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Index of Abbreviations

APC	Antigen presenting cells
AScl	Atherosclerosis
CAD	Coronary artery disease
CR	Caloric restriction
IF	Intermittent fasting
FMD	Fasting-mimicking diet
qPCR	quantitative polymerase chain reaction
CVD	Cardiovascular disease
DM	Diabetes mellitus
DP	Double positive
FACS	Fluorescence activated cell sorting
FFA	Free fatty acids
GALT	Gut associated lymphoid tissue
IBD	Inflammatory bowel disease
LCT	Long-chain triglycerides
LN	Lymph nodes
NAFL	Non-alcoholic fatty liver
ND	Neurodegenerative disease
PP	Peyer's patches
SD	Starch diet
Si	Small intestine
SP	Single positive
Vs	Versus
WAT	White adipose tissue
WSD	Western-style-diet

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1 Abstract

In a modern society, the access to food is exceptionally easy as there are food stores and restaurants on every corner. In addition, the physical activity is reduced due to the sedentary lifestyle, which leads to diseases of affluence such as obesity, diabetes type II, hypertension, cardiovascular diseases and promotes age-related disorders. The stated diseases share a common root, namely an unhealthy diet, which is a modifiable risk factor. Therefore, the possible effects of restrictive dieting on the immune status of the gastrointestinal tract are investigated in this thesis

In order to investigate this premise, an experiment was conducted on mice, including five diet groups and two control groups. The ketogenic groups were fed with high fat paste. The ketogenic control diet contained a higher amount of protein, as opposed to the ketogenic diets. Additionally, the caloric restriction (CR) was restricted to 75% of the daily calorie intake. The intermittent fasting (IF) group was fed every second day and the fasting-mimicking diet (FMD) was divided in cycles where the daily intake was reduced up to 10%. Each dietary group consisted of eight mice.

After the diets were completed, spleen, lymph nodes (LN) and Peyer's patches (PP) were collected for the flow cytometry. Targeted antigens were measured with the use of flow cytometry to detect the immune cells in the investigated tissues.

The gene expression of NOD2, TNFα, OAS1a, RSAD2, IRF1, PPARγ, IRF7, TLR3, MYD88, REG3γ and STAT1 were investigated, which play a role in inflammation processes and can lead to the development of lifestyle diseases, if derailed. For the examination of the gene expression small intestine (Si), LN and PP were used.

This thesis supports the observation that restrictive diets affect the expression of genes, which are involved in inflammation. In addition, the diets also influence immune cells. Ketogenic control diet decreases STAT1 and REG3γ. IF reduces IRF1, MYD88, TLR3 and PPARγ. FMD has the potential to lower REG3γ, TLR3, IRF1 and

OAS1a. CR diminishes OAS1a, REG3γ, IRF1 and TLR3. Ketogenic diet shows on almost all genes a positive effect and outperforms the other diets.

2 Zusammenfassung

In einer modernen Gesellschaft ist der Zugang zu Nahrung einfacher denn je, an jeder Ecke ist ein Lebensmittelgeschäft oder ein Restaurant anzutreffen. Zusätzlich sinkt die körperliche Aktivität durch Berufe im Büro oder Einsatz von Maschinen. Dieser Lebensstil führt zu Zivilisationskrankheiten wie Adipositas, Diabetes Typ II, Hypertonie, kardiovaskuläre Erkrankungen sowie altersbedingte Erkrankungen. Ein gemeinsamer Nenner der zuvor genannten Krankheiten, ist eine ungesunde Lebenshaltung, die einen modifizierbaren Risikofaktor darstellt.

Aus diesem Grund wurde die Auswirkung restriktiver Diäten auf den Immunstatus des Gastrointestinaltraktes untersucht. Hierfür wurde ein Experiment mit Mäusen durchgeführt, das fünf Diäten und zwei Kontrollgruppen beinhaltet. Die zwei ketogenen Gruppen wurden mit einer fettigen Paste gefüttert. Die ketogene Kontrolldiät enthält einen höheren Proteingehalt als die zwei ketogenen Diäten. Die CR Gruppe bekam 75% ihres täglichen Energiebedarfs. Die IF Gruppe wurde jeden zweiten Tag gefüttert und das Scheinfasten bestand aus Zyklen, in denen die tägliche Energiezufuhr auf bis zu 10% reduziert wurde. Jede Gruppe umfasste acht Mäuse.

Nach Abschluss der Diäten wurden Milz, Lymphknoten (LN) und Peyer's Patches (PP) für die Durchflusszytometrie gesammelt. Markierte Antigene wurden mit Hilfe der Durchflusszytometrie gemessen um die Immunzellen zu detektieren.

Die Genexpression von NOD2, TNFα, OAS1a, RSAD2, IRF1, PPARγ, IRF7, TLR3, MYD88, REG3γ und STAT1 wurde untersucht, da sie eine Rolle in Entzündungsprozessen spielt und bei Entgleisung zu Zivilisationskrankheiten führen kann. Für die Untersuchung der Genexpression wurden Dünndarm (Si), LN und PP verwendet.

Diese Arbeit unterstützt die Beobachtung, dass restriktive Diäten einen Einfluss auf die Expression proinflammatorischer Gene haben. Die ketogene Kontrolldiät vermindert STAT1 und REG3γ. IF reduziert IRF1, MYD88, TLR3 und PPARγ. FMD hat das Potential

REG3γ, IRF1, TLR3 und OAS1a zu reduzieren. CR mindert OAS1a, REG3γ, IRF1 und TLR3. Die ketogene Diät zeigt einen positiven Effekt auf alle untersuchten Gene und übertrifft alle anderen Diäten.

3 Introduction

The aim of this thesis was to investigate the impact that restrictive diets had on the regulation of the immune status with respect to the gastrointestinal tract (more formal). In order to do so, an experiment on mice was conducted, which included five dietary groups and two control groups. Each group consisted of eight mice. The ketogenic diet is high in fat and imitates the metabolic status of fasting. This means the metabolism switches, and gains energy from fat catabolism, which results in ketosis. Caloric restriction is a reduction of the calorie *ad lib (ad libitum)* intake. Intermittent fasting is the abstinence of food for a defined period; this can be 16 hours, 24 hours or any other time span. Fasting-mimicking diet is based on macronutrients and micronutrients, which lower glucose and IGF-1 levels, and increase ketone bodies.

The positive effects of some of the diets have been studied across many species from microorganisms to primates. The objective of this experiment is to determine the influence on immune cells and gene expression of proinflammatory genes

After all mice have passed the restrictive diets, their organs were isolated. Spleen, Ln and PP were used for the flow cytometry to detect the immune cells. With the help of the qPCR the gene expression of the chosen genes was investigated in Si, LN and PP.

The next chapter provides an overview of the conducted diets and explains diseases that occur due to unhealthy lifestyle.

4 Diets and diseases

The conducted diets are well investigated diets, which comprises of the ketogenic diet, CR, IF and FMD.

4.1.1 Ketogenic diet (keto)

The ketogenic diet (keto) is a high fat, low-carbohydrates diet that imitates the metabolic status of fasting. The ketosis is achieved by covering the energy requirement with 60-90% dietary fat. With the help of the diet, this condition can be maintained as long as required. Ketone bodies contain at least three carbon atoms and a non-end standing carbonyl group (>C=O). While fasting, ketone bodies are produced by catabolism of fat (ß-oxidation) in the liver and are an alternative energy source for many organs including muscles. Main medical indications for the ketogenic diet are pharmacon resistant types of epilepsy in infancy and adolescence. [1] Since the brain is exclusively dependent on glucose and ketone bodies as source of energy, ketogenic diets are an essential therapy in cerebral metabolic disorders such as Glut1-defect and Pyruvatdehydrogenase-(PDH)-deficit. Ketone bodies most likely have a neuroprotective effect because of their high energy density and the alternative metabolism to glucose. Due to increased cerebral energy reserves and thereby stronger resistance of neurons, reduction of free radicals and modulation of inflammatory activity the use of ketogenetic diets as adjuvants in neurodegenerative diseases is disputed increasingly. [2] Cancer is another possible indication that is investigated for this diet. The idea is to disrupt the metabolism of the tumor by detracting its energy source (glucose). This way, one can suppress the growth of a tumor. [3]

Absolute contraindications for the ketogenic diets are fatty acid-oxidation-disorders (disturbed catabolism of fatty acids inhibits ketone bodies production), ketoneogenese defect/ketolysis defect (disturbed production of ketone bodies/disturbed catabolism of ketone bodies), gluconeogenese defect (GSD I) (life-threatening hypoglycemia by

initiation of ketogenic diet) and hyperinsulinism (same effect as last point- due to insulin). It is important to exclude these factors before starting a ketogenic diet to avoid complications. [4]

4.1.2 Caloric restriction (CR)

CR is a reduction of the daily calorie intake. Most of the time the restriction is between 20-40%, in some rigorous cases it is higher than 50%. [5] As a reaction to CR it comes to weight loss, mostly from the white adipose tissue (WAT), whereas the lean body mass is less affected or unchanged. Therefore, CR is a commonly used strategy to treat adiposity. [6] With weight loss, the body composition changes and the resting metabolic rate drops. Body temperature is decreased and hunger is increased, with the latter not adapting even with a long- term restriction. Due to the reduction in WAT mass circulating adipokines are reduced. Reflecting the reduction of WAT there is a large decrease of insulin and glucose blood levels. The profound metabolism changes in tissue result in a shift from carbohydrate to fat metabolism.

For at least 500 years, this diet has been practiced to increase both the length and quality of life. The success of restricting the intake of calories has been confirmed in experimental work in animals. Evidence was first presented in 1930 by McCay et al. CR slows down aging and extends median and maximal life span. [7] Lifelong CR can extend the life of rodents by up to 50%, with the impact decreasing the later in life it is started. The CR has profound effects on age related diseases, including reduced risk of cancer, cardiovascular disease (CVD), diabetes mellitus type 2 (DM II), autoimmune diseases and neurodegenerative disorders. [8] Surrogate markers that are positively affected by CR: reduced oxidative stress, improved insulin sensitivity and altered neuroendocrine and sympathetic nervous system function in animals. Humans on long-term restriction report similar negative side effects to those observed in animals – perpetual hunger, reduced body temperature leading to a feeling of being cold, and

diminished libido. The last two effects are due to the reduced thyroid and gonadotropic concentrations. [7]

4.1.3 Intermittent Fasting (IF)

IF consists of periods of voluntary abstinence from food. [9] Fasting is partial or total refraining from all foods. There are several types, like the modified fasting regimens (5:2 diet) by Michelle Harvie, which provides 5 days eating *ad libitum* and 2 days with a maximum 20-25% intake of calories. Another diet is the 'eat-stop-eat-method' by Brad Pilon. This consists of fasting two days per week. The abstinence of food is from lunch to lunch or from dinner to dinner to the following day. [10] One known method, which gained popularity over the last years, is the time-restricted feeding. It allows *ad libitum* energy intake for a specific period of time and the rest of the day is fasting time. [11] For example there is the 16:8 method, he time slot pertaining to the eating *ad libitum* amounts to eight hours with prolonged daily or nightly fasting of 16 hours. [10]

Most of the data on intermittent fasting is from research based on animal models, primarily the studies of male rodents. Human studies are largely limited to observational studies of religious fasting. [11]

Health outcomes are changes in weight and metabolic parameters associated with CVD, DM2, renal diseases, cancer and biomarkers of oxidative stress. [12] Studies show that restricting food overnight improves metabolic profiles and reduces risk of obesity and obesity related diseases such as NAFL, hypercholesterolemia, high blood pressure, diabetes, metabolic syndrome and cancer. [9] Hypotheses to the mechanisms are the effects on 1) circadian biology, 2) the gastrointestinal microbiota and 3) modifiable lifestyle behaviors. Circadian rhythm is known as the inner biological clock, which is a mechanism that optimizes the body function in consideration of metabolism, energetic, physiologic indices such as hormonal secretion patterns, physical coordination and sleep. In mammals, the endogenous master clock is located in the suprachiasmatic nuclei of the hypothalamus and is stimulated by light/dark. Similar

circadian rhythms have been found in peripheral tissues, such as the liver, where feeding the timing cue is. Since the functions of the gastrointestinal tract (GIT) are regulated by the circadian rhythm such as gastric emptying, blood flow and speed of metabolic responses to glucose, this has an influence on utilization of food and allocation of energy. [11] It is known that commensal bacterial flora is affected by numerous factors, *inter alia* nourishment. One assumes that IF supports the gut flora and keeps the balance. It has been observed that several human diseases such as obesity and metabolic disorders, infectious and neurological diseases, including inflammatory bowel diseases (IBD) are linked to microbiota dysbiosis. The diet is relatively easy to integrate in daily routine, complicated cooking is not necessary and since the fasting is for a short period, there is no jojo effect. Moreover, it improves handling and increases awareness of food. [11]

It is proven that even a single fasting interval in humans (e.g. overnight) can reduce the concentration of many basal biomarkers, that is why patients are required to fast for eight - twelve hours before blood draws. These steady-state fasting levels can be used for reducing the risk of chronic diseases in humans and is eventually an option to prevent metabolic disorders. [9] Moreover, intermittent fasting resulted in prolong lifespan. [12] However, more studies on humans are needed with long-term follow-up to show maintenance of weight loss and diet adherence in the long run. In addition, future studies should include subgroups of population with specific risk factors such as CVD or DM2 to observe modifying processes of the diseases, in case there is any. [13]

4.1.4 Fasting-mimicking diet (FMD)

FMD is a periodic use of a plant based, low calorie diet that causes the cells to act like the body is fasting. [14] The composition of the aliment consists of macronutrients and micronutrients especially formulated to trigger responses that lower glucose and insulin-like growth factor-1 (IGF-1) levels, and increase ketone bodies. The period varies from 2-7 days every 15-365 days. [15] People who restrict calories for only five

days monthly and eat proper food can reduce the risk for DM2, myocardial infarct (MI) and cancer. It has the positive effects of a regiment that counters aging and reduces the noxious visceral fat without effectuating muscle and bone loss. On the first day, the faster is allowed to consume 50% (1100 kcal) of the daily calorie intake and on the days 2-5, it is ~33% (750 kcal). Participants who underwent the FMD for 3 months showed improved factors, which are associated with DM and CVD. Moreover, glucose levels, visceral fat and C-reactive protein, which is involved in inflammatory processes in the body, decreased. In addition the IGF-1 level dropped. This hormone is essential for growth, especially in youth. Nevertheless, it also rushes aging and promotes cancer. [16] Furthermore, FMD has potential in the therapy of inflammatory bowel diseases (IBD). It reduces intestinal inflammation, increases stem cell number, which promotes intestinal regeneration, and stimulates the growth of protective gut microbiota. In rodents, it was able to reverse intestinal pathology caused by dextran sodium sulfate (DSS). [14] Several studies show that FMD promotes regeneration, enhances cognitive performance and increases lifespan. [16] [17] Beside the mentioned effects it also ameliorates metabolic disorders such as diabetes. There is a significant improvement in beta cell function and insulin sensitivity. [18]

4.1.5 Inflammatory bowel diseases (IBD)

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases with relapse and remission. The disorders occur mostly before the age of thirty and are located on different segments of the intestine. Furthermore, extra intestinal inflammatory processes can be observed. First grade relatives of IBD-patients have a quintuple to twentyfold higher risk to suffer from IBD as well. CD and UC are defined as immune mediated diseases that can be triggered in genetic predisposed persons through particular environmental factors. An imbalance between gut microbiota, mucosa barrier and mucosal immune system operates an aberrant immune response.

A genome-wide association study identified 163 mutated gene loci that cause risk factors for the development of CD and UC. [19]

In CD, the inflammation is transmural and besets all gut wall layers; it mostly appears on the ileum and colon but can affect all regions of the GIT in form of discontinued inflammations. Clinical symptoms include common diarrhea and abdominal pain on the right side, however, abscesses, fistulas and strictures can result in a danger obstruction of the bowels. The fistulas often break through nearby organs. [20]

CU, causes ulcers on the rectal mucosa that cause inflammation and expanse proximal, which explains the bloody diarrhea. Under circumstances, the whole colon is affected. In contrast to CD the UC is mostly restricted to the mucosa and submucosa, but a transmural progression of the ulceration is possible and leads to peritonitis, inflammatory related extension such as toxic megacolon and perforation.

CD and UC both increase the risk for bowel and colorectal carcinoma.

