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„Antinflammatory impact of macronutrient restriction in DSS-induced colitis in mice“

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5-ASA 5-aminosalicylic acid

ad lib ad libitum

BSA bovine serum albumin, bovine serum albumin

cDNA complementary DNA, complementary DNA

CR Caloric restriction

CT computed tomography

Ctrl Control

ddH2O double-distilled water

DNA Deoxyribonucleic acid

DSS Dextran sulfate sodium

EDTA Ethylenediaminetetraacetic acid

ELISA enzyme-linked immunosorbent assay

HTAB hexadecyltrimethylammonium bromide

IBD inflammatory bowel diseases

IL interleukine

Irf1 Interferon Regulatory Factor 1

MPO Myeloperoxidase

MRI magnetic resonance imaging

MyD88 Myeloid differentiation primary response 88

NF-κB nuclear factor-κB, nuclear factor- κB

NOD nucleotide-binding oligomerization domain

PBS Phosphate-buffered saline

PCR polymerase chain reaction, Polymerase chain reaction, polymerase chain reaction

qPCR quantitative real-time PCR

RNA Ribonucleic acid, Ribonucleic acid

RT-PCR reverse transcription polymerase chain reaction

STAT1 Signal Transducer and Activator of Transcription 1

TGF1β transforming growth factor beta 1

Th T-helper cell

TLR Toll-like receptor

TNF-α tumor necrosis factor α

UC ulcerative colitis

Abstract

Nutrition plays an essential role in human life and influences our existence in various ways. Its impact extends across different aspects of our well-being and health. While intensive research efforts and ongoing debates have not definitively clarified the complex relationships between nutrition and health, there is no doubt that nutrition plays a significant role in our overall well-being, especially through its complex interactions with our digestive system.
Ulcerative colitis(UC) is a chronic inflammatory bowel disease(IBD) that affects the colon and rectum and is characterized by periods of remission and relapse. Although the exact cause of this condition is not fully understood, it is believed that genetic, environmental and immunological factors in combination contribute to its development. These factors include genetic predisposition, immune system dysfunction, environmental influences and an imbalanced intestinal microbiome.

This master's thesis is dedicated to an in-depth investigation of the complex relationship between nutrition and ulcerative colitis. Special attention is given to the intake of macronutrients through food, with an analysis of the potentially anti-inflammatory effects of a macronutrient-restricted diet. This work contributes to deepening our understanding of the complex interactions between nutrition and health, particularly in the context of inflammatory bowel diseases such as ulcerative colitis. To discuss the potential anti-inflammatory effects of macronutrients, groups with low macronutrient intake were compared to control groups, both with and without dextran sulfate sodium(DSS)-induced colitis. The results show a promising trend regarding the effects of carbohydrate and protein restriction on cytokine levels. Additionally, significant changes in the muscularis externa, villi length and crypt depth were observed in histological examinations.

This work supports previous research findings that already suggested a possible contribution of restrictive diets reducing the inflammatory process. It thus contributes to deepening our understanding of the complex relationships between nutrition and inflammatory diseases like ulcerative colitis. Despite these promising results, there are still open questions that require further research in this area. It is necessary to explore and understand the mechanisms and potential therapeutic applications more thoroughly. These findings open new horizons for future studies and could ultimately help to develop more effective treatment approaches for ulcerative colitis.

Zusammenfassung

Ernährung spielt eine essenzielle Rolle im menschlichen Leben und beeinflusst unsere Existenz in vielfältiger Weise. Ihr Einfluss erstreckt sich über verschiedene Aspekte unseres Wohlbefindens und unserer Gesundheit. Obwohl intensive Forschungsbemühungen und anhaltende Debatten die komplexen Beziehungen zwischen Ernährung und Gesundheit noch nicht endgültig geklärt haben, steht außer Frage, dass Ernährung eine bedeutende Rolle für unser allgemeines Wohlbefinden spielt, insbesondere durch die komplexen Wechselwirkungen mit unserem Verdauungssystem.
Colitis ulcerosa ist eine chronisch entzündliche Darmerkrankung, die den Dickdarm und den Enddarm betrifft und von Phasen der Remission und Rückfälle gekennzeichnet ist. Obwohl die genaue Ursache dieser Erkrankung noch nicht vollständig verstanden ist, wird angenommen, dass genetische, umweltrelevante und immunologische Faktoren in Kombination zu ihrer Entstehung beitragen.

Diese Masterarbeit widmet sich einer tiefgreifenden Untersuchung der komplexen Beziehung zwischen Ernährung und Colitis ulcerosa. Besonderes Augenmerk liegt auf der Aufnahme von Makronährstoffen über die Nahrung, wobei auch die möglicherweise entzündungshemmenden Auswirkungen einer makronährstoffarmen Ernährung analysiert werden. Diese Arbeit trägt dazu bei, das Verständnis für die komplexen Wechselwirkungen zwischen Ernährung und Gesundheit zu vertiefen, insbesondere im Kontext entzündlicher Darmerkrankungen wie Colitis ulcerosa. Um die potenzielle antiinflammatorische Wirkung von Makronährstoffen zu erörtern, wurden Gruppen mit geringer Makronährstoffzufuhr mit Kontrollgruppen verglichen, die sowohl mit als auch ohne DSS-induzierter Colitis untersucht wurden. Die Ergebnisse zeigen einen vielversprechenden Trend hinsichtlich der Auswirkungen von Kohlenhydrat- und Proteinrestriktion auf die Zytokin-Spiegel. Zusätzlich wurden in histologischen Untersuchungen signifikante Veränderungen in der muscularis externa, der Villilänge und der Kryptentiefe festgestellt. Diese Arbeit stützt frühere Forschungsergebnisse, die bereits auf einen möglichen Beitrag restriktiver Diäten zur Reduzierung des entzündlichen Prozesses hindeuteten. Sie trägt somit zur Vertiefung unseres Verständnisses der komplexen Zusammenhänge zwischen Ernährung und entzündlichen Erkrankungen wie Colitis ulcerosa bei. Trotz dieser vielversprechenden Ergebnisse gibt es noch offene Fragen, die weitere Forschung in diesem Bereich erfordern. Es ist notwendig, die Mechanismen und potenziellen therapeutischen Anwendungen genauer zu erforschen und zu verstehen. Diese Erkenntnisse eröffnen neue Horizonte für zukünftige Studien und könnten letztendlich dazu beitragen, effektivere Therapieansätze für Colitis ulcerosa zu entwickeln.

1 Introduction

1.1 Caloric restriction

Caloric restriction (CR) is considered the gold standard for preventive measures that extend lifespan and enhance health in various organisms, effectively preventing or delaying numerous age-related diseases. In rodents, studies have consistently demonstrated the beneficial effects of caloric restriction on metabolic health and glucose homeostasis. But given the difficulties many people face in adhering to a long-term calorie-restricted diet, there is considerable interest for clarifying the physiological and molecular mechanisms that may contribute to the beneficial outcomes of calorie restriction. So far, it has been generally assumed that the positive effects of a calorie-restricted diet are primarily due to the reduction of total calorie intake. But more recent research suggests that the reduction of certain macronutrients may also be contributing to these effects [1].

Extended life expectancy is a remarkable benefit of caloric restriction, which has been proven by a large number of studies. This dietary intervention significantly strengthens cellular stress resistance and effectively reduces oxidative stress, leading to a longer and healthier life expectancy [2]. Compared to *ad libitum* fed rats, the effects on the cardiovascular system can be seen in lower blood pressure and heart rate. Synaptic plasticity also improves, leading to the survival of neurons and the formation of new neurons [2]. Several factors that increase the risk of cardiovascular disease and stroke have been shown to be significantly impacted by CR. Insulin resistance, also known as impaired glucose regulation, significantly increases the risk of cardiovascular disease and stroke. These diseases are typically associated with elevations in plasma glucose and insulin levels. By inducing an increase in insulin sensitivity in monkeys and rodents through CR treatment, they were less likely to develop diabetes and cardiovascular disease [2]. By lowering LDL levels, CR also influences cholesterol levels. As a result of lowering the leucocyte count and circulating levels of tumor necrosis factor and other inflammatory cytokines, CR decreases inflammatory processes that are suspected of contributing to atherosclerosis. To conclude, calorie restriction has a cardiovascular protective effect. At the same time, it protects against ischemic damage [2]. As well as playing a major function in the ageing process, systemic inflammation affects physical and mental performance and may be caused by chronic overeating. Calorie restriction led to a decrease of these inflammatory processes [3].

CR appears to have antineoplastic potential and decreases cancer incidence. It also minimizes the potential side effects of chemotherapy without loss of treatment efficacy. The effects of caloric restriction on neurons and tissues are well characterized in animal models. These approaches increase stress resistance, promote autophagy, support DNA repair and improve mitochondrial function. In neurodegenerative models such as Alzheimer's and Parkinson's disease, CR has been shown to delay disease onset and progression, accompanied by positive changes at the molecular, physiological and cognitive levels [3].

1.2 Macronutrient diets

Macronutrients such as fat, protein and carbohydrates are essential for provision of energy and vital components. Each macronutrient plays a particular and important role in the functions of our body and overall health. Fat is mainly made up of glycerol and fatty acids, protein consists of amino acids and carbohydrates contain simple sugars such as monosaccharides or chains of monosaccharides linked together. Though it is important to consume a balanced combination of these macronutrients to support longevity and maintain good health, the accurate composition for achieving optimal health is still undefined [4].

