit people for studies in general and even more difficult if stool samples are required. Therefore, it might be difficult to get a number of subjects big enough to prove significant changes. It was also shown, that microbiota has an influence on taurine and GSH levels, what suggests that microbiota has a high impact on oxidative stress and overall health. We were not able to prove significant influences of different fibres and different fibre contents on taurine and glutathione transport in jejunum and it was also not possible to prove significant influences of taurine intake on taurine transport and taurine and GSH levels. However, as there are slight influences between the means of the groups, further investigation might find a different outcome.

11. Abstract in German

Verschiedene Diätinterventionen haben bereits in der Vergangenheit positive Einflüsse auf oxidativen Stress gezeigt. Taurin und GSH sind ebenfalls involviert in Redox-Mechanismen. Deswegen war es von Interesse zu zeigen, ob verschiedene Diätformen Einfluss auf die Konzentrationen dieser Metabolite haben. Zuvor wurde bereits festgestellt, dass kalorienreduzierte Diäten Taurinkonzentrationen im Jejunum erhöhen, währen die GSH-Konzentrationen senken. In den Fäzes wurde ein umgekehrter Effekt festgestellt. Das führte zu der Frage, ob das Microbiom des Darms eine Rolle in diesem Zusammenhang spielen. Um dieser Frage auf den Grund zu gehen, wurde ein Experiment mit Antibiotika-behandelten Mäusen durchgeführt. Um die Mechanismen hinter diesen Veränderungen besser zu verstehen, wurden Taurintransporter und Glutathiontransferasen gemessen. Leider waren wir nicht in der Lage, einen signifikanten Einfluss verschiedener Diätformen auf die Expression der hierfür verantwortlichen Gene zu finden. Allerdings, konnten wir einen signifikanten Einfluss des Mikrobioms auf Taurin- und GSH-Konzentrationen feststellen.

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