4.1.6 Obesity

Obesity is one of the greatest challenges of the 21st century we must deal with. Its incidence has tripled in Europe since the 1980s. Adiposity reached an epidemic extent: at least 2.8 million people die annually due to the consequences of overweight and adiposeness. Regardless of physical disabilities, it increases the risk of developing numerous diseases such as diabetes, atherosclerosis, CVD, cancer and several chronic diseases. [21]

Categories	BMI (kg/m²)
Underweight	< 18,5
Normal weight	18,5 – < 25

Overweight	≥ 25
Pre-adiposities	25 – < 30
Adiposities	≥ 30
Grade I	30 – < 35
Grade II	35 – < 40
Grade III	≥ 40

Table 1) Classification of obesity [20]

BMI is not specified on fat, because it does not include the composition of the body. That is why the waist circumference and clinical features are essential for the diagnosis.

4.1.7 Diabetes

Worldwide there are 422 million people affected by diabetes, the number has almost quadrupled since the 1980's. According to the WHO (World Health Organization), 64 million diabetics live in Europe and the prevalence is increasing. The expanding diabetes epidemic correlates clearly with the rise of overweight and obesity, unhealthy nutrition and sedentary lifestyle. In some European countries the diabetes rate reaches up to 14%. These countries are burdened strongly with this metabolic disorder, and their healthcare systems must face the handling of the disease and its consequences. [22]

Diabetes type II (90%) is mostly diagnosed 4-7 years too late. Non-insulin dependent diabetes mellitus (NIDDM) – also known as adult onset diabetes, is divided in Type IIa: without obesity and Type IIb: with obesity (80% of DM type II).

Insulin resistance: metabolism status with high insulin values despite normal or increased serum glucose because of inefficient insulin effect, which results in higher necessity of insulin. Sedentary lifestyle, fatty dietary and especially abdominal obesity with liberation of mediators such as FFA, TNFα, leptin and adipokinin from adipocytes cause DMII. [23] Through a disruption in the insulin signal cascade hyperinsulinism occurs. People with a healthy metabolism have a plasma glucose between 70-80mg/dl after nocturnal fasting and after an extensive meal; it does not overrun 140mg/dl. [19]

This leads to the next chapter, where the molecular level and the origin of the pathogenesis is explained.

4.2 Genes

Chosen genes that are involved in inflammatory processes were investigated to see the effect of the diets on the gene expression.

NOD2 is a nucleotide binding oligomerization domain containing protein 2. It provides instructions for a protein that has an essential function in the immune system. It is an intracellular receptor belonging to the (NOD)-like receptor family (NLR) and functions as a pattern recognition receptor (PRR). NOD2 receptor, encoded by the NOD2 gene is expressed in macrophages, monocytes, hepatocytes, epithelium of oral cavity, dendritic cells, lungs and intestine, with higher expression in intestinal stem cells and in ileal Paneth cells. [24] It plays an important role through the activation of proinflammatory transcription program and innate immune pathways as a response to foreign bodies such as bacteria, viruses and parasites. For instance, Muramyl dipeptid (MDP) is a conserved motif in bacterial peptidoglycan (PGN) which stimulates NLR and leads to the activation of the nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFKB) protein, mitogen-activated protein kinases (MAPKs), and Caspase-1.

Balanced NOD2 signaling is pivotal for the maintenance of immune homeostasis. [25] An imbalance through abnormal interactions between microbes (either commensal or pathogen) and host lead to the so-called microbiota dysbiosis, which is linked to IBD, including ulcerative colitis (UC) and Crohn's disease CD. [24] Researchers observed the involvement of NOD2 not only in protection against a shift in the composition of the enteric microbiota but also against non-microbiota-related intestinal damages. [26]

TLR3 encodes a protein that is a member of the Toll-like receptor family (TLR). [27] TLR plays a fundamental role in recognizing pathogen-associated molecular patterns (PAMPs) and activating the innate immunity. This receptor is found on plasma membranes, in vesicles inside cells, and is restricted to the dendritic subpopulation of leukocytes. [28] It is believed that TLR3 senses the presence of extracellular pathogens after the lysis of infected cells as well as intracellular pathogens since it occurs both intra- and extracellular. [29] It is known for its recognition of double-stranded RNA structures (dsRNA) such as virus, bacteria or microbial derived ligands, which leads to activation of NFKB and production of type 1 Interferon (INF) (mainly INFα/β). Latorre et al. show that there is a relation between visceral adipose tissue (VAT) and TLR3. TLR messenger RNA (mRNA) was detected in probands after and before liposuction and a significant diminish in TLR3 was visible. [30]

MYD88 encodes a cytosolic receptor protein that acts as a signal transducer in the interleukin-1 (IL-1) and TLR signaling pathways. It functions as an adapter, connecting proteins that receive signals from outside the cell to IL-1R-associated kinase (IRAK). Thus, it activates the nuclear factor-kappa-B (NFKB), mitogen-activated protein kinases (MAPK) and activating protein 1 (AP1) which regulate the activity of inflammatory reactions and immune responses. [31] MYD88 deletion prevented western style diet (refined grain, cholesterol and high in saturated fat) (WSD)-induced adipose inflammation. [32] Age-related inflammation was linked to altered gut flora and showed reduction in cytokine expression through MYD88 ablation. [33]

Interferon regulatory factor 1 (IRF1) gene has the instruction for a protein that is a transcriptional regulator in cell proliferation, apoptosis, DNA damage response and immune response. As the name indicates, it regulates IFN and IFN-inducible genes but is also involved in many gene expressions, amongst others during hematopoiesis, inflammation and cell differentiation. The encoded protein by this gene has a versatile function, including tumor suppression, antiviral activities and maturity of immune cells. Indeed, IRF1 is concerned with the control of adipogenesis, which contributes to up-regulation of inflammatory processes. [34] It is demonstrated that IRF1 plays a role in obesity-related inflammation and metabolic dysregulation. [35]

Interferon regulatory factor 7 encoded by IRF gene is alike IRF1 a regulatory transcription factor. It has similar functions as IRF1 but its inducible expression is largely restricted to lymphoid tissue. [36] Wang et al. investigated the role of IRF7 in the regulation of energy metabolism as well as its involvement in the etiology of obesity and type 2 diabetes. In this study, wild type (WT) mice were compared to IRF7 knockout (KO) mice. 24 weeks after high fat diet (HFD) IRF7 KO mice showed less weight gain and obesity in comparison to WT mice. Moreover, their fasting blood glucose was only slightly increased versus the greatly higher levels in WT. IRF7 levels were increased in WAT and other tissues such as insulin target organs, which explain why the insulin sensitivity was reduced in WT but not in IRF7 KO mice. KO mice also showed less macrophage infiltration into several organs and no indication of local or systemic inflammation. This investigation highlights IRF7 as a target for a new strategy in the treatment of adiposity and related metabolic disorders. [37]

TNFα belongs to the tumor necrosis factor (TNF) superfamily and plays a role in multifunctional proinflammatory cytokines. It is mainly secreted by macrophages and regulates a wide spectrum of biological processes such as coagulation, apoptosis, lipid metabolism, cell proliferation and differentiation. Therefore it is associated with

diverse diseases including cancer, insulin resistance and autoimmune diseases. [38] Hussain et al. verified high TNFα levels due to high fat diet. A possible explanation is that the high fat intake results in the impairment of the intestinal epithelium, which up-regulates cytokines and induces colonic inflammatory. [39]

REG3γ belongs to the regenerating islet-derived genes and is encoded by the REG family gen. It is known for its antibacterial activity, acute-phase response, and positive regulation of wound healing and cell proliferation. [40] Up-regulation of REG3γ expression, suggests altered bacterial-epithelial signaling and innate defense responses. [41]

STAT1 (Signal transducer and activator of transcription 1) provides a construction plan for a protein that is participating in various immune system functions. It keeps the balance of the immune system through inhibition of IL-17 pathway and in contrast to its inhibitory function, the STAT1 protein promotes apart from this INFα/β and INFγ. [42] Obesity increases STAT1 signaling and hence inflammation. [43] In patients, who suffered from Crohn's disease (CD) STAT1 led to cell death and Paneth cell death. [44]

OAS1a encodes a protein that synthesizes 2', 5'-oligoadenylates (2-5As), which mediates antiviral responses. However, polymorphisms in this gene are associated with DM. [45] In the strict sense it is associated with 1h postprandial plasma glucose, which correlates with DM. [46]

RSAD2 is radical S-adenosyl methionine domain containing 2. This gene is involved in processes that regulate type 1 INF signaling pathway, T-cell activation and differentiation as well as defense response to viruses. Western diet (WD) is associated with increased RSAD2 that causes epicardial adipose tissue (EAT) inflammation. Thus, it promotes the development of coronary artery disease (CAD). [47]

PPARγ is a member of the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors, which acts as a transcription factor. Its product plays a role in the differentiation of adipocytes. [48] Moreover, it modulates the transcription of acetyl-CoA oxidase and therefore participated in the peroxisomal β-oxidation pathway of fatty acids. [49] For this reason PPARγ is associated with the etiology of various diseases, including atherosclerosis, diabetes, obesity, cancer and colon inflammation. [50]

EEF1A1 is the eukaryotic translation elongation factor 1 α 1, which encodes the elongation factor-1 complex that induces the enzymatic delivery of aminoacyl tRNA to the ribosome. [51] This gene was used as reference gene for normalization.

4.3 Intestinal immune system

A short overview of the intestinal immune system is provided, in order to gain a better understanding of the relevant background.

Lymph nodes and spleen are the best-known lymphatic organs and play a crucial role in the initiation of immune response when antigens enter through skin or get in contact with blood. Therefore, it is no surprise that these organs are well researched and described in immunological literature. The major exposition to foreign antigens is neither across skin nor blood but the mucosal surfaces, especially of the intestine.

The intestine accommodates the biggest immune organ of the body. Immunocompetent cells can be found in the mucosa's epithelial layer and in the underneath lamina propria, where two thirds of all lymphocytes are located (Figure 1). According to Prof. Dr. Reinhard, this shall avoid the penetration of potentially dangerous bacteria from the intestinal lumen into the body. [52] Concurrently, food components have to be absorbed effectively. The intestinal immune system has to

conciliate these two contrary functions. Due to those, anatomic barriers, unspecific defense mechanisms and a specific immune system are involved to achieve this goal.

- Antigen presenting cells (APC), these include epithelial cells (mainly M-cells) and dendritic cells (DC) of the lamina propria
- Lymphocytes, particular in lamina propria and epithelium or unionized as lymph follicle and Peyer's Patches
- Inflammatory cells (macrophages, mast cells, granulocytes)

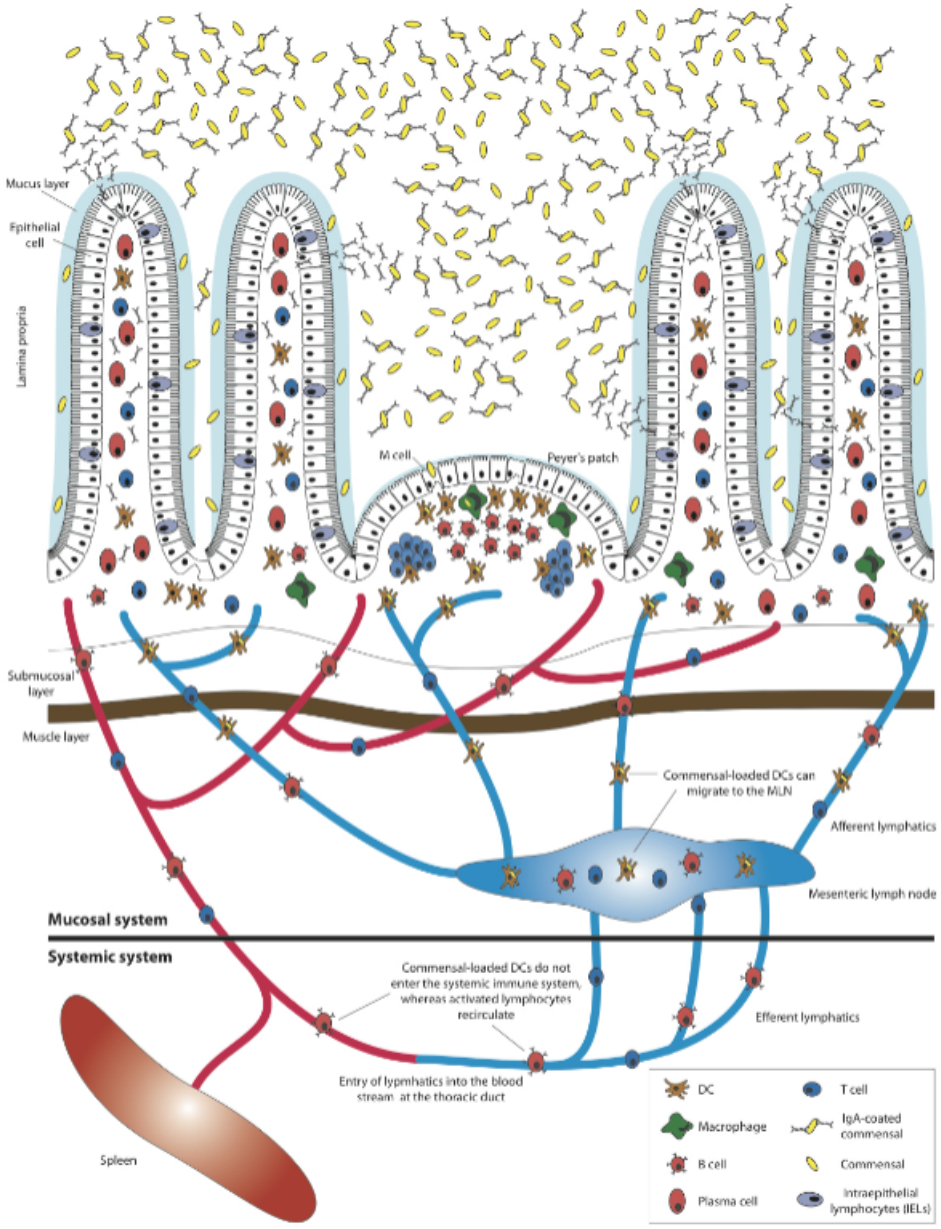


Figure 1) Structure and function of the intestine as immune organ [53]

Via nutrition, the average human takes over 100g of foreign proteins daily. Parts of them reach the blood intact and can trigger an immune reaction. At the same time, the intestine accommodates a tight accumulation of commensal and mutualistic intestine bacteria. The bacterial count surmounts our own body cells. The gut flora itself acts as a “metabolic organ” through breakdown dietary carbohydrates and proteins,

fermenting and generating vitamins. [24] The consideration is how our immune system manages to tolerate harmless nutrition antigens and essential intestine bacteria and at the same time fight pathogen bacteria, viruses and parasites effectively. The basis for this purpose is the intestinal immune system: directly in the intestine there are more immune cells located than in all lymph nodes together. The composition and function of these immune cells are strongly embossed by the conditions in the intestine and differ substantially from the peripheral lymph nodes and spleen.

The immune system of the small intestine divides in gut associated lymphoid tissue (GALT) and intestine draining mesenteric lymph nodes (mLN) as well as diverse immune cells distributed in the tissue (Figure 1). In GALT and mLN antigens are presented via APC to lymphocytes and initialize the adaptive B- and T-cell- responses. In comparison, the intestine epithelium and the underneath lying lamina propria accommodate substantially the so-called effector cells. These comprises mainly of antibody secreting plasma cells, macrophages, dendritic cells and a big population close akin to the epithelium, the intraepithelial lymphocytes (IEL).