1.2.1 Proteins in nutrition

Proteins are fundamental constituents of the human body, playing a vital role in numerous essential functions. They are involved in cellular structure, growth, and repair, as well as regulating various biological processes [5]. Amino acids serve as the structural units of proteins and are essential for various vital biochemical processes in the human body. Proteins can fulfill both structural and functional roles, contributing to the formation of muscles, organs, glands, ligaments, tendons, nails, hair and bones. Additionally, proteins can act as hormones and enzymes, playing a crucial role in catalyzing and regulating numerous essential processes within the body. Certain amino acids, known as functional amino acids have specific functions in regulating important metabolic pathways. These pathways are integral to immunity, reproduction, growth and the overall maintenance of the body. Proteins and amino acids also play a vital role as the sole source of nitrogen for humans. Since the body does not possess protein stores, the growth, maintenance of cellular structures, wound healing and the proper functioning of enzymes and hormones rely on a consistent supply of external proteins to compensate for daily losses [6].

In recent years, high-protein diets have gained popularity not only among athletes but also among the general population as a method for weight reduction. One of the most well-known high protein diets is the low-carbohydrate, high-protein, high-fat diet promoted by Atkins. However, concerns have been raised regarding the safety of high-protein diets. These risks include metabolic, cardiac, renal, bone and liver diseases. A major concern with high protein, low-carbohydrate diets is the possibility of developing metabolic ketosis. When carbohydrate intake is reduced, the body shifts its energy source to fat. This can result in an increased production and release of ketone bodies into the bloodstream. Elevated levels of ketone bodies can lead to metabolic acidosis, an imbalance in the body's acid-base levels. In severe cases, this condition can lead to coma or even death. High-protein diets are associated with possible negative impacts on the blood lipid profile and blood pressure, increasing the risk of cardiovascular disease. These effects are primarily due to the higher intake of saturated fats commonly associated with such diets. The concern about the impact of high-protein diets on renal function is primarily related to the potential strain on the kidneys due to increased nitrogen excretion [7].

Low-protein diets have been shown to have positive effects on lifespan and cardiometabolic health, primarily observed in rodents but likely applicable to humans as well. These diets significantly impact caloric intake and energy expenditure, leading to reduced body weight and adiposity in animals [8].

1.2.2 Carbohydrate consumption

Carbohydrates are the main source of energy for the human body. They are needed for the forming of connective tissue substances and cell membranes. Also, they provide the basic substance for the building of glycoproteins, hormones and transport proteins. A major part of the taken carbohydrates is converted into energy via the citrate cycle, the respiratory chain and via glycolysis. The part of glucose that is not used for energy purposes is stored in the liver as glycogen [9].

Low-carbohydrate diets were originally introduced and investigated primarily in the context of epilepsy management. However, their potential applications have expanded as increasing evidence suggests their effectiveness in managing a range of other health conditions, including diabetes, neoplasms, gastrointestinal and lung diseases, cardiovascular conditions and obesity [10]. Low-carbohydrate diets have gained popularity as a weight loss strategy and continue to be of interest in the field of nutrition. While these diets share the common characteristic of reducing carbohydrate intake, there is currently no widely accepted consensus on the precise definition of a low-carb diet. Based on the hypothesis that reducing insulin levels can enhance cardiometabolic function and facilitate weight loss, low-carb approaches have gained traction [11].

Referred to as the carbohydrate-insulin model, these diets exhibit greater initial weight loss compared to other methods. While the precise mechanisms of their effectiveness are still debated, reducing carbohydrates often prompts an increase in dietary fat and protein intake as compensation [11]. A carbohydrate-rich dietary pattern has been linked to the development of dysbiosis in mouse models. Furthermore, a diet high in carbohydrates is associated with obesity, which is connected to increased inflammation and heightened intestinal permeability [12]. Adverse effects are linked to both excessive and insufficient carbohydrate consumption. Increased carbohydrate intake leads to obesity because of increased energy intake and excessive glucose intake induces lipogenesis [9].

1.2.3 Dietary Fats

Apart from being energy suppliers, fats also serve as structural building blocks and facilitate the absorption of fat-soluble vitamins. In the body, fats can be stored in adipose tissue and released as fatty acids when energy is required [13]. Fatty acids are classified into saturated, monounsaturated and polyunsaturated based on the number of double bonds they possess. Animal fats tend to be high in saturated fatty acids, while vegetable fats, except for palm and coconut oils, are higher in polyunsaturated fatty acids [14]. Saturated fatty acids have long been associated with adverse health effects, especially cardiovascular disease, due to their role in elevating cholesterol levels and increasing the risk of heart-related issues. Consequently, diets abundant in these saturated fats, as commonly found in Western-style diets, have also been linked to other detrimental health outcomes, including obesity, diabetes and even inflammatory bowel disease [14, 15]. In contrast, specific polyunsaturated fatty acids have shown promising effects as biological mediators in cardiovascular conditions like myocardial infarction. Moreover, diets abundant in unsaturated fatty acids, notably those adhering to the Mediterranean diet, are recognized for their anti-inflammatory properties [14, 15]. A high-fat diet can impair tight junctions, the protein complexes that maintain the integrity of the intestinal barrier, leading to decreased tightness and increased permeability of the intestinal epithelium. The increased permeability allows luminal components such as bacteria, toxins and undigested food particles to penetrate the lamina propria, enter the bloodstream and provoke an immune response, ultimately leading to intestinal inflammation [15]. The strategy of reducing overall fat intake is commonly employed for weight loss due to the higher calorie content of fat compared to carbohydrates or protein. A low-fat diet usually includes a moderate amount of fat and saturated fatty acids [16]. However, randomized studies have failed to show superior long-term weight loss results when compared to alternative dietary approaches. It is mentioned that a low-fat diet can lower LDL cholesterol levels in individuals with obesity, but at the same time, triglyceride levels may increase and HDL cholesterol levels may decrease [16].

1.3 Colitis

Ulcerative colitis is a chronic inflammatory bowel disease affecting the colon. It leads to persistent inflammation of the mucosa of the colon, beginning in the rectum and reaching to varying degrees to the proximal colon. Phases of relapses and remissions mark the disease. The leading pathogenic factors for UC include genetics, environmental triggers, autoimmunity and the composition of the intestinal microbiota. The clinical signs of UC include bloody diarrhoea, sometimes followed by mucus, rectal urination, tenesmus and varying degrees of abdominal pain, often resolved by defecation. A distinctive hallmark of ulcerative colitis is the presence of persistent inflammation in the colon, characterized by symptoms such as erythema, disruption of the normal vascular pattern, granularity, erosions, friability, haemorrhage and ulceration. These findings are combined with a clear differentiation between the inflamed and non-inflamed sections of the bowel [17].

Ulcerative colitis and Crohn's disease are the two primary forms of inflammatory bowel disease. Although there are certain similarities between these conditions, they can be differentiated based on variations in genetic predisposition, risk factors, as well as clinical, endoscopic and histological features. The exact underlying cause of inflammatory bowel disease remains a subject of ongoing research and is not yet definitively known. However, individuals who are genetically susceptible display an abnormal immune response in the intestinal mucosa when exposed to the normal gut microbiota, leading to inflammation in the intestines. This dysregulation of the immune system is widely recognized as a pivotal factor in the pathogenesis of inflammatory bowel disease, contributing significantly to its development and progression. In ulcerative colitis, the inflammation primarily affects the mucosal surface of the colon. The disease typically begins in the rectum and gradually extends continuously towards the proximal parts of the colon. This characteristic pattern of progression distinguishes ulcerative colitis from other forms of inflammatory bowel disease [18]. Indeed, ulcerative colitis has a higher prevalence compared to Crohn's disease. Specifically, regions such as North America and northern Europe exhibit the highest reported incidence and prevalence rates of ulcerative colitis. The prevalence of ulcerative colitis tends to be lower in the southern hemisphere and eastern countries. However, an interesting trend has been observed in countries that have adopted an industrialized lifestyle, as they have experienced an increase in the incidence of ulcerative colitis, indicating that environmental factors may play a crucial role in triggering the onset of the disease. In general, ulcerative colitis exhibits a dual-peak incidence pattern, with peaks commonly observed during two age ranges: 15-30 and 50-70. The gender distribution in studies on ulcerative colitis shows conflicting results, with a lack of consensus [18].

While some studies find no significant gender preference, others suggest a slight tendency towards male predominance [18]. Ulcerative colitis is a complex disorder with multiple factors contributing to its development. Genetic predisposition, abnormalities in the epithelial barrier function, dysregulated immune responses and environmental factors, all play a role in the pathogenesis of the disease [19]. Dietary factors have also been found to influence the risk of ulcerative colitis. A diet high in refined sugar, fat and meat has been associated with an increased risk, while a diet rich in vegetables has been shown to reduce the risk. This highlights the potential impact of dietary choices on the development of the disease [20].

1.3.1 Pathophysiology

The mucus-covered epithelial barrier serves as the first line of defense to prevent the invasion of pathogens. Defects in this barrier layer lead to dysfunction and microbial permeability, which is a possible pathophysiological starting point for the pathogenesis of ulcerative colitis. This imbalance in the barrier results from a disturbed tight junction arrangement [18].