4.4 Induction of antibody response

The best-known structures of GALT are the Peyer's Patches (PP). They belong to the secondary lymphatic organs that are divided alike the lymph nodes (LN) in B- and T-cell areas. Unlike LN, the PP do not have access to afferent lymphatic vessels, instead of that the antigens are directly gripped from the intestinal lumen. This active antigen intake occurs via specialized enterocytes, where diverse antigens – including commensal and pathogen bacteria, viruses as food components – are transported to the PP. [54] Hence, it is obvious that the intestinal immune system does not seek the best possible isolation from luminal antigens, but a regulated immune response to keep the healthy balance between immune tolerance and protective immune reaction. The main task of the PP is the production of immunoglobulin A (IgA) producing plasma cells. [55]

There are constitutive huge germinal centers in the PP, which is a sign for enduring B-cell activation. In the germinal centers the affinity maturation and the category change of the antibody building plasma cells takes place, whereas in the PP the production of the IgA antibodies is preferred. New built plasma cells leave the PP, reach the blood via mLN, and finally migrate back to the intestine. This process demonstrates the relevant characteristics of the GALT: in GALT induced immune responses act specific in the intestine. Therefore, the activated B-cells in the PP are equipped with address molecules, which allow them later to migrate back as plasma cells into the intestine. Relevant surface molecules that are involved in this process are β7-Integrin and the chemokine receptor CCR9. These two factors bind ligands that are almost exclusively produced in the intestine and permit selective roam into the intestine. For the development of this address-code, the vitamin A metabolite retinoic acid is necessary, which is produced in a high concentration by the immune cells of the GALT. Therefore, for the effectiveness of an immune response it is important in which compartment it takes place. If plasma cells are produced under lower retinoic acid concentration in skin draining LNs or in the spleen, they do not have the address information to migrate into the intestine. This principle might be useful in the future for the development of vaccines against enteropathogenic microbes.

To detect the immune cells in the GIT, the following antigens were targeted. CD45 is a surface protein that is expressed on all leukocytes (granulocytes, lymphocytes and monocytes) and assists in cell activation. CD8 is a protein that is a co-receptor of the T-cell receptor (TCR), which interacts with MHC I complex (MHC I receptor + intracellular foreign peptide). Essential representatives are cytotoxic T-cells, but also found on nk cells (natural killer cells), thrombocytes and DC (dendritic cells). CD4 is a glycoprotein that is found on the surface of monocytes, macrophages and T-helper cells. It is a co-receptor of the TCR and plays a role in the recognition of MHC II complex (MHC II receptor + extracellular foreign peptide). [56] $CD4^+CD8^+$ double positive (DP) where both structures are expressed on immature thymocytes before they differentiate into $CD4^+$ or $CD8^+$ SP (single positive). [57] However, several studies have shown that

$CD4^+CD8^+$ T-cells are a distinct existing mature population. [58] Although associated with multiple human diseases and disorders, the function of these cells is poorly described. But $CD4^+CD8^+$ DP cells seem to play a pivotal role as potent immune suppressors or as cells with high cytotoxic abilities. In IBD, they function as suppressors and in cancer (e.g. breast, colorectal) they are both suppressive and cytotoxic. $CD8^+CD4^-$ SP and $CD8^-CD4^+$ SP belong to the thymocytes and are an intermediate step of premature cells, which will differentiate into $CD8^+$ and $CD4^+$. γδT Is a subset of T-cells (belongs to IEL), which is generally enriched in epithelial and mucosal tissues where it is thought to be the first defense barrier. [59] Moreover, it plays a role in phagocytose tumor antigens and can induce DC maturation. Overall, these regulatory cells can process a wide range of antigens for presentation and stimulate other immune cells. [60]

4.5 Flow Cytometry

4.5.1 What is Flow Cytometry?

With the help of flow cytometry big populations of loose cells in a liquid medium can be detected rapidly. For instance, in immunology it is used to separate and profile immune-cell subtypes due to their size and morphology.
When further information is required, antibodies tagged with fluorescent dyes and directed against highly specific surface antigens (e.g. cluster of differentiation or CD-markers) can be used to better identify and isolate specific subpopulations within large groups. Compare Figure 2 for the following description.

- Sample cells pass through a narrow channel one by one
- Light is used to illuminate the cells in the channel
- A series of sensors record the light types that are refracted and emitted from the cells

- The detected data from the sensors are integrated to reflect an extensive picture of the samples

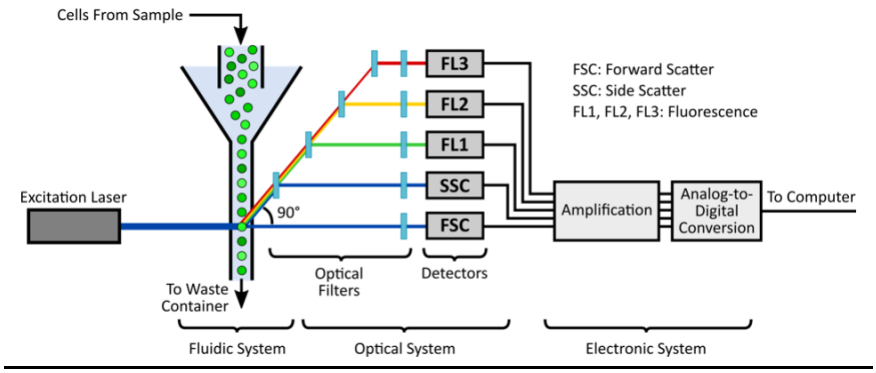


Figure 2) Schematic illustration of flow cytometry [61]

4.5.2 Light types

The technique uses refracted and emitted light to count and identify cells.

Forward scattered light is refracted by a cell in the flow channel and continues in the same direction where it was originally travelling. It is detected by sensors in the light path, and typically used to identify the particle size. The amount of scattered light correlates with the size of a cell and its complexity. Granulocytes, who have a rough surface and contain many vesicles, scatter more light than the smooth B- and T-cells.

Sideward-scattered light passes from the illumination source into the flow channel and is refracted by cells in a direction that is outside of its original light path. Sideward scatter is usually used to determine granularity of a cell, size and structure of its nucleus as well as the amount of the intracellular vesicles. Cells with high inner complexity, such as neutrophils produce a lot of sideward scattered light.

Fluorescent molecules emit fluorescent light after excitation by a compatible wavelength laser. Fluorescent light can be from native fluorescing materials of a cell,

or from fluorescent dyes or fluorescent tagged antibodies that are used to mark a particular surface structure. [62]

4.5.3 Multiparameter analysis

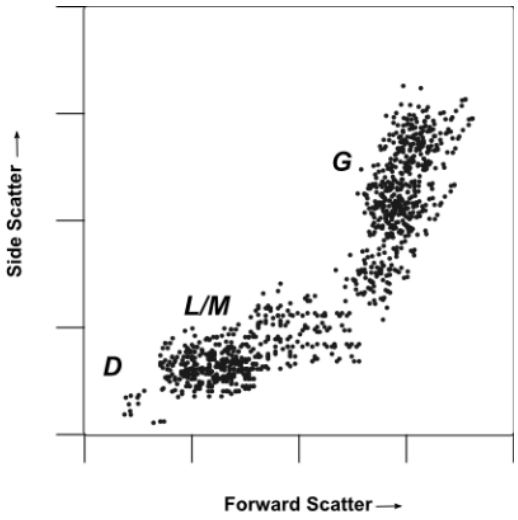


Figure 2.1) Illustration of a dot plot [60]

Dot-plot: each dot represents a cell depending on its FSC and SSC. The outcomes of this are populations, which are characterized by their probable identity. D shows cell debris. L/M represents leucocytes and monocytes, small to medium cells with low inner complexity/granularity (medium FSC and low SSC). G seems to be granulocytes, large cells with high complexity/granularity (both high FSC and SSC).

For specific cell determination FSC and SSC are not sufficient, for example granulocytes include three categories (neutrophil, basophil and eosinophil), which are similar in size and structure. Therefore, the tagging with cell-specific marker provides greater resolution and certainty in profiling complex populations of cells. [62]

5 Materials and methods

5.1 Materials

5.1.1 Bedding and chow

- **Lignocel select**, poplar, cubic; J. Rettenmaier & Söhne GmbH + Co KG (Vienna, Austria)
- **ssniff® R/M-H**, complete feed for rats and mice – maintenance; ssniff Spezialitäten GmbH (Soest, Germany)

5.1.2 Equipment

- **Centrifuge 5418R**; 100 rpm –14000 rpm; 0°C to +40°C; Eppendorf AG (Hamburg, Germany)
- **MACSQuant® Analyzer 10** fluorescence-activated cell sorting (FACS); Miltenyi Biotec (Germany)
- **Heating block**
- **Nanodrop one**; Thermo Fisher scientific (Madison, US)
- **peqGOLD MicroSpin Total RNA Kit**; VWR International GmbH (Erlangen, Germany)
- **peqGOLD Total RNA Kit**; VWR International GmbH (Erlangen, Germany)
- **QuantStudio™** 6 Flex Real-Time PCR System**;** Contains the OptiFlex™ Optics System; applied biosystems® by life technologies™ (California, US)
- **Stirrer** IKAMAG® RCT; Janke & Kunkel GmbH & Co. KG; Ika®-Labortechnik (Staufen i. Breisgau, Germany)
- **ThermoFischer** scientific incubator; Thermo Electron LED GmbH (Zweigniederlassung Osterode am Kalkberg, Germany)
- **Vortex mixer,** lab dancer; VWR Chemical (Fontenay-sous-Bois, France)
- **VWR Micro Star 17R** Microcentrifuge; VWR International bvba; (Leuven, Belgium)

5.1.3 Accessories

- **18 G needle** 100 Sterican B|BRAUN (Hessen, Germany)
- **384-well PCR plate**; Thermo scientific (UK)
- **Centrifuge tubes** 15 mL, 50mL; Corning Inc. (Corning, New York, USA)
- **MACS®SmartStrainers** (30µm); MACS Miltenyi Biotec (Bergisch Gladbach, Germany)
- **MACS®SmartStrainers** (70µm); MACS Miltenyi Biotec (Bergisch Gladbach, Germany)
- **Microcentrifuge tubes** (1,5ml & 2ml) Eppendorf Austria GmbH (Vienna, Austria)
- **MicroPipette**, Research plus; 2.5µl, 10µl, 20µl, 100µl, 200µl, 1000µl; Eppendorf Austria GmbH (Vienna, Austria)
- **Pipette tip**; 10ul, 50ul, 200ul, 1000ul SARSTEDT AG & Co. KG (Nuembrecht, Germany)
- **Pipette with tip** 10mL, 20mL sterile single packed; greiner bio-one (Kremsmuenster, Austria)
- **qPCR Seal** adhesive seal sheet; 4titude® (Surrey, UK)
- **Syringe** 1ml, 10ml; Omnifix®-F B|BRAUN (Hessen, Germany)

5.1.4 Reagents

- **Aprotinin** (C284H432N84O79S7) from bovine lung; CAS: 9087-70-1; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)
- **BSA** ≥98%, Bovine Serum Albumin; CAS: 9048-46-8; Sigma-AldrichChemie GmbH (Steinheim, Germany)
- **DNase I** grade II, from bovine pancreas; CAS: 9003-98-9; Roche Diagnostic GmbH (Mannheim, Germany)
- **Dispase II,** Protease from Bacillus polymyxa; CAS: 42613-33-2; Sigma-AldrichChemie GmbH (Steinheim, Germany)

- **EDTA** ≥98.0%, (Ethylenediaminetetraacetic acid, (HO2CCH2)2NCH2CH2N(CH2CO2H)2); CAS: 60-00-4; Sigma-AldrichChemie GmbH (Steinheim, Germany)
- **EtOH 70% denatured** (C2H6O); CAS: 64-17-5; Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
- **FBS**; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)
- **HEPES** (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, (C8H18N2O4S)); CAS: 7365-45-9; Sigma-AldrichChemie GmbH (Steinheim, Germany)
- **PBS**; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)
- **Primers**; Introgen; ThermoFisher scientific

	Forward		Reverse
NOD2	GGCAACAGTGTAGGTGATAAGGG		TAGTGACTTGTTCTTCTCCAGCATC
Myd88	GCACCTGTGTCTGGTCCATT		TGTTGGACACCTGGAGACAG
IRF7	GAGACTGGCTATTGGGGGAG		GACCGAAATGCTTCCAGGG
IRF1	CCCAGCTCTTGCTTTCGGA		AAGCCCAGTAGTTCACGACC
TNF	CAGGCGGTGCCTATGTCTC		CGATCACCCCGAAGTTCAGTAG
Reg3β	TGGGAATGGAGTAACAATG		GGCAACTTCACCTCACAT
STAT1	CAGTATGATGAGCACAGTA		AAGTCCTTCAGAGTAACAG
Oas1a	ATGGAGCACGGACTCAGGA		TCACACACGACATTGACGGC
Rsad2	TGCTGGCTGAGAATAGCATTAGG		GCTGAGTGCTGTTCCCATCT
PPAR	AGACCCAGCTCTACAACAGGCC		CAGACTCGGCACTCAATGGCCA
Eef1a1	CCTGGCAAGCCCATGTGT		TCATGTCACGAACAGCAAAGC
mTLR3	AGCATCAAAAGAAGCCGAAA		CTTGCTGAACTGCGTGATGT

- **Propidium iodide** (C27H34I2N4); CAS: 25535-16-4; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)
- **RPMI-1640 Medium**; Sigma-AldrichChemie GmbH (Steinheim, Germany)
- **Sodium acetate**; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)
- **Sybr green**; PerfeCTa® SYBR® Green FastMix®; Quantabio (Massachusetts, US)
- **Trypan blue**
- **Viability antibodies**
  - CD45 Monoclonal Antibody (30-F11), eFluor 450, eBioscience™; Life Technologies Austria Zweigniederlassung (Vienna, Austria)
  - Ly6G FITC Rat anti-Mouse Ly-6G Clone 1A8; BD Biosciences (San Jose, US)

- Ly6C Monoclonal Antibody (HK1.4), PerCP-Cyanine5.5, eBioscience™; Life Technologies Austria Zweigniederlassung (Vienna, Austria)
- CD3 PE-CF594 Hamster Anti-Mouse CD3e Clone 145-2C11; BD Biosciences (San Jose, US)
- CD4 APC Rat Anti-Mouse CD4 Clone RM4-5; BD Biosciences (San Jose, US)
- CD8 PE-Cy™7 Rat Anti-Mouse CD8a Clone 53-6.7; BD Biosciences (San Jose, US)
- γδT FITC Hamster Anti-Mouse γδ T-Cell Receptor Clone GL3; BD Biosciences (San Jose, US)

- **Water** for molecular biology, DEPC-treated and sterile filtered; Sigma-Aldrich Chemie GmbH (Steinheim, Germany)

5.2 Preparation of solvents for extraction and digestion

Extraction media (per small intestine): 30ml RPMI + 46,5µl 10% (w/v) dithiothreitol (DTT) + 60µl 0,5 M EDTA + 500µl fetal bovine serum (FBS). DTT added immediately before use.

Digestion media (per small intestine): 22,5ml RPMI + 0,375µg DNase + 0,375µl HEPES + 18,75mg collagenase II + 5ml 6% FBS. Enzymes added immediately before use.

5.3 Animals and experimental protocol

For the experiment, male C57BL/6NRj mice were purchased from Janvier Inc. Labs (Le Genest, France). They were kept in cages in an animal house, under standard SPF (specific-pathogen-free) conditions using Tecniplast IVC system (cage type 2L, blue line). The room temperature and its humidity were electronically controlled and constantly supervised. The lighting was set to a 12-hour light-dark cycle. The chow, SD (keto control), keto and keto (LCT) mice lived in groups of maximum four per cage, with free access to food and water. The IF, FMD and CR mice were kept in single cages to control the food intake and prevent fights of aggressive mice. The animals were

divided in two control and five diet groups. Each group comprises of eight mice. Thus, in total 56 mice were investigated.

Chow diet lasted for eight weeks. This group had food access *ad lib* (=*ad libitum*) and the body weight was measured weekly. The food (ssniff® R/M-H) was ordered by ssniff Spezialdiäten GmbH. The composition is listed in Table 2. For detailed composition of the food, see Table 4.

Food composition	[%]
Dry matter	87,7
Crude protein	19,0
Crude fat	3,3
Crude fibre	4,9
Crude ash	6,4
Starch	36,5
Sugar	4,7

Table 2) Main composition of chow

SD (keto control) lasted for eight weeks. This group had *ad lib* food access and the body weight was measured weekly. Composition of the food described in Table 3

Keto (LCT) diet lasted for eight weeks. This group either had *ad lib* food access and the body weight was measured weekly. For the composition of the food, see Table 3.

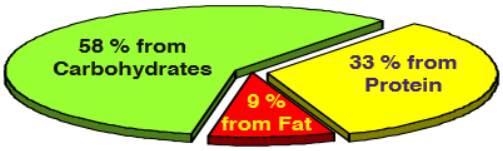
Keto diet lasted for eight weeks. Similar to the previous groups *ad lib* dietary. For the composition of the food, see Table 3.

	SD	Keto (LCT)	Keto
	Control 16% protein	**100% LCT**	**70%LCT/30% MCT**
Crude Protein %	16,1	13,9	13,9
LCT %	7	66,9	36,7
MCT8 %	0	0	15
MCT10 %	0	0	15
Sugar %	6	1	1
Starch %	51,2	1,8	1,8
Crude fiber%	9,9	9,9	9,9
Crude ash%	4,4	4,4	4,4
	per Kg	per Kg	per Kg
Vitamin A (IU)	15000	15000	15000
Vitamin D_3 (IU)	1500	1500	1500
Vitamin E (mg)	150	150	150
Vitamin K_3 (mg)	20	20	20
Vitamin C (mg)	30	30	30
Copper (mg)	11	11	11

Table 3) Main components of the diets (SD, keto (LCT) and keto)

Crude Nutrients	[%]
Dry matter	87.7
Crude protein (N x 6.25)	19.0
Crude fat	3.3
Crude fibre	4.9
Crude ash	6.4
N free extracts	54.1
Starch	36.5
Sugar	4.7

Energy	[MJ/kg]
Gross Energy (GE)	16.3
Metabolizable Energy (ME) *	12.8



Minerals	[%]
Calcium	1.00
Phosphorus	0.70
Sodium	0.24
Magnesium	0.22
Potassium	0.91

Fatty acids	[%]
C 14:0	0.01
C 16:0	0.47
C 16:1	0.01
C 18:0	0.08
C 18:1	0.62
C 18:2	1.80
C 18:3	0.23
C 20:0	0.01
C 20:1	0.02
C 20:5	——
C 22:6	——

Amino acids	[%]
Lysine	1.00
Methionine	0.30
Met+Cys	0.65
Threonine	0.68
Tryptophan	0.25
Arginine	1.14
Histidine	0.44
Valine	0.88
Isoleucine	0.76
Leucine	1.30
Phenylalanine	0.85
Phe+Tyr	1.43
Glycine	0.80
Glutamic acid	3.90
Aspartic acid	1.61
Proline	1.25
Alanine	0.79
Serine	0.89

Vitamins	per kg
Vitamin A	15,000 IU
Vitamin D_3	1,000 IU
Vitamin E	110 mg
Vitamin K (as menadione)	5 mg
Thiamin (B_1)	18 mg
Riboflavin (B_2)	23 mg
Pyridoxine (B_6)	21 mg
Cobalamin (B_{12})	100 µg
Nicotinic acid	135 mg
Pantothenic acid	43 mg
Folic acid	7 mg
Biotin	525 µg
Choline-Chloride	2,990 mg
Inositol	100 mg

Trace elements	per kg
Iron	179 mg
Manganese	69 mg
Zinc	94 mg
Copper	16 mg
Iodine	2.2 mg
Selenium	0.3 mg
Cobalt	2.1 mg

Feed composition
descending order of feedingstuffs (FMV)
Grain and grain by-products, oil seed products, minerals, vegetable oils, vitamins, trace elements.

Table 4) Detailed composition of chow

CR diet was performed for two weeks. Mice were fed with ssniff® R/M-H (Table 4) 75% of their daily food intake. Since the daily intake was restricted, these mice were in single cages. The measured portion of food was given daily at 5 pm after measuring the mice's body weight.

IF diet, for the experiment the complete alternate-day fasting was used, which means 24 hours eating *ad lib* followed by 24 hours abstinence of energy-containing food or beverages. IF-protocol was performed for two weeks. The animals were kept in single cages to prevent fights between aggressive mice. The body weight was measured before the food was delivered.

The FMD protocol consisted of three cycles, the first cycle involves four fasting mimicking days and seven days refeeding with chow. Cycle number two and three included five days fasting mimicking followed by seven days refeeding. First day food intake restricted to 50%, on day two-four or rather five, intake reduced to 10%, followed by seven days refeeding, and then the cycle started again. Mice were housed in single cages. Ingredients were: hydrogel, broth, vegetable mix, extra virgin olive oil (EVOO), essential fatty acids and glycerol. Diet provided by Prof. Valter D. Longo.

5.4 Tissue collection

After all diets were successfully completed, the mice were euthanized with isoflurane and blood was drawn by cardiac puncture then the mice were dissected. The blood was mixed with 10µl ethylenediaminetetraacetic acid (EDTA) to ensure blood in fluid form. Then 20µl aprotinin and 10µl dipeptidyl peptidase (DPP) IV were added and the sample was centrifuged for 10min at 3,600rpm 4°C. The plasma was sampled and collected in liquid nitrogen and after the dissection stored at –80°C.

The following organs were collected: spleen, PP, LN and Si. The eWAT's (epididymal white adipose tissue) weight was measured while dissection of the mice.

5.5 Sample preparation for flow cytometry

Based on the Protocol 'Isolation and Flow Cytometric Characterization of Murine Small Intestinal Lymphocytes' by Cheryn J. Couter and Neeraj K. Surana following work was done:

5.5.1 Extraction of immune cells from spleen, PP and LN

Spleen was cut in small pieces on 30µm strainer membrane, while PP and LN were directly placed on the strainer. In two to three steps, alternately 1-2ml PBS was added and the organ minced, continued by rinsing the organ with PBS to reach a volume of 10ml. The samples were centrifuged for 5min at 500g 4°C. After removing the supernatant, the cells were resuspended in 10ml PBS and the last step was repeated to wash the sample. To eliminate the erythrocytes lysis buffer was added to the spleen sample. After washing, the cells were resuspended in 1ml PBS. For the cell count 5µl Trypan blue was added to 45µl sample.

5.5.2 Staining

10^6 cells were pipetted to 96 well plate, for L/D control half of the cells were killed by incubating for 1min 90°C. Live/dead stain (DAPI/Propidiumiodid) in a dilution of 1:2000 in 200µl PBS was added and the plate incubated for 30min RT (room temperature) in darkness. Afterwards the plate was centrifuged, the supernatant removed and the samples washed with 100µl PBS/BSA 5%. Upon adding 25µl PBS/BSA 5% and incubating again for 30min, dark 4°C, the cells were washed and resuspended in 100µl PBS/BSA and ready for the FACS measurement.

Target	Channel
Viability	APC-Cy7
CD45	Bv421
Ly6G	FITC
Ly6C	PerCP-Cy5.5
CD3	PE-Texas Red
CD4	APC
CD8	PE-Cy7

gdT	FITC
Fcy Block (aCD16 + aCD32)	Unlabelled

Table 5) Antibodies that were used for flow cytometry

5.5.3 Extraction of IEL and LP from the Si

After the Si was isolated, liberated from fat tissue, flushed and cut in approximately 8cm segments the inside tissue was turned out (the inside surface was outside). The tissue segments were placed in a cup containing 30ml of extraction media and a stir bar on a stirrer at 500rpm for 15min at 37°C. Afterwards the tissue pieces were strained from the IEL containing supernatant (saved for further analyze) and exempted from residual extraction media and mucus. Tissue fragments were minced with a scissor and incubated in 25ml digestion media on a stirrer at 500rpm for 30min at 37°C. Halfway through the digestion (around 15min), the solution containing the Si fragments was pipette up and down to break up any clot of tissue. The digested tissue and saved supernatant containing IEL were filtered through a 100μm cell strainer each into a 50ml tube (Si IEL and Si LP). Then the strainer were rinsed with 20ml of RPMI containing 10% FBS and the filtered solutions centrifuged at 500g for 10min at 4°C. Subsequently the pellet was resuspended in 1ml of RPMI containing 10% FBS and the resuspended cells filtered through 40μm cell strainer into a 50ml tube (of course apart from each other IEL and LP). After rinsing the strainer with 20ml RPMI containing 10% FBS, centrifuging the filtered solutions and resuspending the pellet in 1ml RPMI containing 2% FBS, the cells were ready for counting. The well plate was maximal with two million cells/well (100-200μl) loaded. Then spinned for 5min at 300 rpm at 4°C, resuspended in live/dead stain (DAPI/Propidiumiodid) and incubated for 30min in the dark RT. Furthermore, the plate was spinned, washed with FACS buffer twice and resuspended in 25μl FACS buffer plus Ig (Immunoglobulin). After incubating for 30min at dark 4°C, washing and adding buffer the plate was ready for FACS measurements. During the whole work the tissue was stored at 4°C.

5.6 peqGOLD total RNA isolation protocol for Si

Before starting, 1,5ml tubes (Eppendorf tubes) with the lettering RNA, Si and mouse ID for each mouse were prepared as well as 1ml syringes (with blue needles G23) and dry ice for the storage of the samples.

Homogenization and lysis: a small piece (about 40mg) of the frozen tissue was cut and placed in a tube containing 600µl RNA Lysis Buffer. The sample was pipette up and down with the syringe and needle, till the whole tissue was lysed. Then the lysate was directly transferred into a DNA Removing Column (green) placed in a 2ml CT (Collection Tube) and centrifuged for 1min at 12.000g

Load and bind: The flow-through lysate (~550µl) was transferred into a new 1,5ml tube plus an equal volume of 70% EtOH (Ethanol) and mixed thoroughly by vortexing. The PerfectBind RNA Column (orange) in a new 2ml CT was loaded with lysate and centrifuged at 10.000g for 90sec. The flow-through liquid and CT were discarded.

Wash: Into the PerfectBind RNA Column, placed in a new 2ml CT 500µl RNA Wash Buffer I were added and then centrifuged at 10.000g for 30sec. the flow-through liquid was trashed and the CT reused for the second washing step. Into the PerfectBind RNA Column 600µl RNA Wash Buffer II (concentrate + 4x volume of EtOH 100%) was added and centrifuged at 10.000g for 30sec. The flow-through liquid was discarded and the washing step with RNA Wash Buffer II repeated.

Dry: The PerfectBinding RNA Column containing the RNA was placed in the empty CT and centrifuged for 2,5min at 10.000g. This step is essential to completely dry the column matrix and remove the EtOH.

Elution: RNA containing Column was placed in a 1,5ml Tube and eluted with 100µl sterile RNAse-free dH_2O for 90sec at 5.000g.

Subsequent, the RNA was quantified and its purity checked with the help of NanoDrop spectrophotometry.

5.7 peqGOLD MicroSpin total RNA isolation protocol for LN and PP

This kit is for the isolation of up to 50μg of total RNA from small amounts of tissue. Before starting it is necessary that the samples and buffer are equilibrated to RT. All steps were carried out at RT.

Homogenization and lysis: About 5mg of the tissue were placed in a tube containing 300μl RNA Lysis Buffer. The sample was pipette up and down with the syringe and needle, till the whole tissue was lysed.

DNA removal: The lysate was directly applied to a DNA Removing Column (green) placed in a 2ml CT (Collection Tube) and centrifuged for 1min at 12.000g. The DNA Removing Column was discarded. 200μl EtOH 70% were added to the filtrate and mixed thoroughly with a pipette.

Load and bind: The PerfectBind RNA Column (blue) in a new 2ml CT was loaded with lysate and centrifuged at 10.000g for 90sec. The flow-through liquid and CT were discarded.

Wash: Into the PerfectBind RNA Column, placed in a new 2ml CT 500μl RNA Wash Buffer I were added and then centrifuged at 10.000g for 1min. The flow-through liquid was trashed and the CT reused for the second washing step. Into the PerfectBind RNA Column 500μl RNA Wash Buffer II (concentrate + 4x volume of EtOH 100%) was added and centrifuged at 10.000g for 1min. The flow-through liquid was discarded and the washing step with RNA Wash Buffer II repeated.

Dry: The PerfectBinding RNA Column containing the RNA was placed in the empty CT and centrifuged for 2min at 10.000g. This step is essential to completely dry the column matrix and remove the EtOH.

Elution: RNA containing Column was placed in a 1,5ml Tube and eluted with 100μl sterile RNAse-free dH_2O for 1min at 6.000g.

For the quality control nanodrop was used.

5.8 NanoDrop

Based on the Beer-Lambert Law the following absorbances were measured and compared. [63]

Absorbance at 260nm: Nucleic acids absorb UV light at 260nm due to the aromatic base within its structure. Purines (adenine and guanine) and pyrimidines (thymine, cytosine and uracil) both have peak absorbances in this wavelength. Therefore it is standard for quantification of nucleic acid samples.

Absorbance at 280nm: Proteins and phenolic compounds have usually a strong absorbance at 280nm. Responsible for the absorbance are aromatic amino acid moieties such as tryptophan, tyrosine, phenylalanine and histidine. In the same way phenol groups of organic compounds absorb strongly in this wave range.

Absorbance at 230nm: Many organic compounds plus TRIzol, chaotropic salts and peptides absorb light in this wave range.

A260/280 ratio is generally used to determine protein contamination of a sample. A low ratio indicates a contamination by residual reagents used in extraction protocol. For a good purity quality the ratio should not be under 1,8. [64]

A260/230 ratio is used to indicate the presence of organic contaminants, for example chaotropic salts, peptides or other compounds. The ratio should be above 2 and is necessary for the further reverse transcription. [65]

5.9 How to concentrate and purify extracted RNA to higher 260/239 ratio

For 100µl RNA, 10µl sodium acetate 3M in H_2O and 300µl -20°C EtOH 99% were used. The whole sample was incubated for 2-16 hours in pre-cooled racks at -20°C and afterwards centrifuged for 30min at 12.000rpm 4°C. Then 300µl 4°C EtOH 75% were added and the sample vortexed, followed by 15min centrifuging at 4°C. Supernatant was carefully decanted and the residual drops removed with a tip. Then dry the

remaining whitish pellet (RNA). Before it turns completely white, 50µl RNase free H_2O were added. (If it turns totally white, it dissolves very hard). Quality was checked with the help of NanoDrop.

5.10 Converting isolated RNA to cDNA

Labeled Tubes (tissue, ID, cDNA) were prepared for each sample. Three heating blocks were prepared at 22°C, 42°C and 85°C. With the help of the NanoDrop quantification sample amount in µl was calculated that was necessary to get 1mg RNA. Then it was calculated how much µl RNase free H_2O was necessary to reach a defined volume of 20µl, whereof 20% should be buffer. The last component is the RT (reverse transcriptase) from which 1µl is required to transcript 1mg RNA. This means in total: 1mg RNA + Master Mix (1µl RT + buffer (20% of final volume)) + the amount of RNase free H_2O that is necessary to reach the defined volume (20µl). Generally a Master Mix consisting of 1µl RT + 4µl buffer was used, except for LN, since the RNA amount was so little more sample was used and the amount of buffer was adapted to 20% of total volume. The Master Mix was prepared daily fresh by pipetting QScript Reaction mix Buffer first in a tube and then adding the QScript RT (because RT is viscose). First of all the RNase free H_2O was pipette in a tube, then the sample containing RNA was pipetted on the tube-wall and the Master Mix was pipetted on the other side of the tube-wall. Thus, the tubes were shortly centrifuged to mix thoroughly the content. Incubated 5min at 22°C, 30min at 42°C and 5min at 85°C then let the sample cool down for 5min before adding 380µl H_2O (RNase free). The samples were centrifuged and then stored at -20°C.

5.11 Relative quantification

The start of the exponential phase is used for the quantification and represents the cycle where the fluorescence is first significant above the background-fluorescence. It

is defined as Ct (threshold cycle). In the relative quantification the gene-expression of the target gene is normalized with a reference gene. This means the gene of interest is related to an ubiquitary and homogeneous expressed gene. The advantages are reduction of variance of expression-results - due to different RNA isolation efficiency, which leads to mistakes of RT - since both target and reference gene are likewise affected. The calculation is carried out via $\Delta\Delta$Ct (delta-delta Ct) method. In the first step, for every investigated sample the Ct value of the reference gene is subtracted from the Ct of the target gene (ΔCt = Ct target gene – Ct reference). After this normalization the ΔCt value of a control (chow-group) is subtracted from the ΔCt of the experimental treated samples (other diets), which leads to the so-called $\Delta\Delta$Ct method of computation. The relative expression difference of a sample between treated and control, normalized with a reference results in the arithmetic formula $Ratio = 2^{-\Delta\Delta Ct}$. [66] For each group the average and standard deviation is computed and the groups are compared with each other by using the t-test.

5.12 Statistical analysis

Statistical analysis was performed with Microsoft Excel. T-Tests and correction for multiple testing were used to show the significant difference between the groups. Significance was defined by a p-value < 0,0071.