Ulcerative colitis is marked by an imbalanced response of the immune system to the intestinal flora, leading to chronic inflammation. Normally, the immune system maintains a balance between tolerance to beneficial bacteria and protection from harmful pathogens. However, evidence from animal models and human studies suggests that non-pathogenic gut bacteria may play a determining role in the development of ulcerative colitis. This mucosal immune response dysfunction results in an abnormal reaction against harmless bacteria and triggers chronic inflammation in the gut [18].

The immune response in ulcerative colitis is a complex interplay between the innate and adaptive immune systems. Antigens stimulate macrophages and dendritic cells, which activate adaptive immune responses. In patients with ulcerative colitis, there is an increase in activated dendritic cells with enhanced stimulatory capacity, suggesting their involvement in initiating and sustaining inflammation. Dendritic cells express pattern-recognition receptors like Toll-like receptors (TLR) and NOD-like receptors, which recognize microbial components. Aberrant expression and genetic variations in TLRs, particularly TLR4, have been linked to ulcerative colitis and altered immune responses to commensal bacteria. TLR activation triggers the release of pro-inflammatory molecules and activates transcription factors like nuclear factor-κB (NF-κB), which have complex roles in inflammation depending on the cell type involved. Overall, the immune response in ulcerative colitis is characterized by an imbalance between the innate and adaptive immune components, leading to chronic intestinal inflammation [18].

In patients with ulcerative colitis, there is a disruption of the balance between regulatory and effector T-cells, particularly the atypical Th2 response mediated by non-classic natural killer T-cells. These T-cells produce interleukins 5 and 13, with interleukin 13 playing a significant role in inducing cytotoxic effects on epithelial cells and affecting tight junction proteins. The increased presence of natural killer T-cells in the inflamed colon and their production of Th2 cytokines, particularly interleukin 13, further contribute to tissue injury and inflammation. Alterations in these immune responses are believed to be key factors in the pathogenesis of ulcerative colitis. Additionally, abnormalities in interleukin-10 signaling and elevated levels of tumor necrosis factor (TNF)-α have been observed in patients with ulcerative colitis, further supporting their involvement in the disease process. Blockade of interleukin-13 and depletion of natural killer T-cells have shown potential for preventing colitis development, while anti-TNF-α treatment has proven effective in managing ulcerative colitis. These findings highlight the importance of immune dysregulation and specific cytokines in the pathogenesis and treatment of ulcerative colitis [18].

1.3.2 Symptoms and differential diagnosis

Clinical symptoms of UC typically involve bloody stools and diarrhea. There are also other symptoms such as urinary urgency, incontinence, fatigue, increased bowel motions, mucous discharge, nocturnal urination and abdominal complaints. Abdominal distress seems to be less prominent than in Crohn's disease, whereas fever and weight loss may occur. Clinical presentation varies depending on the disease extent, with proctitis patients primarily experiencing urgency and tenesmus, while those with pancolitis may have bloody diarrhea and abdominal pain. Physical examination may reveal signs of anemia, abdominal tenderness and blood during rectal examination. Clostridium difficile infection should be ruled out as it can trigger flares and increase the risk of surgery and mortality. Prompt diagnosis and evaluation are important to ensure appropriate management of ulcerative colitis [21]. Crohn's disease and infectious colitis caused by bacterial, viral or parasitic organisms are part of the differential diagnosis of ulcerative colitis. In Crohn's disease, there may be areas of normal mucosa between the affected areas, whereas ulcerative colitis is specifically identified by continuous inflammation. Another condition to consider is microscopic colitis, which is manifested by non-bloody diarrhoea, abdominal pain and weight loss. An endoscopic biopsy is performed to diagnose microscopic colitis. If ulcerative colitis is suspected, a bacterial stool culture should be obtained and a test for Clostridium difficile toxin should be performed in people who have recently taken antibiotics [20].

A precise diagnosis of ulcerative colitis is important for proper treatment. Current diagnostic principles consist of clinical symptoms, endoscopy, tissue examination and exclusion of other causes. The medical history is decisive. The initial endoscopy is essential for diagnosis and shows typical signs such as increased blood flow, inflammation and ulcers in the colon. In severe cases, imaging procedures such as CT or MRI are considered as an alternative [22].

Histopathology takes a significant lead in the diagnosis and characterization of ulcerative colitis. By examining tissue biopsies, histopathology provides valuable information about the changes in the colonic mucosa. These changes include morphological changes in the colonic crypts such as irregularities and decreased crypt density. There is also impairment of the cellularity of the lamina propria with increased infiltration of immune cells, as well as the occurrence of crypt abscesses and basal plasmacytosis. Epithelial abnormalities such as metaplasia and loss of goblet cells are also common. In addition, histopathology helps classify UC and determine the severity of the disease. It helps to assess the extent and severity of inflammation. This influences treatment decisions and the assessment of prognosis [22]. To assess the severity of ulcerative colitis, the Mayo Score is commonly used. It incorporates clinical symptoms, endoscopic findings and physicians' assessments to provide an objective measure of disease activity. Scores range from 0 to 12, with lower scores indicating remission or mild disease activity and higher scores representing moderate to severe or severe disease activity. The Mayo Score is a valuable tool for guiding treatment decisions and monitoring the effectiveness of therapies in UC patients [22].

1.3.3 Therapy

The main goal in the treatment of ulcerative colitis is to achieve and maintain remission to minimize disability, colectomy and risk of bowel cancer. Remission goals include the disappearance of symptoms such as rectal bleeding and better bowel habits. Endoscopic cure with a Mayo score of zero or one is targeted. Direct assessment of bowel inflammation by colonoscopy is critical, correlates with long-term remission and reduces corticosteroid use. The medication regimen depends on severity and extent, with early use of biologics in severe or steroid-resistant colitis. After remission, medication can be adjusted. Decisions on treatments are based on the extent of disease; topical therapy is often sufficient for proctitis, while systemic therapy is more appropriate for more extensive disease. The ultimate goal is individualized treatment for symptom control and remission maintenance [21].

As there are no curative drugs for ulcerative colitis, the focus of treatment is on achieving and maintaining remission, as well as minimizing the side effects of treatment to maintain a certain quality of life [23].

Among the drugs used in the treatment are 5-aminosalicylic acids (5-ASA), systemic and topical corticosteroids and immunomodulators. Topical anti-inflammatory treatments are mainly based on 5-ASA, which are effective when they come into direct contact with the inflamed tissue. Representatives of this group include sulfasalazine, osalazine and mesalamine [23]. 5-ASA medications are recognized as the gold standard treatment for individuals with mild to moderate ulcerative colitis. Specifically, for UC proctitis, 5-ASA suppositories are the preferred first-line therapy, surpassing both oral 5-ASA monotherapy and rectal corticosteroids in terms of effectiveness. In cases where the response to suppositories is suboptimal, the next course of action typically involves adding oral 5-ASA therapy to the treatment regimen [21].

Corticosteroids are administered when 5-ASA therapy proves ineffective. Their primary advantage is their systemic anti-inflammatory effect, eliminating the necessity for direct contact with the inflamed tissue. Moreover, they typically offer a faster onset of action compared to 5-ASA. Corticosteroid therapy, such as prednisone, is started at a dose of 40-60 mg and is then gradually reduced as the symptoms improve. The gradual reduction serves two main purposes: firstly, to reduce the risk of adrenal insufficiency and secondly, to avoid the possibility of colitis relapse associated with abrupt discontinuation. The side effects of corticosteroids can vary based on the dosage and the duration of their use. Short-term use, such as prednisone, is usually well tolerated and has only minimal adverse effects. Long-term and high-dose corticosteroid use can indeed result in significant and often predictable adverse effects, such as facial rounding, acne, increased body hair, diabetes, weight gain, hypertension, cataracts, glaucoma, heightened susceptibility to infections, muscle weakness, and even depression [23].

Patients who do not respond to the anti-inflammatory treatments mentioned above are treated with immune-modulating drugs. These include 6-mercaptopurine, azathioprine, methotrexate and cyclosporine. Immunomodulators are drugs that regulate the immune system to reduce inflammation. In conditions such as ulcerative colitis, the immune system becomes excessively active. These medications work by decreasing inflammation through methods such as reducing the number of immune cells and disrupting the production of inflammatory proteins [23].

In the management of moderate to severe colitis, patients can be treated with thiopurines, biological drugs or a combination of both. Thiopurines like azathioprine or 6-mercaptopurine are used to maintain remission in steroid-dependent patients. The efficacy of methotrexate in ulcerative colitis is still under investigation. Anti-TNF-α drugs, including infliximab, adalimumab and golimumab, are highly effective in inducing and maintaining remission in moderate to severe disease [21].

Infliximab is commonly used in hospitalized patients with severe ulcerative colitis and remains the most widely used biological treatment. Combining azathioprine with infliximab yields better results than azathioprine alone in achieving clinical remission and endoscopic healing. A newer class of biological drugs known as anti-adhesion molecule inhibitors has emerged. Vedolizumab, an example of this class, blocks the α4β7 integrin and is approved for the treatment of moderate to severe ulcerative colitis that is unresponsive to standard medications. Considering its efficacy and safety profile, vedolizumab can be considered as a first-line biological treatment option for ulcerative colitis [21].

Patients with acute severe ulcerative colitis, characterized by frequent bloody bowel movements and specific clinical indicators, should be admitted to a specialized center. Initial treatment involves intravenous corticosteroids, with approximately 65% of patients responding to this therapy. If patients do not respond within 3 to 5 days, rescue medical therapy using either ciclosporin or infliximab can be attempted, as both have shown equal efficacy. Prompt action is crucial to avoid complications and increased mortality rates [21].

Surgical options for acute severe ulcerative colitis, when medical therapy fails, include colectomy as a life-saving procedure. The choice of surgery depends on disease severity and quality of life. Options include subtotal colectomy with ileostomy or restorative procedures like ileoanal pouch or ileorectal anastomosis [24].

2 Materials

2.1 General Equipment

- **Centrifuge** 5418R, 100 rpm – 14 000 rpm, 0°C to 40°C, Eppendorf Ltd. (Hamburg, Germany)
- **Centrifuge tubes**; 15 ml, 50ml, Corning Ltd. (Corning, New York, USA)
- **Microcentrifuge tubes**, 1.5 ml and 2.0 ml, Eppendorf Austria Ltd. (Vienna, Austria)
- **Pipette**, Research plus; 2.5µl, 10µl, 20µl, 100µl, 200µl, 1000µl, Eppendorf Austria Ltd. (Vienna, Austria)
- **Tips** TipOne®; 10µl, 200µl, 1000µl, STARLAB International Ltd. (Hamburg, Germany)
- **Vortex mixer**, lab dancer, VWR® International Ltd. (Radnor, USA)

2.2 qPCR Materials

2.2.1 RNA-Extraction

- Omnifix-F, 1 ml, Braun Melsungen Ltd. (Bad Arolsen, Germany)
- Disposable cannulas: "Sterican® Gr. 14, G 23 x 1 1/4"" / ø 0,60 x 30 mm; Braun
- **Nano drop**: NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer; ThermoFisher Scientific
- **RNA**-Kit: peqGOLD HP Total RNA Kit; peQlab; VWR International GmBH (Erlangen)

2.2.2 Reverse Transcriptase

- **Block 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

2.2.3 qPCR

- **384-well PCR plates**: Thermo Scientific Ltd. (Rockford, USA)
- **qPCR seal:** 4titude® via Brooks Life Science
- **Dispenser:** 0,5 – 10µL, 5-100µl, Eppendorf Xplorer®; Eppendorf
- CFX Connect Real-Time PCR Detection System, Bio-Rad Laboratories Ltd. (Hercules, USA)
- **Software:** QuantStudio (TM) 6 Flex System

2.3 Histology Materials

Embedding

- **Embedding station** AP 250 W, Microm Ltd. (Walldorf, Germany)
- **Histology cassettes**, VWR® International Ltd. (Radnor, USA
- **Peltier-cooled incubator** IPP110, VWR® International Ltd. (Radnor, USA)

Histological cuts:

- **Low Profile Microtome Blades** DB80 LS, Leica Biosystems Ltd. (Nussloch, Germany)
- **Microscope slides** (pre-cleaned/ready-to-use/ground edges) Superfrost® Plus, Thermo Scientific Ltd. (Braunschweig, Germany)
- **Slide holders**, VWR® International Ltd. (Radnor, USA)
- **Slideboxes** (100 lames), VWR® International Ltd. (Radnor, USA)
- **GFL Paraffin stretching bath** (1052), Lauda-GFL Ltd. (Burgwedel, Germany)
- **Semi-motorized rotary microtome** (RM2245), Leica Biosystems Ltd (Nussloch, Germany)

Staining

- **Dyeing troughs for manual dyers** (12 pieces), VWR® International Ltd. (Radnor, USA)
- **Stainless steel caps for dyeing troughs** (12 pieces), VWR® International Ltd. (Radnor, USA)
- **Micro cover glasses** (20x20 mm)

Microscopy

- Axiocam ERc 5s, Carl Zeiss Microscopy Ltd. (Göttingen, Germany)
- Microscope Axio Lab 5x H SF 22, Carl Zeiss Microscopy Ltd. (Göttingen, Germany)

Softwares

- Excel 2013 software
- ImageJ processing program
- Zeiss efficient Navigation Software Program (ZEN)

2.4 Protein Array Materials

2.4.1 Protein extraction

- Omnifix-F, 1 ml, Braun Melsungen Ltd. (Bad Arolsen, Germany)
- Disposable cannulas: "Sterican® Gr. 14, G 23 x 1 1/4"" / ø 0,60 x 30 mm; Braun

2.4.2 Protein concentration

- **96-well plate:** Sarstedt Ltd. (Nümbrecht, Germany)
- **Pipette:** Eppendorf™ Research plus™ Adjustable Volume Multichannel Pipetters
- Peltier-cooled incubator IPP110, VWR® International Ltd. (Radnor, USA)
- **Photometer**

2.4.3 Protein array

- Quantibody® Mouse Inflammation Array 1 RayBiotech Ltd. (Norcross, USA)

2.5 MPO-Assay Materials

- Omnifix-F, 1 ml, Braun Melsungen Ltd. (Bad Arolsen, Germany)
- **Disposable cannulas:** "Sterican® Gr. 14, G 23 x 1 1/4"" / ø 0,60 x 30 mm; Braun
- Peltier-cooled incubator IPP110, VWR® International Ltd. (Radnor, USA)
- 96-well plate: Sarstedt Ltd. (Nümbrecht, Germany)
- **Photometer**
- **Pipette:** Eppendorf™ Research plus™ Adjustable Volume Multichannel Pipetters

3 Reagents

3.1 Histology Reagents

- ddH2O, produced in the lab with Arium®pro
- Eosin Y disodium salt ≥85%, Sigma Aldrich Ltd. (Steinheim, Germany)
- Ethanol ≥96%, 80%, 70%, 30%
- Eukitt® Quick-hardening mounting medium for microscopy, Sigma Aldrich Ltd. (Steinheim, Germany)
- Mayer's hematoxylin solution (Gill No.2), Sigma Aldrich Ltd. (Steinheim, Germany)
- Xylene, histological grade, Sigma Aldrich Ltd. (Steinheim, Germany)

3.2 MPO Assay Reagents

- Potassium phosphate buffer (regular chemicals), 500ml, 50mM pH 6, monobasic, dibasic
- ddH2O, produced in the lab with Arium®pro
- HTAB (hexadecyltrimethylammonium bromide, solid, powder, toxic), Sigma Aldrich Ltd.
- O-dianisidine (D3252), Sigma Aldrich Ltd.

3.3 qpCR Reagents

3.3.1 RNA-Extraction reagents

- TRK Lysis Buffer
- Ethanol: 70%, 80%
- RNA nuclease free water

3.3.2 Reverse Transcription

- RNA nuclease free water
- Reaction mix
- Reverse transcriptase

3.3.3 qPCR

- PerfeCTa® SYBR® Green FastMix®, Quanta bio (Beverly, USA)
- RNA nuclease free water
- Primer (fw&rv):

IL7	•**fw:** TCTGCTGCCTGTCACATCATC •**rv:** GGACATTGAATTCTTCACTGATATTCA
Eef1a1	•**fw:** CCTGGCAAGCCCATGTGT •**rv:** TCATGTCACGAACAGCAAAGC
TNFa	•**fw:** CCACAGGGCTGCAATTTTCC •**rv**: CCACAGGGCTGCAATTTTCC
IL1b	•**fw:** TCCTGTGTAATGAAAGACGGC •**rv:** GGTGCTGATGTACCAGTTGGG
Irf1	•**fw:** CCCAGCTCTTGCTTTCGGA •**rv:** AAGCCCAGTAGTTCACGACC
STAT1	•**fw:** GCTGAGTGCTGTTCCCATCT •**rv:** AAGTCCTTCAGAGTAACAG
Ifng	○**fw:** ATGAACGCTACACACTGCATC ○**rv:** CCATCCTTTTGCCAGTTCCTC
Tgf1b	•**fw:** CCACAGGGCTGCAATTTTCC •**rv:** CCACAGGGCTGCAATTTTCC
MyD88	•**fw**: GCACCTGTGTCTGGTCCATT •**rv:** TGTTGGACACCTGGAGACAG
Tlr3	•**fw:** GTATTGCCTGGTTTGTTAATTGG •**rv:** AAGAGTTCAAAGGGGGCACT
Reg3g	•**fw:** CTCCCCACCTGTCGTGTAGT •**rv:** CTCCCCACCTGTCGTGTAGT
Il33	•**fw:** TGAGACTCCGTTCTGGCCTC •**rv:** CTCTTCATGCTTGGTACCCGAT

Table 1 List of primers and corresponding sequences: fw=forward; rv: reversed

3.4 Protein array reagents

3.4.1 Protein extraction reagents

- RIPA Lysis Buffer, 10x, EMD Millipore Corp. (Billerica MA, USA)
- Phenylmethylsulfonyl fluoride (PMSF), Sigma Aldrich Ltd. (Steinheim, Germany)
- Roche tablet

3.4.2 Protein concentration materials

- BSA
- BCA
- ddH2O

4 Methods

4.1 Animals Intervention

In the conducted experiments, C57Bl/6 male 4-weeks old mice were purchased from the Janvier Labs. The division was made into 5 different groups with 8 mice per group. The first group was the control group (Ctrl) and the second group was treated with DSS food. The three remaining groups were assigned reduced macronutrient diets, resulting in the following group configurations. Each of these groups had already been exposed to DSS: low-carbohydrate, low-fat and low-protein.