6 Results

6.1 Body weight

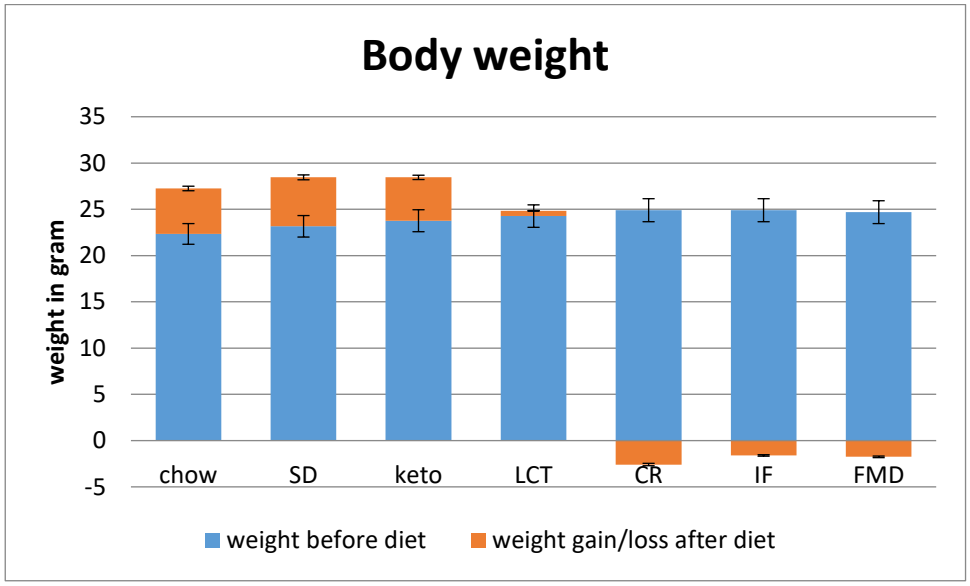


Figure 3) The weight of each group before and after diet

The chow control group fed *ad lib* shows a continuous weight gain. The average weight gain is 23%.

The keto control group was also fed *ad lib* but with different chow (Table 3). Continuous weight gain is observed. Total weight gain is averagely 20%.

The keto-diet group averagely gained 24% weight.

The keto (LCT) group gained about 3% weight.

In the CR group a continuous body weight loss can be observed. In mice a two weeks CR is sufficient to see beneficial effects. The average loss of weight amounts ~15%.

The IF mice show alternately weight gain in the feeding period and weight loss in the fasting period. After two weeks the averagely weight loss on the fasting day was approximately 7%.

This apparent fasting diet (FMD) group lost weight in each cycle but gained it back in the refeeding phase. The last cycle ends with an average weight loss of 11%.

6.2 Weight of eWAT

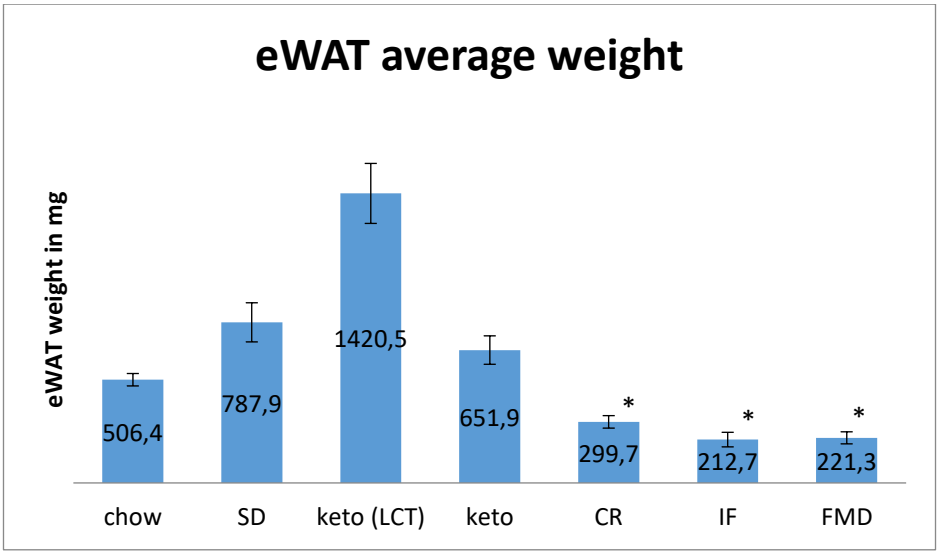


Figure 4) The average eWAT weight of each group

While dissecting the weight of epididymal white adipose tissue (eWAT) was scaled in mg. The keto (LCT) diet with 100% LCT share of fat shows the highest eWAT proportion. While in CR- ($p<0,0001$), IF- ($p<0,0009$) and FMD ($p<0,0002$) group less eWAT is observed in comparison to chow, SD and keto groups.

6.3 Flow Cytometry

6.3.1 Spleen

CD8 is significantly increased in CR ($p<0,0001$) compared to chow, SD and keto. IF either exhibits a significant amount of CD8 compared to SD ($p=0,0047$) and keto ($p=0,0035$), see Figure 7.

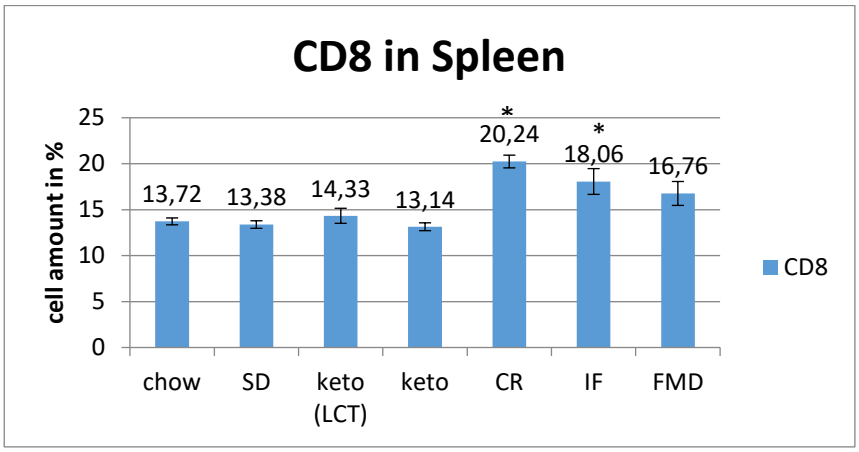


Figure 5) Chart of CD 8 cell amount in each group

SD (p=0,0029) and FMD (p<0,0001) show a significant reduction in CD4 compared to chow. FMD (p<0,0001) exhibits the lowest count of CD4. While CR shows the highest production in comparison to chow (p=0,0006), keto (p=0,0001) and FMD (p<0,0001). Followed by IF, which is higher than chow (p=0,0008), SD (0,0002) and keto (0,0001) as illustrated in Figure 8.

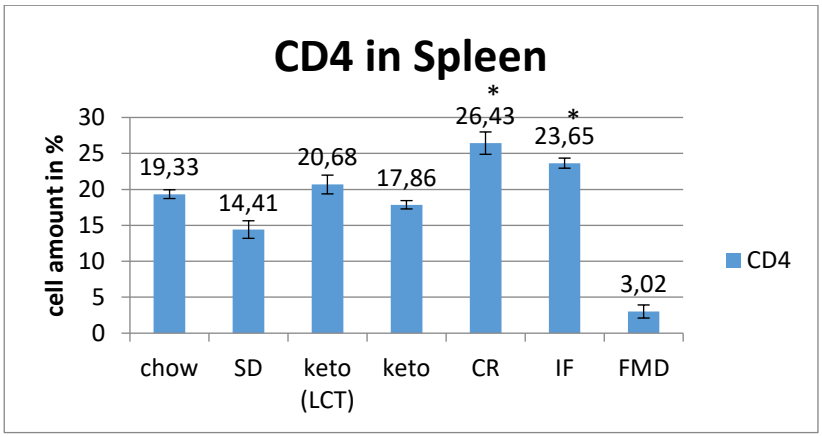


Figure 6) The cell count of CD4 for each group

FMD deliver significant results of $CD8^{+}CD4^{+}$ compared to chow (p=0,0032), keto (p=0,0066) and CR (p=0,0042) as shown in Figure 9.

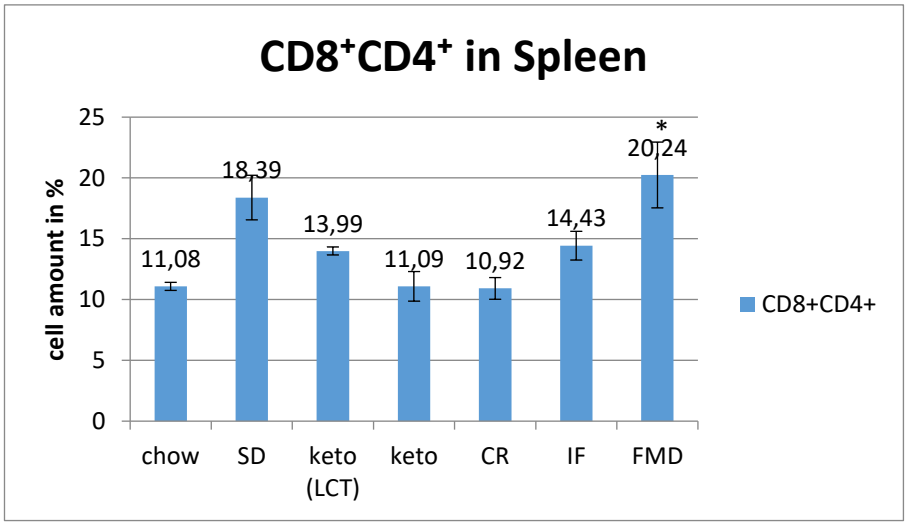


Figure 7) The cell count of $CD8^+CD4^+$ each group

CR and IF possess high presence of $CD8^+CD4^-$ in comparison to chow ($p<0,0001$ for both), SD ($p=0,0001$; $p=0,0018$) and keto ($p<0,0001$ for both), as demonstrated in Figure 10. In addition is $CD8^+CD4^-$ in CR higher than in FMD ($p=0,0032$).

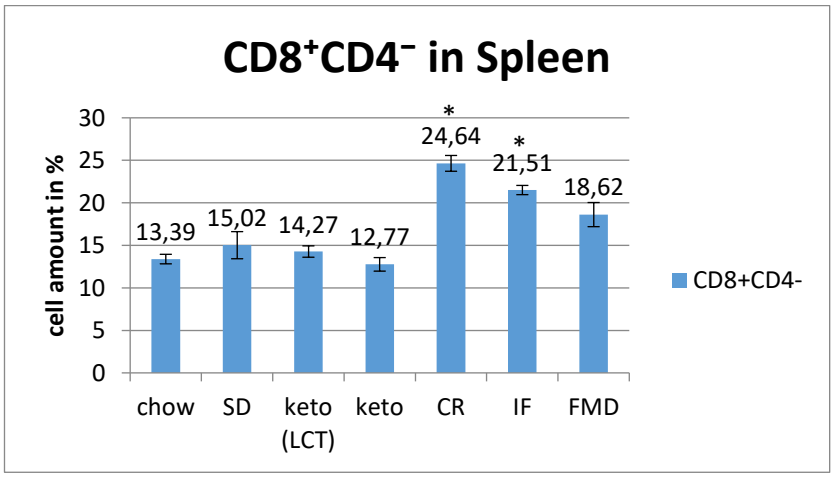


Figure 8) The cell count of $CD8^+CD4^-$ for each group

FMD shows the lowest amount of $CD8^-CD4^+$ compared to all other groups $p=\leq 0,0001$, see Figure 11.

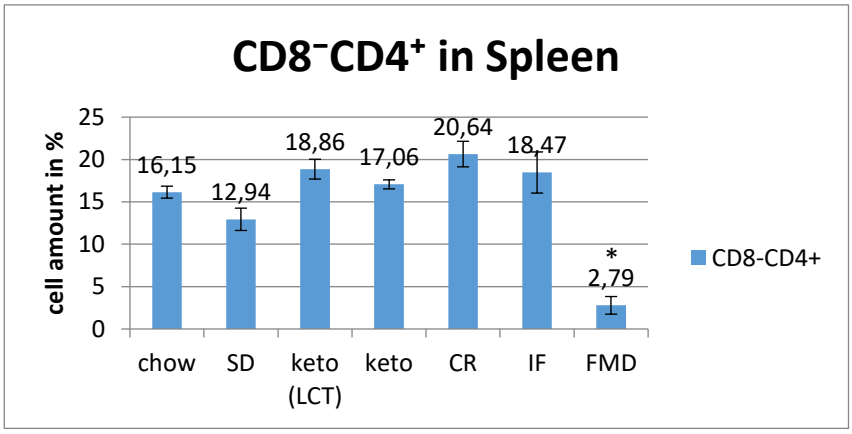


Figure 9) The cell count of CD8⁻CD4⁺ for each group

6.3.2 LN

Keto shows the lowest count of CD8⁺CD4⁺ in comparison to chow (p=0,0003) and FMD (p=0,0014) as shown in Figure 11.

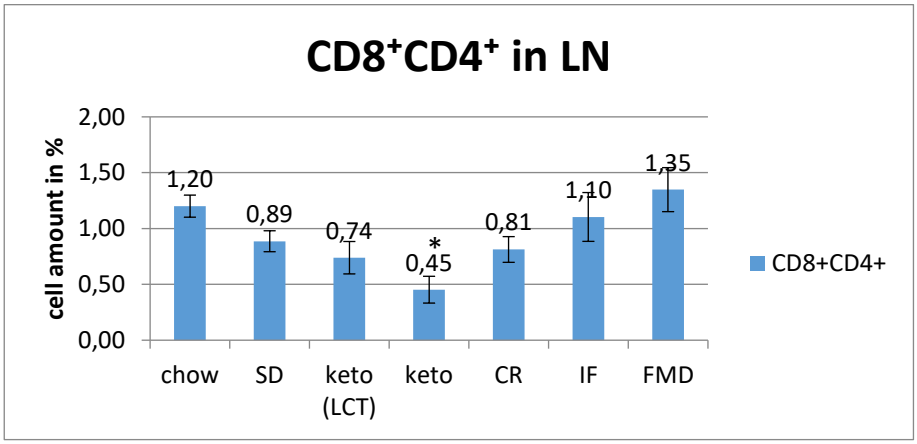


Figure 10) The cell count of CD8⁺CD4⁺ for each group

It is apparent that keto has a lower expression of the CD8⁺CD4⁻ antigen compared to chow (p=0,0022), SD (p=0,0025), keto (LCT) (p=0,0001), CR (p=0,0001) and FMD (p=0,0003). Moreover, FMD is higher than chow (p=0,0004) and keto (LCT) (p=0,0002) and CR is higher than chow (p=0,0005), see Figure 13.

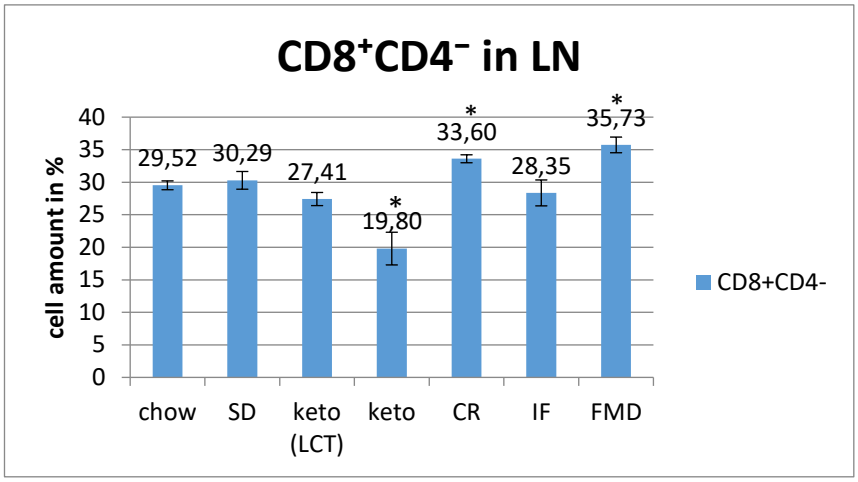


Figure 11) The cell count of $CD8^{+}CD4^{-}$ for each group

Keto exhibits the lowest expression of CD8 and is significant to chow (p=0,0004), SD (p=0,0012), CR (p<0,0001) and FMD (p<0,0001). Contrarily FMD and CR show higher expression of the antigen in comparison to chow (p=0,0006; p=0,0009) and keto (LCT) (p=0,0002; p=0,0001), see Figure 14.