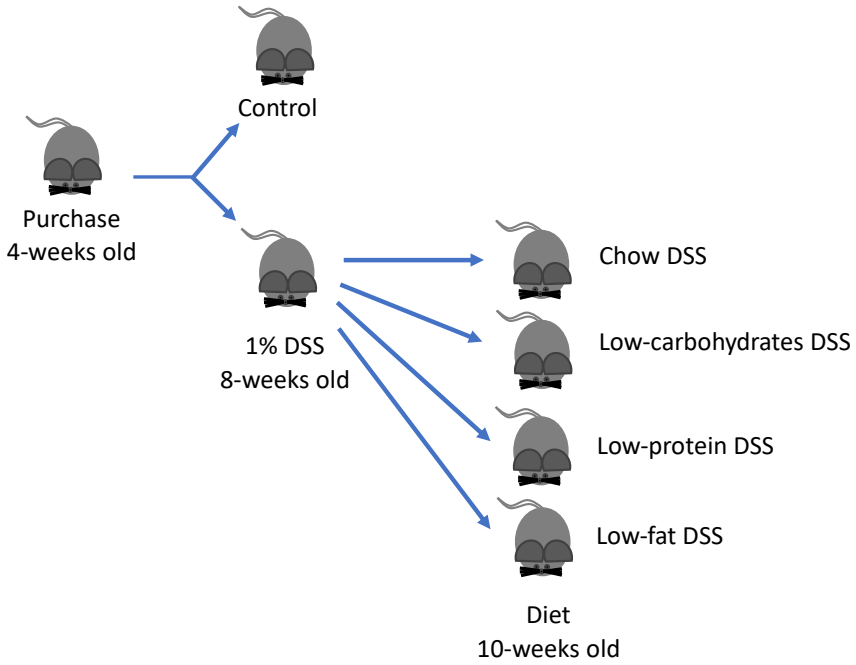


Figure 1 Experimental setup: An overview of the classification into the 5 groups
Source: Project design document by Kalina Duszka [25]

The dietary intervention lasted four weeks, starting when the mice were 10 weeks old. The treatment with DSS (1 % in the drinking water) was started two weeks prior to the dietary intervention. The development of inflammation was monitored daily. This included assessments of physical activity, body weight, stool consistency and the presence of blood in the stool. Once the mice were 14-weeks old, they were then dissected for the following experiments. Throughout the diet interventions, the mice were treated under *ad libitum* conditions, allowing them to consume the diet at any time without any restrictions on the amount or frequency of consumption [26].

The following table provides detailed information about the diet interventions and the amount of the macronutrients administered.

Ingredients and energy	Chow	Low-carbohydrates	Low-protein	Low-fat
Carbohydrates (gm%)	65	**36**	79	74
Protein (gm%)	20	44	**5**	25
Fat (gm%)	7	11	7	**35**
Fibre (gm%)	5	5	5	5
Vitamins and minerals (gm%)	1	1	1	1
Choline	3	3	3	3
Total energy (kcal/g)	4	4.2	4.0	3.7
% energy from carbohydrates	65	**35**	80	74
% energy from protein	20	42	**5**	25
% energy from fat	15	23	15	**1**

Table 2 Composition of the low-macronutrient diets: The exact details of the feed supplied are given here in a list. Source: Project proposal document by Kalina Duszka [25]

4.2 Dissection and tissue collection

On the day of dissection, the mice were individually removed from their cages and anesthetized using isoflurane. Following this, they were weighed and blood samples were collected, which were treated with aprotinin and EDTA as protease inhibitors. Subsequently, tissue samples including the liver, cecum, small intestine and lymph nodes were taken. The collected tissue samples were frozen and stored at -150°C until further processing. Tissue samples from the small intestine and proximal colon were placed in cassettes and were preserved in 4% PBS-buffered for later histological analysis.

4.3 qRT-PCR-experiments

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess changes in messenger ribonucleic acid (mRNA) gene expression. The qPCR experiments were divided into several steps, starting with RNA extraction, followed by reverse transcription and concluding with the qPCR measurement. The QuantStudio 6 Flex Real-time PCR System by Life Technologies Holding was utilized for the measurements.

4.3.1 RNA-Extraction

For the RNA extraction, tissue samples from the small intestine, Peyer's patches, lymph nodes and proximal colon were used. Each sample, weighing 10-15 µg, was cut on dry ice. To begin the extraction, 350 µl of lysis buffer was added to each sample, which was then disrupted using syringes. After complete dissolution of the tissue in the buffer, the samples were transferred to DNA removing tubes and centrifuged at 13 000 x g for 1 minute at room temperature. The filtrate was collected and the column was discarded. Next, 350 µl of 70% ethanol was added to the filtrate and mixed thoroughly by pipetting. This mixture was then transferred to a mini column, which is a tube that binds RNA. After centrifugation at 10 000 x g for 1 minute, the filtrate was discarded. The washing steps started with 500 µl of wash buffer, followed by two washes with 80% ethanol. Between each wash, the mixture was centrifuged, the filtrate was trashed and the tubes were dried thoroughly. After the final wash with ethanol, the mini column was centrifuged at 13 000 x g for 2 minutes to remove any residual ethanol. The mini columns were then placed on new Eppendorf tubes and 50 µl of RNAse-free water was added. After centrifugation, the RNAse-free water was collected from the bottom and reapplied to the columns for a final centrifugation step. The mini columns were then removed and the RNA concentrations were measured using NanoDrop. RNAse-free water was used as the blank value.

4.3.2 Reverse Transcription

The first step in RNA extraction is to put in the previously calculated amount of nuclease-free water and then pipette the pre-extracted amount of RNA for each sample. In the meantime, the samples should be kept on ice to ensure the stability of the RNA and minimize the risk of degradation. To each sample, 5 µl of the master mix is added, which is composed of 4 µl of reaction mix buffer and 1 µl of nuclease-free water. After centrifugation, the samples are placed in a heating block at 22°C for 5 minutes. They are then transferred to a heating block at 42°C for 30 minutes, followed by a final incubation at 85°C for 5 minutes. Once the thermal cycling steps are completed, the samples are placed back on ice and 380 µl of water is added to each one. The resulting cDNA is stored at -20°C for later PCR analysis.

4.3.3 qPCR- process

The PCR analysis is performed using 384-well plates. Following a predetermined plate layout, 2 µl of the cDNA sample and 8 µl of the master mix are added to each well in a specific pattern. The master mix used for the plates was composed as shown in the following table. The specific primers used are listed in *Table 1.*

The plate is then covered to prevent contamination and centrifuged to ensure that the samples remain in their designated wells. After the plate is prepared, the samples undergo real-time PCR analysis using the Quant studio™ 6 Flex Real-time PCR System.

components	Amount per well (in µl)
SYBR Green	5
Forward primer (fw)	0,3
Reversed Primer (rv)	0,3
ddH2O	2,4

Table 3 Master mix configuration

4.4 Histological tissue protocol

4.4.1 Preparation and embedding process

The histological tissue samples were taken from the small intestine and proximal colon and placed in histological cassettes. They were stored in a 4% formalin solution buffered with PBS. After two days, the solution was removed and the samples were rinsed under running cold tap water for 15 minutes. Subsequently, the samples were stored in a 75% ethanol solution. This solution was removed and replaced with a solution of 85% ethanol. The samples were then shaken at room temperature for one hour. After one hour, this solution was also removed and the samples were stored overnight in a solution of 95% ethanol and 5% methanol in a cold storage room. The next day, the samples underwent three washing steps using 96% ethanol. During each washing step, the samples were shaken in the ethanol solution for one hour. After each hour, the ethanol solution was discarded and replaced with fresh 96% ethanol. This process was repeated three times to ensure thorough washing of the samples. After the ethanol washing steps, the samples were transferred twice to xylene for 45 minutes each. In the final step, the paraffin was heated in an incubator at 60°C. The paraffin had already been melted at 60°C the day before for the next steps. The melted paraffin was labeled as Paraffin I, Paraffin II and Paraffin III. Each of these paraffins was added to the samples for 30 minutes, one after the other and heated in the incubator. In between, the samples were shaken every 5 minutes.

After the last paraffin solution was drained, the samples were embedded in paraffin using a special embedding machine. For this, the samples were divided into multiple sections according to the height of the cassette, with the aim of creating cross-sections of the tissue later. Then, warm paraffin was released from the embedding machine and guided to a special position where it was allowed to solidify. It was important to ensure that the embedded samples remained in their correct positions to create optimal slices. After embedding, the tissue blocks are allowed to cure overnight at room temperature and then stored in a cool room until the cutting process.

4.4.2 Tissue sectioning

The tissue blocks are cooled in a freezer at -20°C for at least 10 minutes prior to sectioning. First, the excess paraffin is trimmed away using the *"TRIM"* function set at a thickness of 16µm. This allows for approaching the sample and removing the excess paraffin. Once close to the tissue, the *"SECT"* function is used to cut sections at a thickness of 4µm per step. Three sections are taken from each sample, with typically 1-2 sections per slide. The cut sections are transferred to a water bath and then mounted on slides. They are dried in an oven at 37°C for 48 hours.

4.4.3 Hematoxylin&Eosin staining

The Hematoxylin and Eosin staining (H&E) is usually performed in multiple steps to achieve optimal staining. Samples are typically placed in staining dishes to ensure even distribution of the dyes.
The staining protocol is illustrated with a diagram to provide a clear guide to follow.

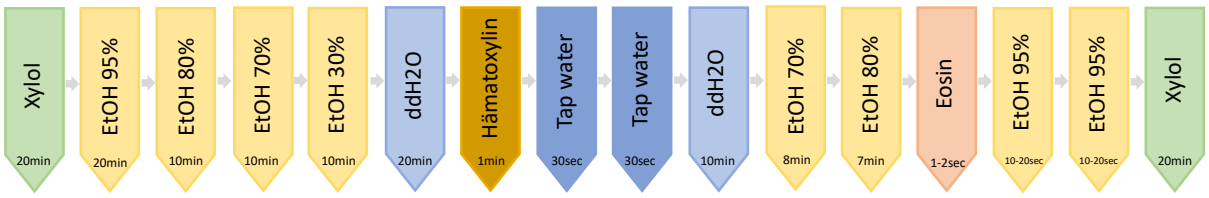


Figure 2 Staining protocol overview: The figure presents a clear overview of the chemicals utilized and their respective timeframes.

The staining process starts with dehydration using xylene and various dilutions of ethanol. During incubation in the different solutions, the samples are gently agitated to ensure optimal penetration of the solutions. Next, the samples are thoroughly rinsed with distilled water before undergoing the first staining step with hematoxylin. Further rinses with tap water and distilled water are performed before dehydrating the samples with 70% and 80% ethanol, respectively.

The samples are then stained with eosin and dehydration is repeated using previously used 95% ethanol and fresh 95% ethanol. After immersion in xylene, the samples are covered with a coverslip using Entellan and left to air-dry completely. This allows for optimal preservation of the stained samples and facilitates further examination under the microscope.

4.4.4 Microscopic analysis

To facilitate further analysis, images of the samples were captured at 5x and 10x magnification using a microscope that was connected to a camera system. These images were later analyzed using the software ImageJ to determine the muscle thickness, villi length and crypt depth of the samples.

4.5 Protein array experiment

The use of protein arrays, such as the Quantibody® Array, allows for the simultaneous detection and quantification of multiple cytokines. Based on a sandwich ELISA platform, this array combines the sensitivity and specificity of ELISA with the high throughput capabilities of arrays. The process involves immobilizing a capture antibody to a glass surface, then trapping the target cytokine on the solid surface after incubation with the sample. Next, a biotin-labeled detection antibody is added, which recognizes a different epitope of the target cytokine. Finally, the cytokine-antibody-biotin complex can be visualized using a streptavidin-conjugated Cy3 equivalent dye and a laser scanner. Unlike traditional ELISAs, protein arrays use an array format, allowing for quantitative, multiplex detection of cytokines in a single experiment by arraying multiple cytokine-specific capture antibodies onto a glass support [27].

4.5.1 Protein extraction with RIPA

In this experimental setup, samples from the small intestine and proximal colon were used. Samples weighing 10-20ug were cut and mixed with 10 times the amount of RIPA buffer. The samples were then disrupted using syringes and incubated on ice, with intermittent disruption. After centrifugation at 10,000g and 4°C, the supernatant was transferred to a fresh Eppendorf tube.

4.5.2 Protein concentration measurement

For the protein concentration measurement, the first step is to prepare the standards. Seven standards are prepared using BSA and ddH2O, as shown in the image below. The BSA stock solution and the ddH2O blank value also serve as standards, making a total of nine standards.

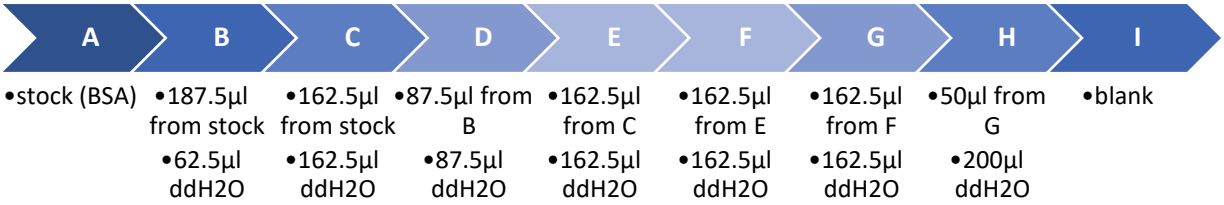


Figure 3 BSA Standards composition

To prepare the working reagent for the BCA protein concentration measurement, BCA reagent A and BCA reagent B were mixed in a 50:1 ratio. The total volume of the working reagent was calculated based on the number of samples and standards and multiplied by 200 µl, which is the volume of working reagent added to each sample. As part of the sample preparation procedure, a pre-established layout was followed to transfer 25 µL of standards and 10 µL of test samples into designated wells of a 96-well microplate. To ensure complete mixing, 200 µL of a working reagent was then dispensed into each well and gently shaken for 30 seconds. Subsequently, the plate was sealed and placed in a preheated incubator at 37°C for 30 minutes to allow the reaction to proceed. After the incubation period, the plate was removed from the incubator and left to cool to room temperature. Finally, the absorbance of each well was measured at a wavelength of 562 nm using a spectrophotometer.

4.6 Myeloperoxidase Activity Assay

Myeloperoxidase (MPO) forms a key component of the innate immune system and is mainly released by neutrophils to fight off incoming pathogens[28]. It serves as a prominent inflammatory marker, with increased MPO levels detected in a variety of pathological conditions, including inflammatory bowel disease (IBD), cardiovascular disease, obesity, liver disease, arthritis, multiple sclerosis, Alzheimer's disease, stroke and cancer [29].

The enzyme is predominantly found in the azurophilic granules of neutrophilic granulocytes, constituting approximately 5% of their dry mass. It performs several functions, including catalyzing the conversion of hydrogen peroxide into hypochlorous acid, generating highly reactive molecules like tyrosyl radicals and assisting in the cross-linking of proteins [30].

Tissue samples weighing a total of 10-15 µg were used in the experiment to analyze a section of the small intestine. Dibasic and monobasic potassium phosphate buffers were first dissolved in 400 mL of deionized water. Next, a 0.5% solution of HTAB (hexadecyltrimethylammonium bromide) was prepared by calculating the required amount (20 times the weight of the samples) and mixing it with the potassium phosphate buffer. Then, 20 µl of 0.5% HTAB was added to each sample, followed by disruption and centrifugation at 15,000g for 15 minutes at 4°C. For the working reagent, O-dianisidine was mixed with H2O2. The number of samples was multiplied by 200 µl to calculate the required amount of O-dianisidine, which totaled 8 ml. Approximately 1.3 mg of O-dianisidine was diluted in potassium buffer to make 8 ml. Finally, 1% H2O2, which was diluted from a 30% H2O2, was added after all samples were pipetted onto the plate. For each sample, 12.5 µl of the centrifuged supernatant was used. The working reagent was added just before measurement to enable the measurement of a zero-time point. The spectrophotometer was set to 37°C and a wavelength of 450 nm, with measurements recorded every 5 minutes.

5 Results

5.1 qPCR results

The mRNA expression of IL-1b in the small intestine tissues showed statistical significance between the Ctrl DSS group and the DSS low-fat group (p=0.000000228), as well as between the Ctrl DSS group and the DSS low-protein group (p=0.000357).

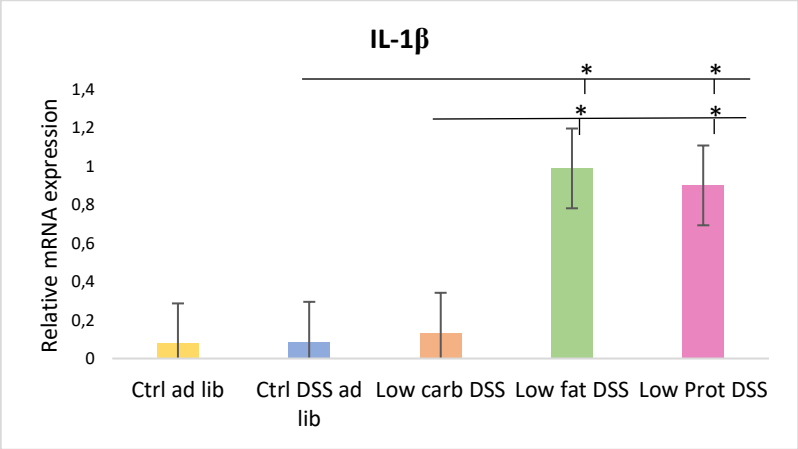


Figure 4 Small intestine IL-1ϐ gene-expression: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

The gene Interferon Regulatory Factor 1 (Irf1) showed significant differences in expression levels between experimental groups. Specifically, significant differences were observed between the Ctrl DSS group and the low-fat group (p=0.0000031) as well as between the Ctrl DSS group and the low-protein group (p=0.0000155). There were also significant differences between the low-carb group and the low-fat group (p=0.00000022) as well as between the low-carb group and the low-protein group (p=0.0000015).