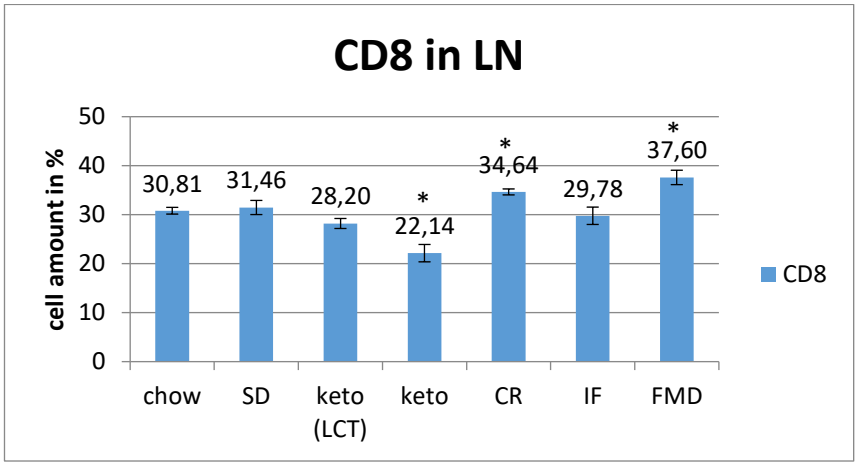


Figure 12) The cell count of CD8 for each group

Keto seems to have lower CD4 than chow (p=0,0066) and CR (p=0,0022), as demonstrated in Figure 15.

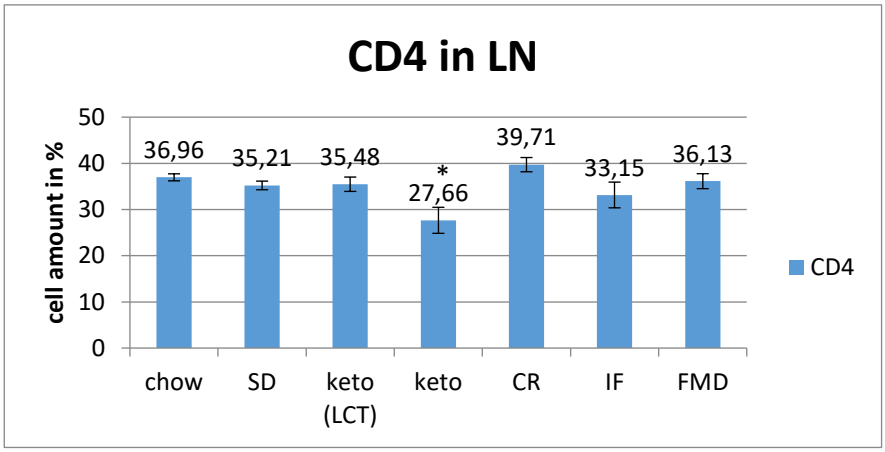


Figure 13) The cell count of CD4 for each group

6.3.3 PP

Significant reduction of CD45 in keto and keto (LCT) compared to chow ($p<0,0001$; $p=0,0004$), SD ($p=0,0001$; $p=0,0057$), IF ($p<0,0001$; $p=0,0003$) and FMD ($p<0,0001$; $p=0,0001$). In addition, CD45 in keto is lower than CR ($p=0,0048$) and CR shows lower amount of the antigen in comparison to chow ($p=0,0039$), IF ($p=0,0031$) and FMD ($p=0,0006$) as demonstrated in Figure 16.

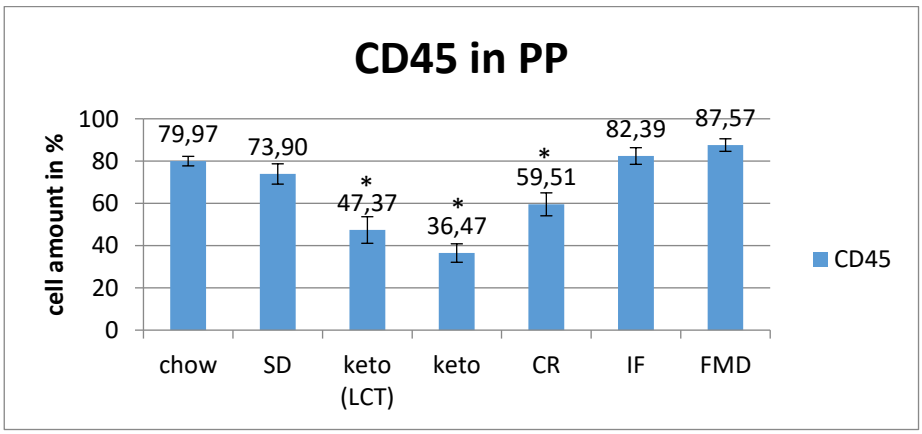


Figure 14) The cell count of CD45 for each group

Keto shows the lowest expression of the $CD8^{-}CD4^{+}$ antigen, compared to chow ($p=0,0021$), SD ($p=0,0001$), keto (LCT) ($p<0,0001$) and FMD ($p=0,0022$) as illustrated in Figure 17.

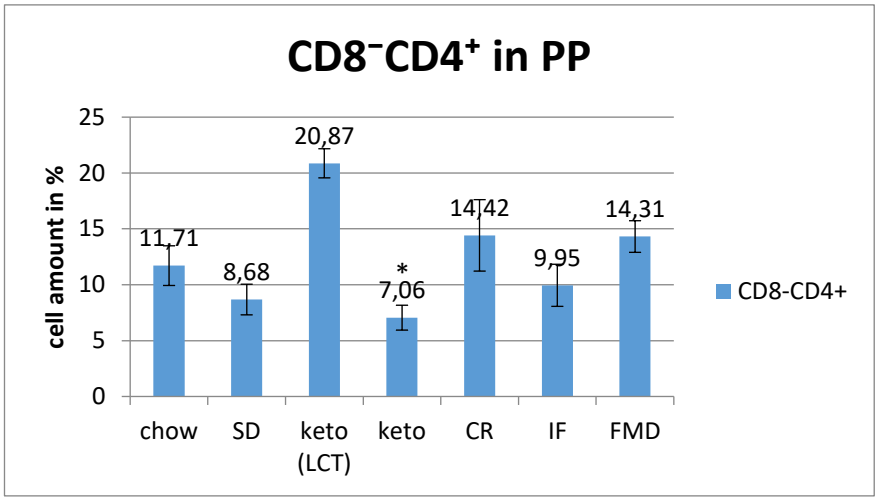


Figure 15) The cell count of CD8⁻CD4⁺ for each group

CD8 is higher expressed in CR compared to chow (p=0,0003), keto (LCT) (p=0,0030) and keto (p=0,0003). Furthermore, it is higher expressed in FMD compared to chow (p=0,0004) and keto (0,0014) as pointed in Figure 18.

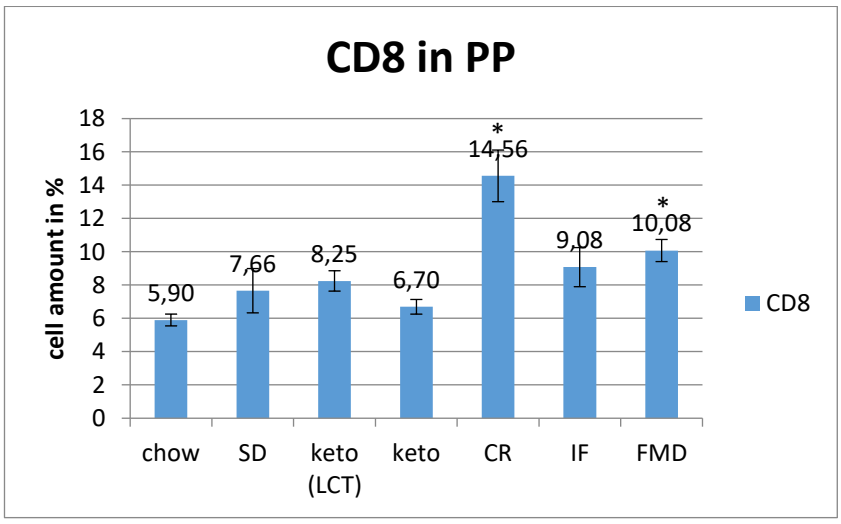


Figure 16) The cell count of CD8 in each group

γδT antigen is increased in keto compared to chow (p=0,0036), SD (p=0,0021), keto (LCT) (p=0,0019), CR (p=0,0014), IF (p=0,0003) and FMD (p=0,0004, see Figure 19.

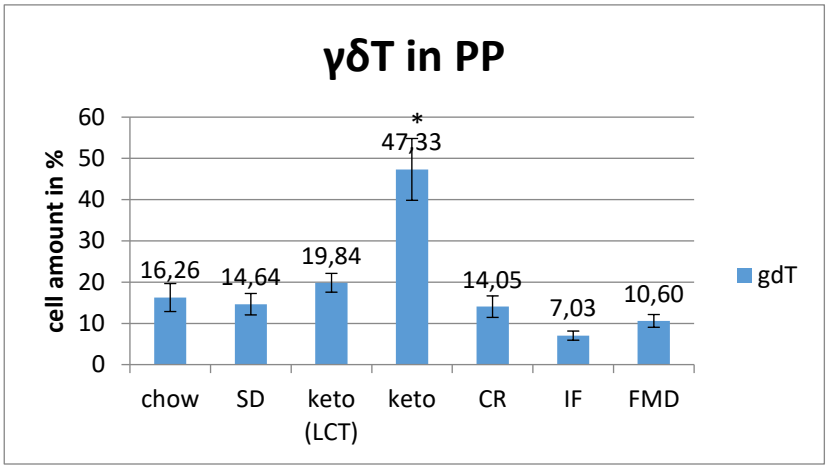


Figure 17) The cell count of γδT in each group

Keto has the lowest expression of CD4 compared to chow (p=0,0023), keto (LCT) (p=0,0001) and FMD (p=0,0011), see Figure 20.

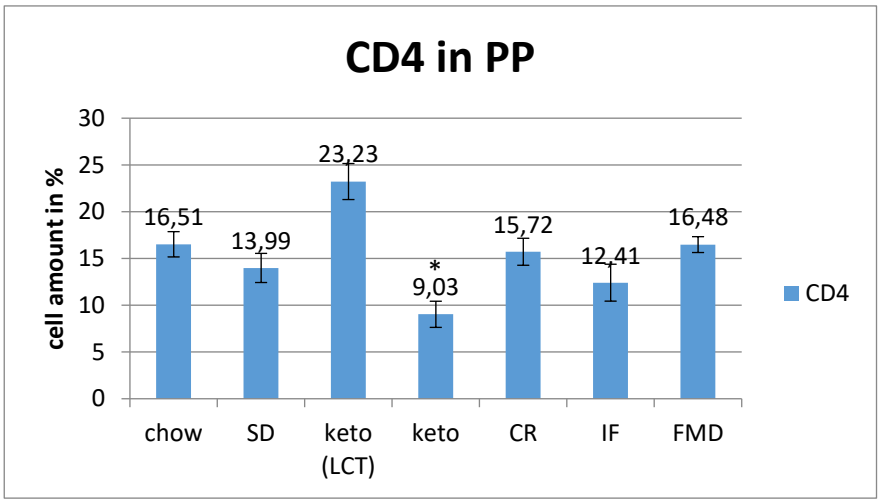


Figure 18) The cell count of CD4 for each group

6.4 qPCR

6.4.1 Si

STAT1 is lower expressed in SD (p=0,0037), keto (LCT) (p=0,0048) and keto (p=0,0056) compared to FMD, see Figure 21.

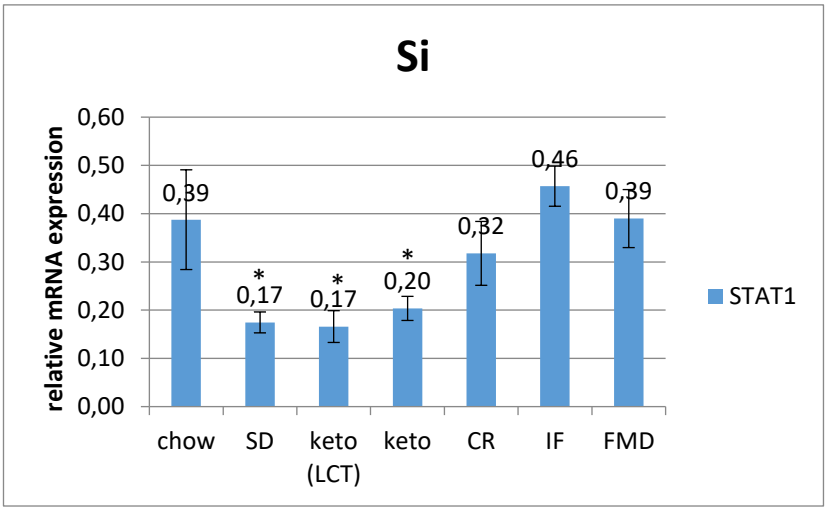


Figure 19) The gene expression of STAT1 in all groups

Both keto (LCT) (p=0,0033) and keto (p=0,0026) lower the expression amount of MYD88 compared to SD as shown in Figure 22.

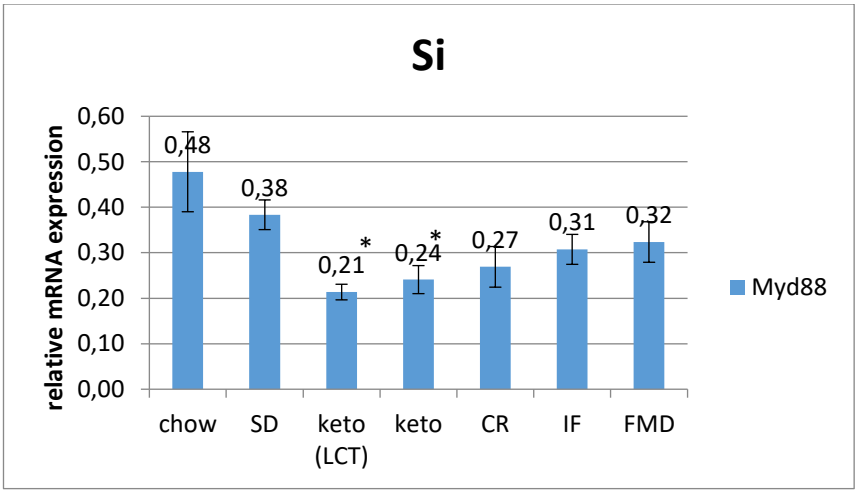


Figure 20) The gene expression of MYD88 in all groups

Compared to chow there is a significant down-regulation of REG3γ in SD (p=0,0009), keto (LCT) (p=0,0013), keto (p=0,0013) and CR (p=0,0022) as demonstrated in Figure 23.

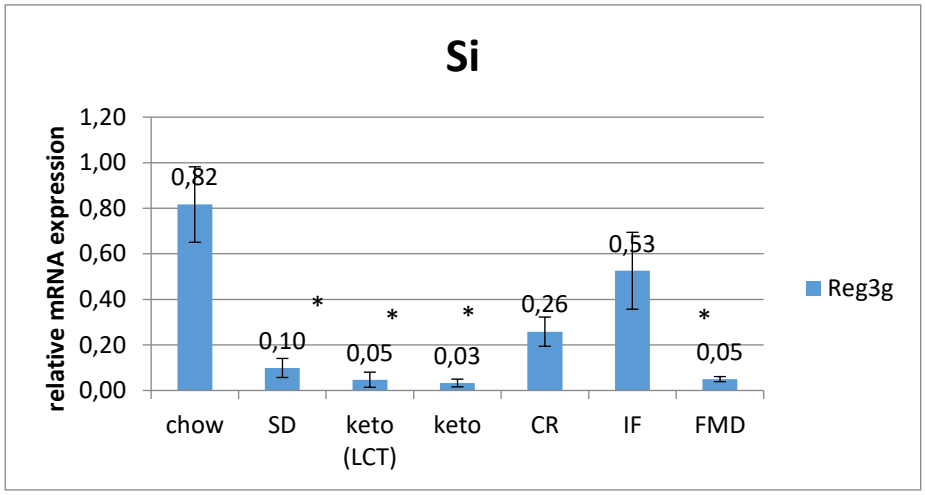


Figure 21) The gene expression of REG3γ in all groups

Keto shows a reduced expression of IRF7 in comparison to chow (p=0,0033), IF (p=0,0036), SD (p=0,0001) and FMD (p=0,0058). Furthermore, keto (LCT) either shows a reduction of IRF7 compared to chow (p=0,0051) and SD (p=0,0008), see Figure 24.