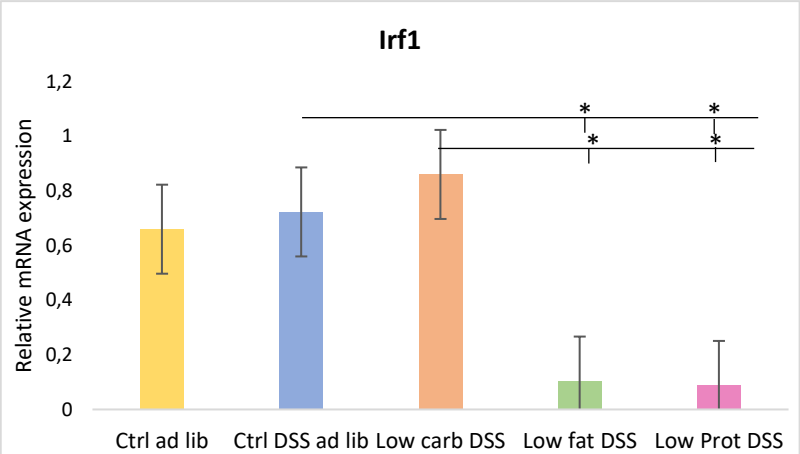


Figure 5 Small intestine gene-expression of Irf1: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

A statistically significant difference was observed between the Ctrl DSS group and the DSS low-carb group in terms of MyD88 gene expression (p=0.00905).

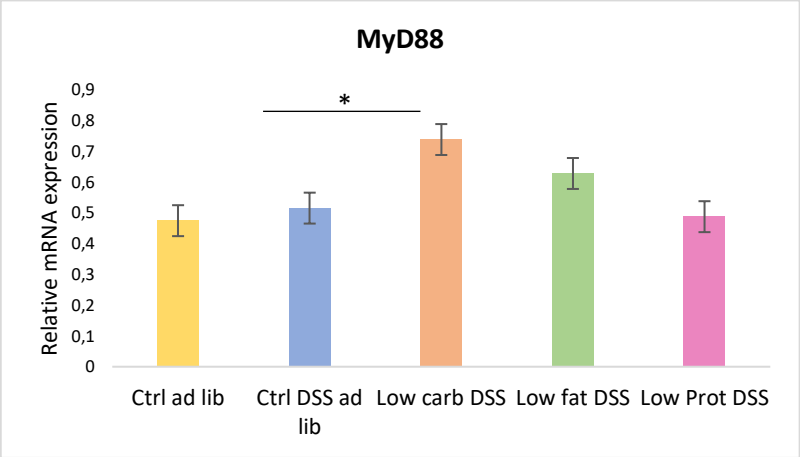


*Figure 6 Small intestine gene-expression of MyD88: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: * implies that the t-tests showed a statistically significant difference with p≤0.01.*

The gene expression analysis of proximal colon samples revealed a statistically significant difference in the expression of the Signal Transducer and Activator of Transcription 1 (STAT1) gene between the Ctrl DSS group and the DSS low-protein group (p=0.0059).

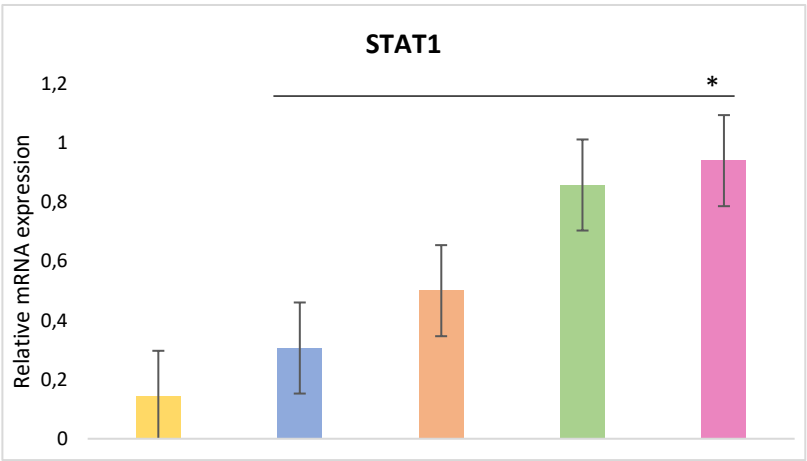


Figure 7 Gene-expression of STAT1 in the proximal colon samples: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

A statistically significant difference was observed in the expression of TGF1β between the Ctrl *ad libitum* group and the Ctrl DSS group (p=0.00165).

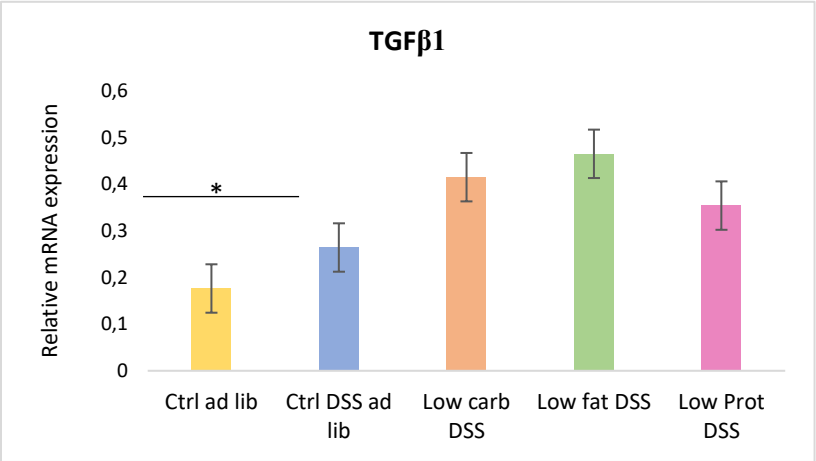


Figure 8 Expression of transforming growth factor beta 1(TGFβ1) in the proximal colon: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

The expression of MyD88 exhibited a statistically significant difference between the Ctrl *ad libitum* group and the Ctrl DSS group (p=0.00743).

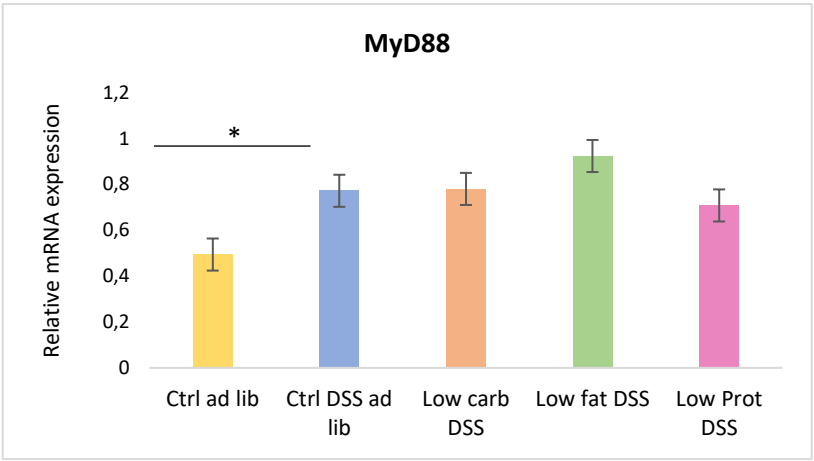


Figure 9 Expression of MyD88 gene in the proximal colon: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

Regarding the mRNA-expression of Il33, there was a statistically significant difference observed between the Ctrl DSS group and the DSS low fat group (p=0.00403).

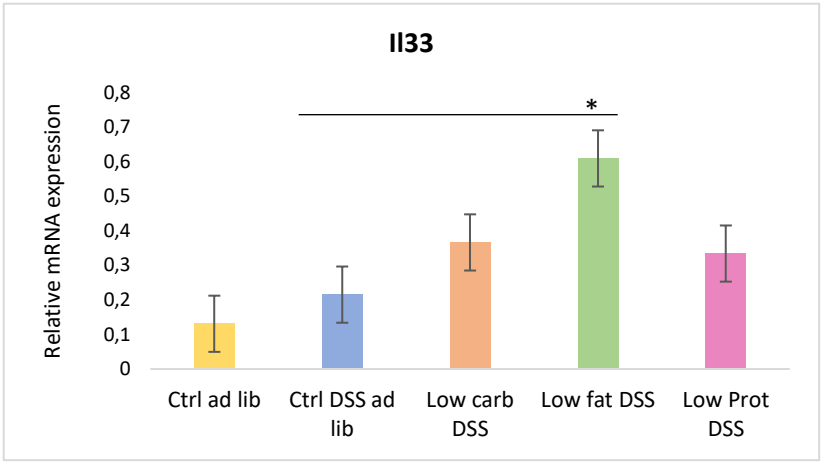


*Figure 10 Il33 gene-expression in proximal colon: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: * implies that the t-tests showed a statistically significant difference with p≤0.01.*

Among the lymph node samples, a statistically significant difference was found in the gene expression of IL-7 between the Ctrl *ad libitum* group and the Ctrl DSS group (p=0.0000374).