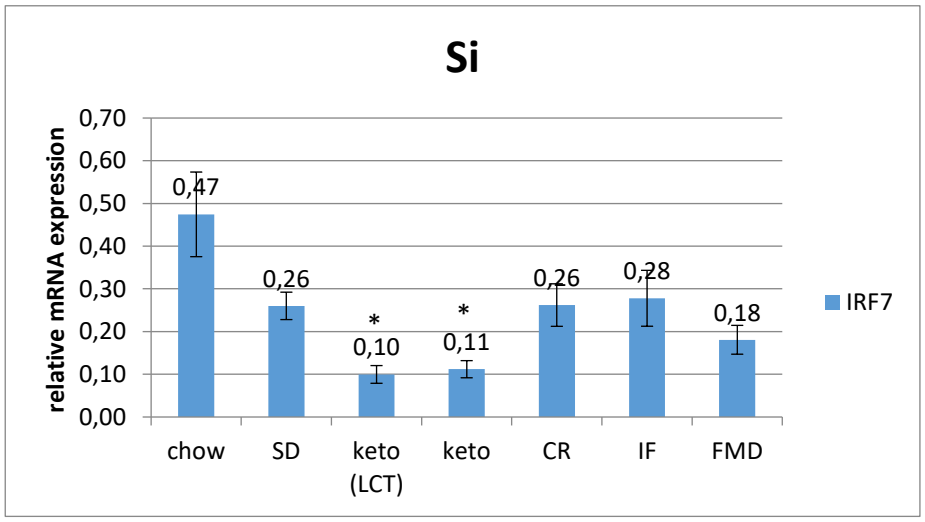


Figure 22) The gene expression of IRF7 in all groups

Keto exhibits reduction of TLR3 compared to SD (p=0,0047) and IF (p=0,0002) as well as keto (LCT) in comparison to SD (p=0,0011), IF(p=0,0001) and FMD (p=0,0042), see Figure 25.

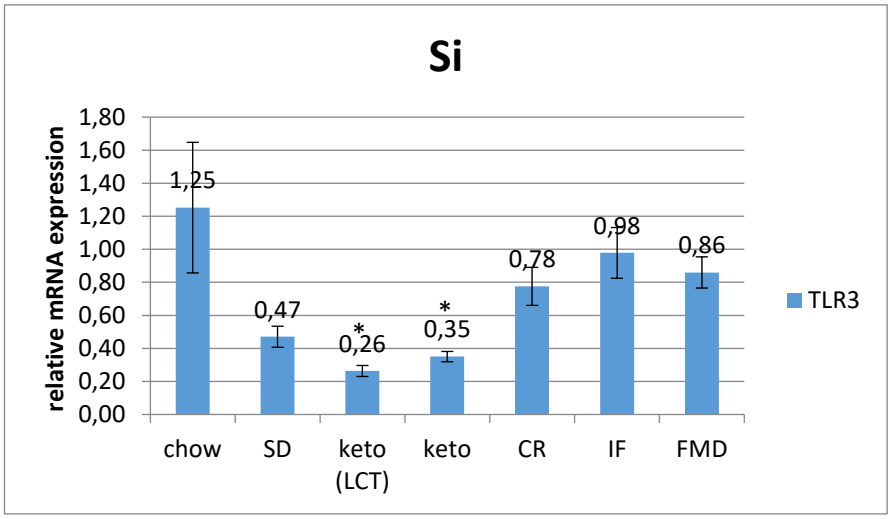


Figure 23) The gene expression of TLR3 in all groups

NOD2 expression is reduced in keto and keto (LCT) compared to IF (p=0,0001 for both) as illustrated in Figure 26.

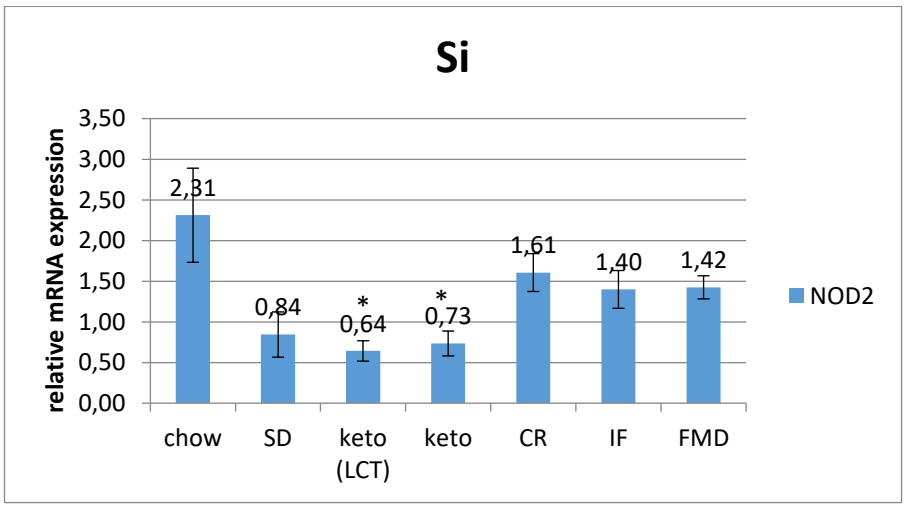


Figure 24) The gene expression of NOD2 in all groups

Keto and keto (LCT) show significant lower expression of TNFα compared to IF ($p<0,0001$; $p=0,0003$), see Figure 27.

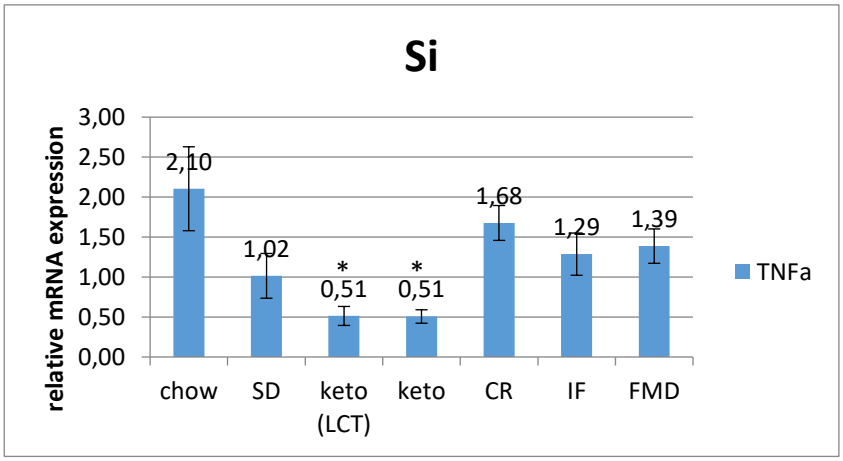


Figure 25) The gene expression of TNFα in all groups

Compared to chow, keto (LCT) (p=0,0010), keto (p=0,008), CR (p=0,0030) IF(p=0,0058) and FMD (p=0,0016) show a reduction in IRF1 as demonstrated in Figure 27.

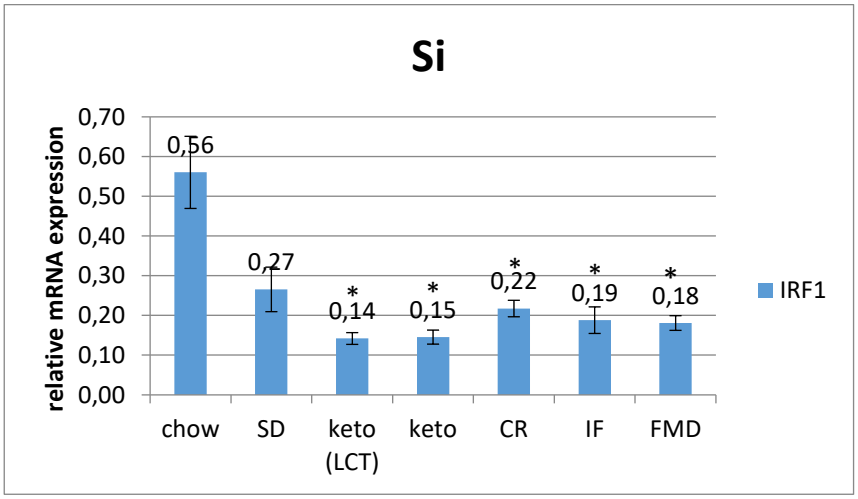


Figure 26) The gene expression of IRF1 in all groups

Keto and keto (LCT) show significant lower amounts of OAS1a in comparison to chow (p=0,0010; p=0,0026) and SD (p=0,0001; p=0,0040)- in addition keto is also significant to FMD (p=0,0010). Another reduction of OAS1a is observed in CR (p=0,0054) and FMD (p=0,0068) compared to chow, as shown in Figure 29.

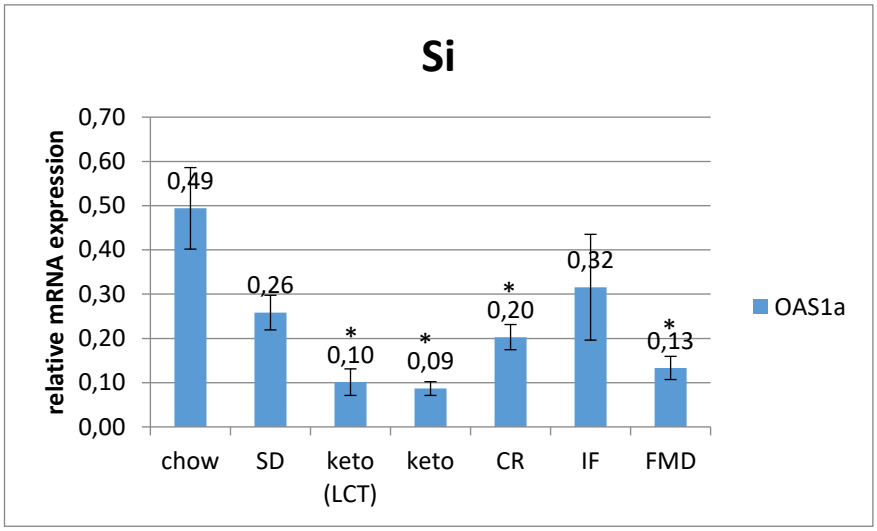


Figure 27) The gene expression of OAS1a in all groups

Keto expresses less RSAD2 than chow (p=0,0008), SD (p=0,0052) and FMD (p=0,0064). Moreover, Keto (LCT) shows also a reduction compared to chow (p=0,0030), see Figure 30.

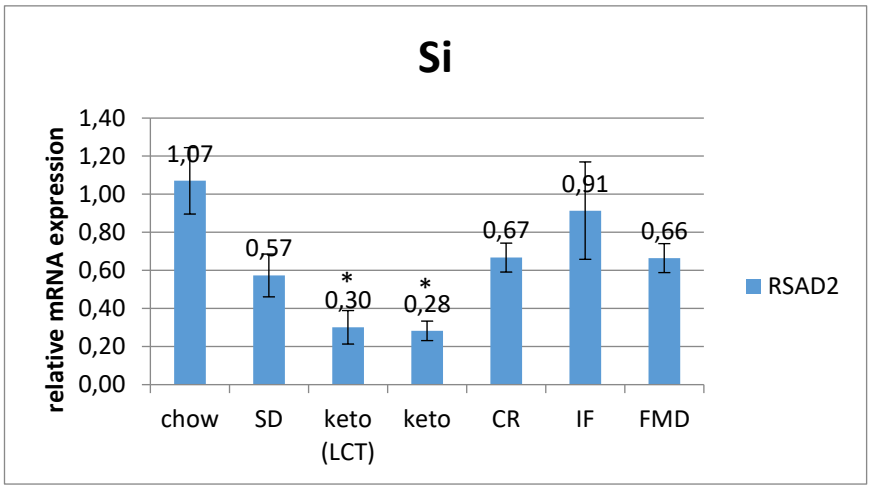


Figure 28) The gene expression of RSAD2 in all groups

6.4.2 LN

Keto (LCT) shows a reduction in the gene expression of REG3γ compared to chow (p=0,0041), as shown in Figure 31.

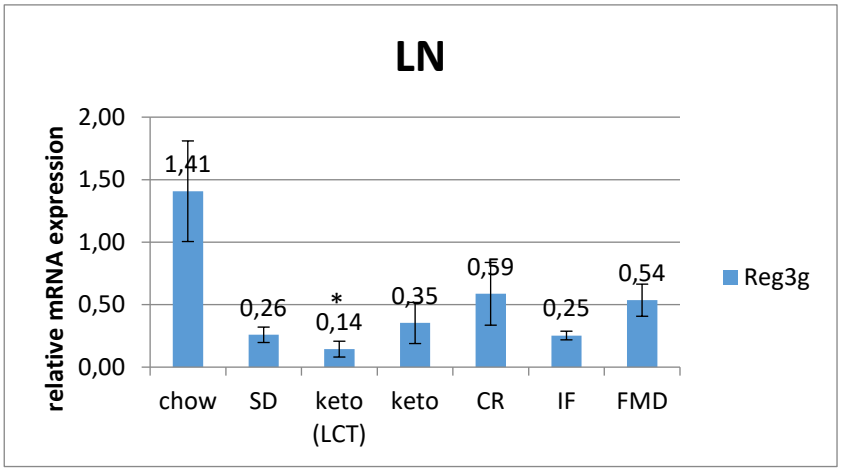


Figure 29) The gene expression of REG3γ in all groups

Significant reduction in keto (LCT) compared to chow as demonstrated in Figure 32.

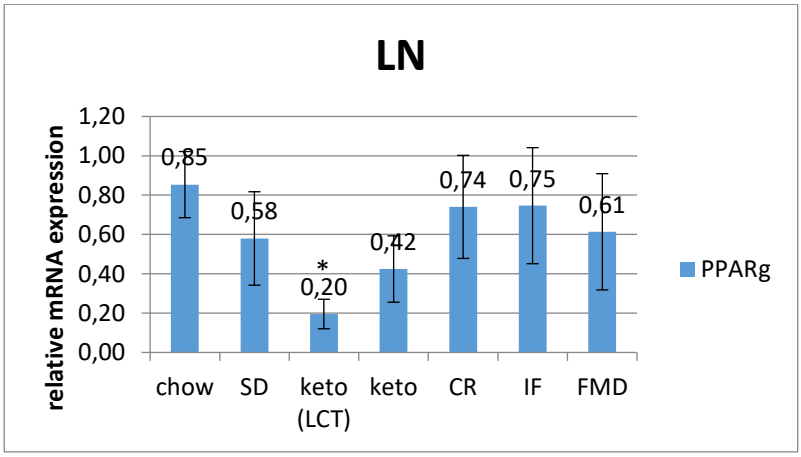


Figure 30) The gene expression of PPARγ in all groups

Keto (LCT) shows a reduction of IL6 compared to chow (p=0,0042), IF (p=0,0043) and FMD (p=0,0031), see Figure 33.

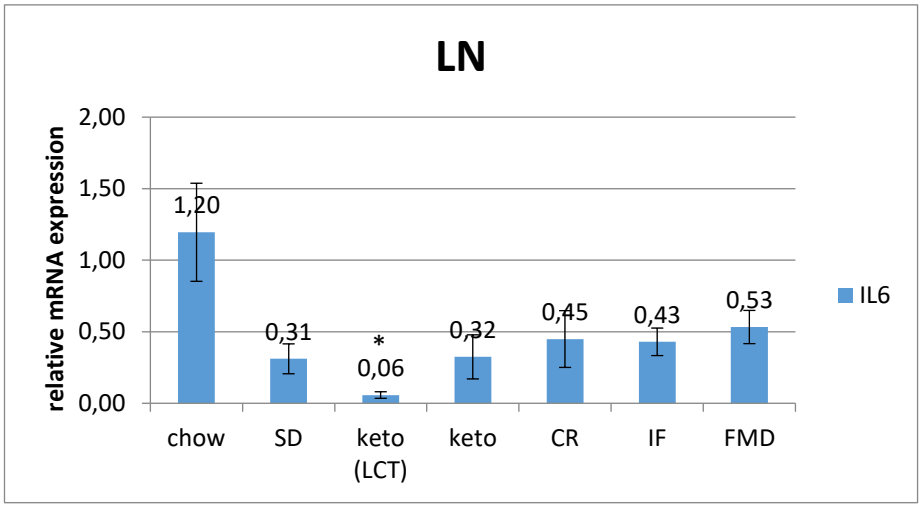


Figure 31) The gene expression of IL6 in all groups

6.4.3 PP

SD exhibits a reduction of STAT1 compared to FMD (p=0,0018), see Figure 34.

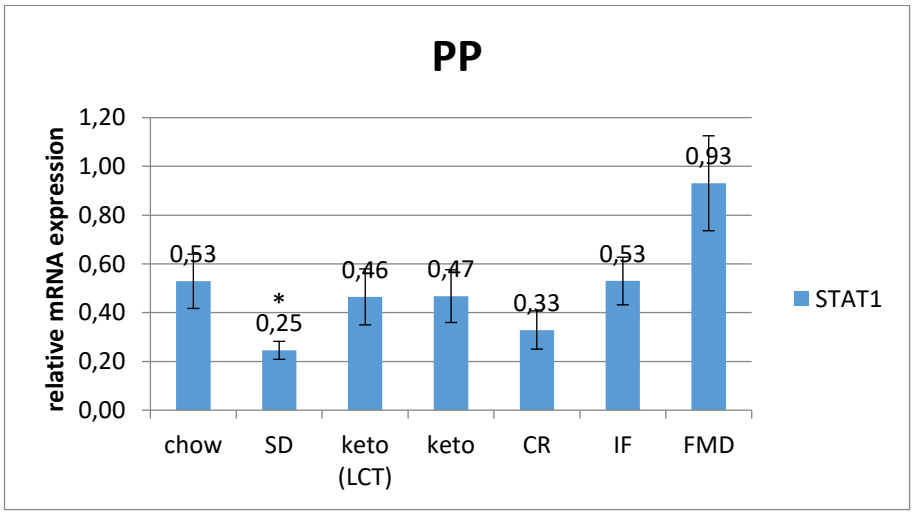


Figure 32) The gene expression of STAT1 in all groups

IF reduce MYD88 expression significantly compared to SD (p=0,0042) as demonstrated in Figure 35.

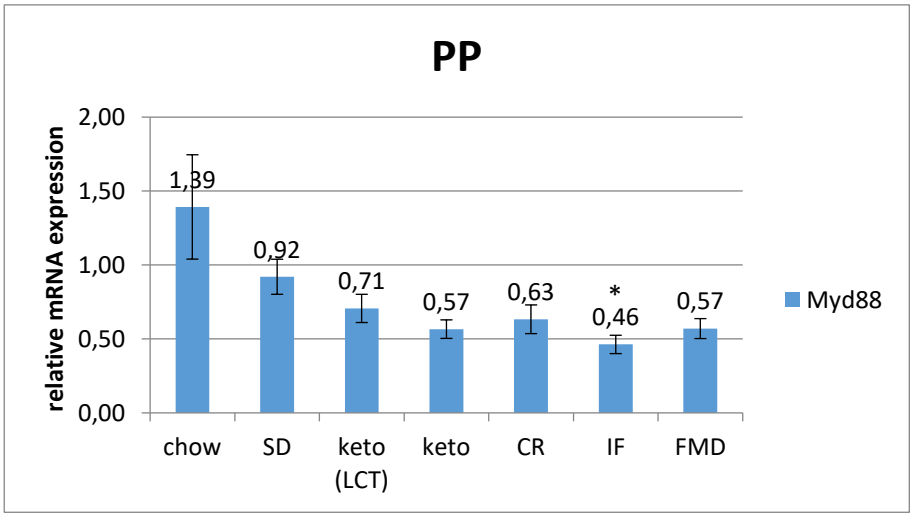


Figure 33) The gene expression of MYD88 in all groups

Keto (p=0,0003), CR (p=0,0020), IF and FMD (p<0,0001 for both) show a reduction of TLR3 compared to SD. In addition, IF shows either a reduction in comparison to chow (p=0,0002) and keto (p=0,0020). TLR3 is lower in FMD than chow (p=0,0014), see Figure 36.

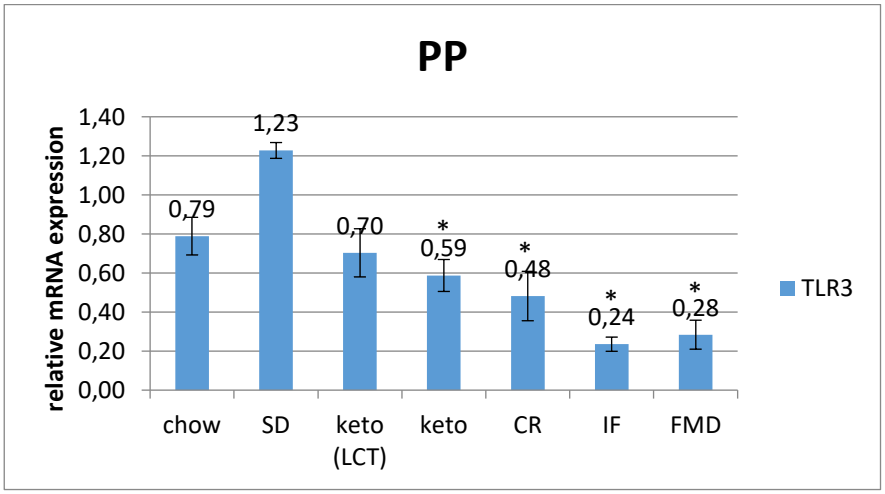


Figure 34) The gene expression of TLR3 in all groups

FMD exhibits the lowest expression of IRF1 compared to chow (p<0,0001), SD (p<0,0001), keto (LCT) (p=0,0049) and keto (p=0,0007). Followed by IF, which operates

a reduction of IRF1 in comparison to chow (p=0,0001), SD (p=0,0001) and keto (p=0,0018). Keto (LCT) and CR show a reduction of IRF1 compared to chow (p=0,0028;p=0,0056), see Figure 37.

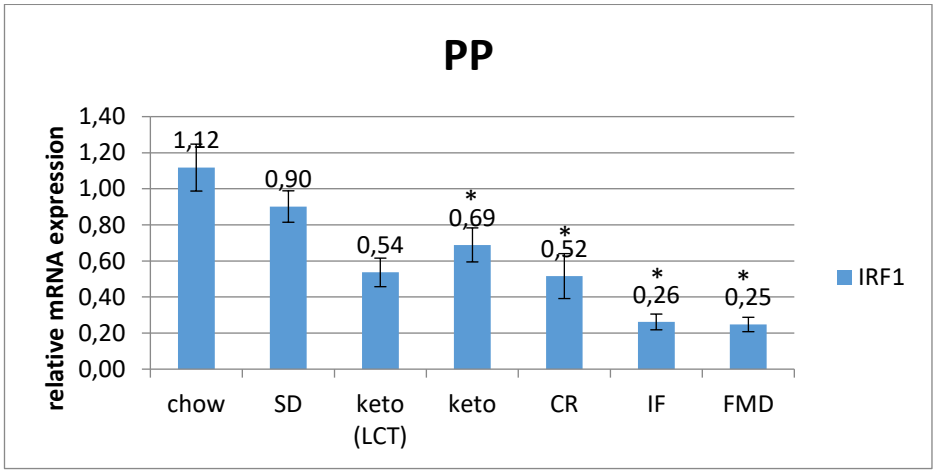


Figure 35) The gene expression of IRF1 in all groups

FMD causes a reduction in OAS1a compared to chow (p=0,0010) and SD (p=0,0007). Moreover, keto (LCT) results in lower expression of OAS1a in comparison to chow (p=0,0065) and SD (p=0,0054) as shown in Figure 38.

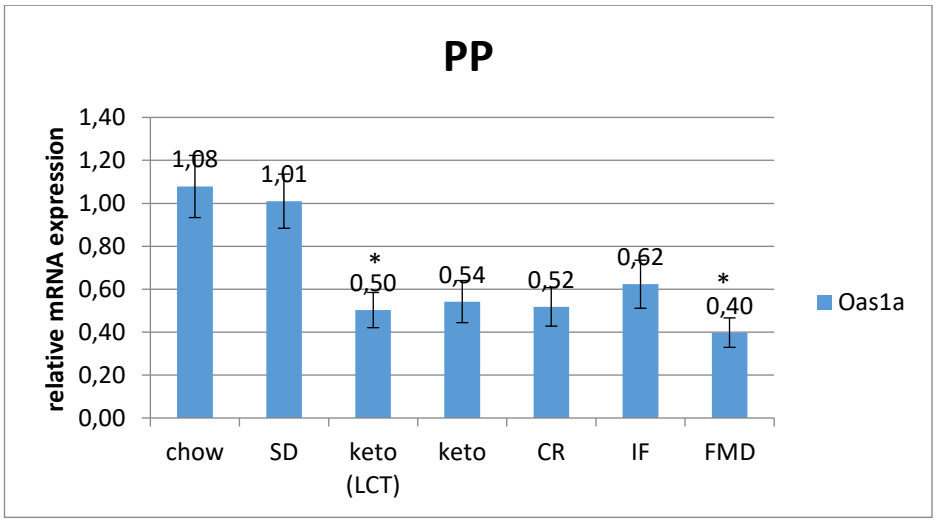


Figure 36) The gene expression of OAS1a in all groups

IF obtains reduction of PPARγ compared to SD (p=0,0038), see Figure 39.

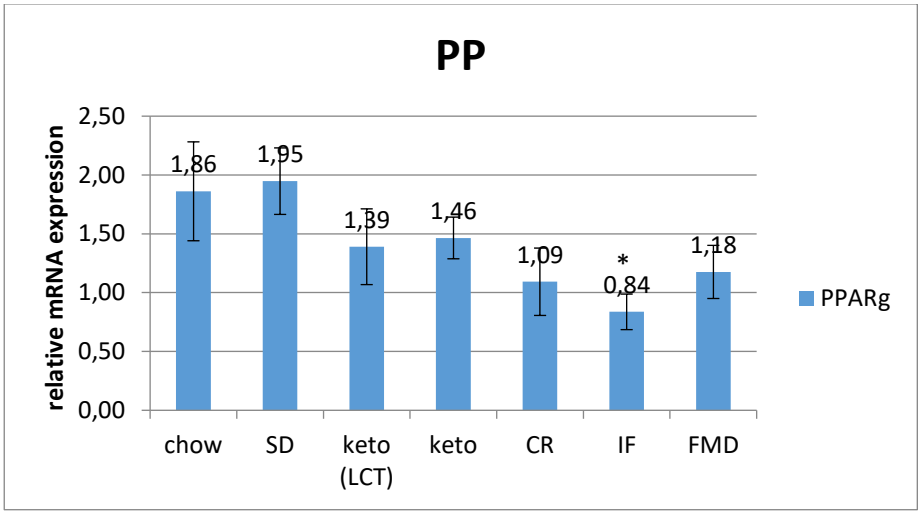


Figure 37) The gene expression of PPARγ in all groups

7 Discussion

7.1 eWAT

Chow group represents the mean average of 506mg eWAT. SD (keto control) and keto groups, who were fed *ad lib*, result in higher weights of eWAT 788mg and 652mg but not significant to chow. The highest eWAT significantly measured in keto (LCT) group (1421mg), whose food contained 100% LCT of the fat part. CR, IF and FMD obtain a reduction in eWAT, 300mg, 213mg and 221mg. This is the first positive effect of these diets. WAT, in the narrower sense visceral fat, is associated with several diseases such as DM II, Ascl, hypertension and CVD that have serious consequences. It causes obesity, which restricts activity, burdens the joints, induces inflammation and reduces quality of life. First step to fight obesity is a diet, compared to medications such as statins and fibrats, it fights the problem at its origin. Beside that it has no side effects and interactions with necessitate medications e.g. antibiotics during an infection. Best

combination to get rid of obesity is a healthy dietary and exercise to achieve a negative energy balance.

7.2 Effect on spleen

CR shows the highest amount of immune competent cells, which can be an advantage to enhance the defense readiness. This effect may references to the autophagy that is stimulated by this diet. [67] CR shows high amount of $CD8^+CD4^-$, CD8 and CD4 cells. This increases the elimination of endogenous defect cells and exogenous foreign structures. [56] Similar effect but weaker pronounced in the IF diet. FMD reaches high count of $CD8^+CD4^+$ cells, which mainly mature to $CD8^+CD4^-$ cells and finally result in cytotoxic T-cells. Less T-helper cells are visible in this diet, which conform to the low amount of $CD8^-$ $CD4^+$ cells. FMD may focus the elimination of endogenous defect cells. A reason for the low CD4 cells might be the reduced presence of foreign structures due to the reduced intake.

7.3 Effect on LN

There is no significance concerning the multitude of leucocytes. CR and FMD show high quantity of $CD8^+CD4^-$, which result in higher amount of cytotoxic T-cells. Thus, it may focus the elimination of degenerated cells. [68] Keto shows less DP cells in LN, this may connected to the fact that DP cells act suppressive while keto has an antimicrobial character. [57] Other observation also belongs to the keto diet, which additionally reduces $CD8^+CD4^-$, CD4 and CD8 cells, which justifies the antibacterial and antiviral ability of FFAs. [69]

7.4 Effect on PP

High expression of CD8 determined in CR and FMD, same effect observed in spleen and LN. Contrarily reduced expressions of $CD8^{-}CD4^{+}$ and CD4 are perceivable in keto, which concur with the effect in LN. Besides, keto exhibits the highest amount of γδ T-cells, which enhances the immune defense in the gut mucosa. [60] That raises the question, whether the γδ T-cell boost combined with lack of glucose antagonizes the genesis and progression of tumor cells. [59] CR, FMD and IF show high amounts of cytotoxic cells, which eliminate degenerated cells. That might explain why IF results in low amount of the regulatory γδ T-cells.

7.5 Effect of the genes

7.5.1 Genes in Si

Keto (LCT) and keto obtains best beneficial effects on the expression of all ten investigated proinflammatory genes. The ketogenic diets reduce WSD and obese induced inflammatory and cell death. [33] One can expect prevention in IBD, AScl, CAD, insulin resistance and DM. [37] Followed by FMD and CR, which diminish expression of REG3γ, IRF1 and OAS1a. [46] Accordingly FMD and CR reduce alert innate responses and obesity, which results in a prevention of IBD, insulin resistance and DM and AScl. [41]

SD reduces the expression of STAT1 and REG3γ, which decrease inflammation, alert gut response and cell death. [44] This diet has potential to prevent IBD and inflammation in obese people. The last positive event observed in Si, is the mitigation of IRF1 by the IF dietary. That precludes AScl and insulin resistance. [35], [70]

7.5.2 Genes in LN

Only keto (LCT) dietary shows an effect in LN. Abatement of REG3γ, PPARγ and IL6 is proven. Expected events are reduction of alert innate response, gut microbiota dysbiosis and endothelial damage. [71] It can be concluded that keto (LCT) prevents IBD, DM, endothelial dysfunction and AScl.

7.5.3 Genes in PP

FMD, CR and keto attain a significant reduction of TLR3 and IRF1, which leads to a reduction of inflammation and insulin resistance. [70] These dietaries are supposed to obviate inflammation in obese people and DM II. In addition FMD and keto (LCT) diminish OAS1a, which reduces the risk for DM II. [46] SD degrades the expression of STAT1 and avoids WSD induced and age-related inflammation. Furthermore IF effectuate a decrease of MYD88, TLR3, IRF1 and PPARγ, which detract WSD induced and age related inflammation, insulin resistance and colon inflammation. [33] [30] For that reason it precludes DM and CAD.

8 Conclusion

In conclusion, restrictive diets have positive impacts on the immune status of the gastrointestinal tract. It was observed that the restrictive diets affect both the immune cells and the gene expression of mediators that are involved in inflammatory processes. CR and FMD enhance the immune cells not only in the circulatory system but also local in the gut mucosa. Furthermore, the ketogenic diet has the ability to enhance the γδT cells, which have a broad display. Although each diet has its own advantages, the ketogenic diet seems to supervise the others by effectuating more gene expressions and acting multifariously. According to the current scientific studies

and the observation in this work, this field of activity is assessed crucial for a future therapeutic approach.

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