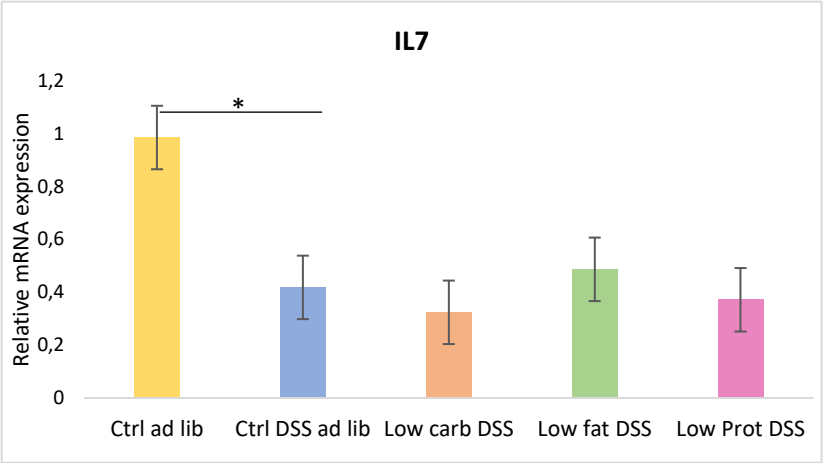


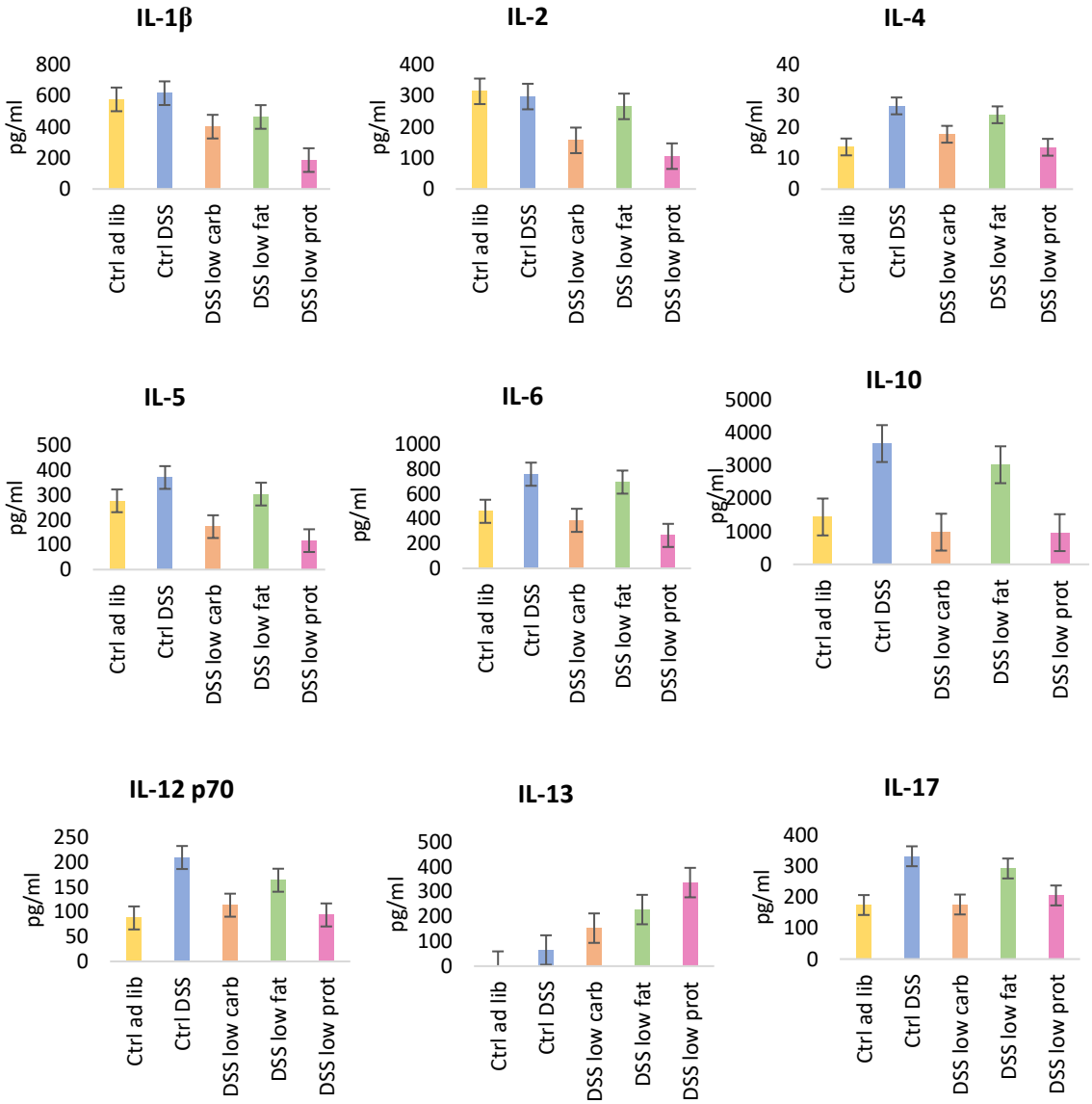
Figure 11 Lymph node samples gene IL-7 expression: Statistical analysis is performed with one-way ANOVA analysis, followed by T-test analysis: implies that the t-tests showed a statistically significant difference with p≤0.01.*

The qPCR results of the Peyer's patches samples revealed no statistically significant differences in gene expression between the groups. This indicates that the expression levels of the genes analyzed were similar across the experimental groups in the Peyer's patches.

5.2 Protein array results

In the protein array analysis, a total of 18 cytokines were examined using the TH17 array. The comparison of the groups revealed a statistically significant difference for all cytokines, as determined by the one factorial ANOVA-Test.

5.2.1 Protein array analyisis for small intestine



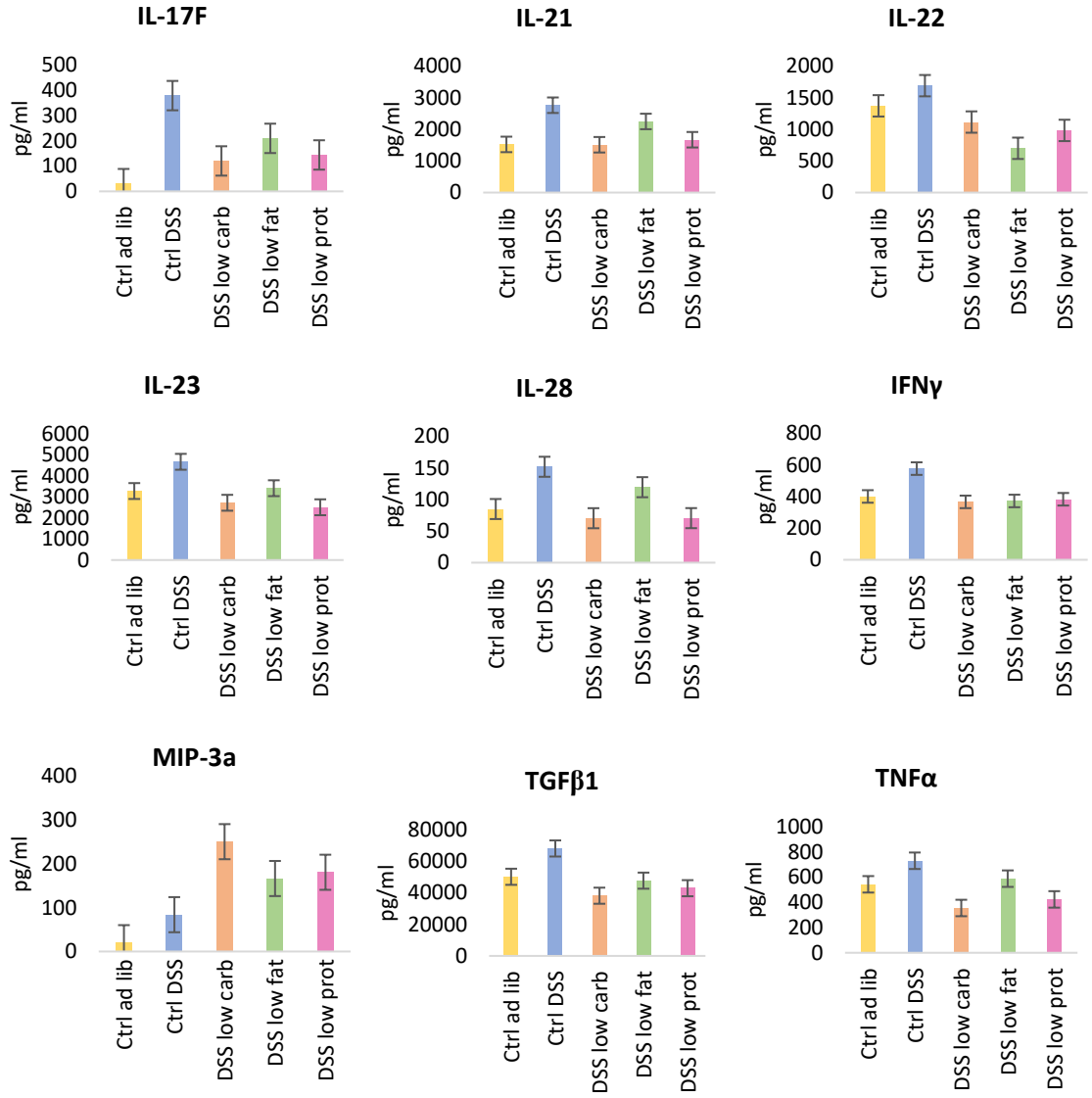


Figure 12 Protein array cytokine expression results: Cytokines of interest were examined with TH17-array. Statistical analysis using ANOVA, followed by T-test to figure out statistical significant differences among the groups: p≤0,01

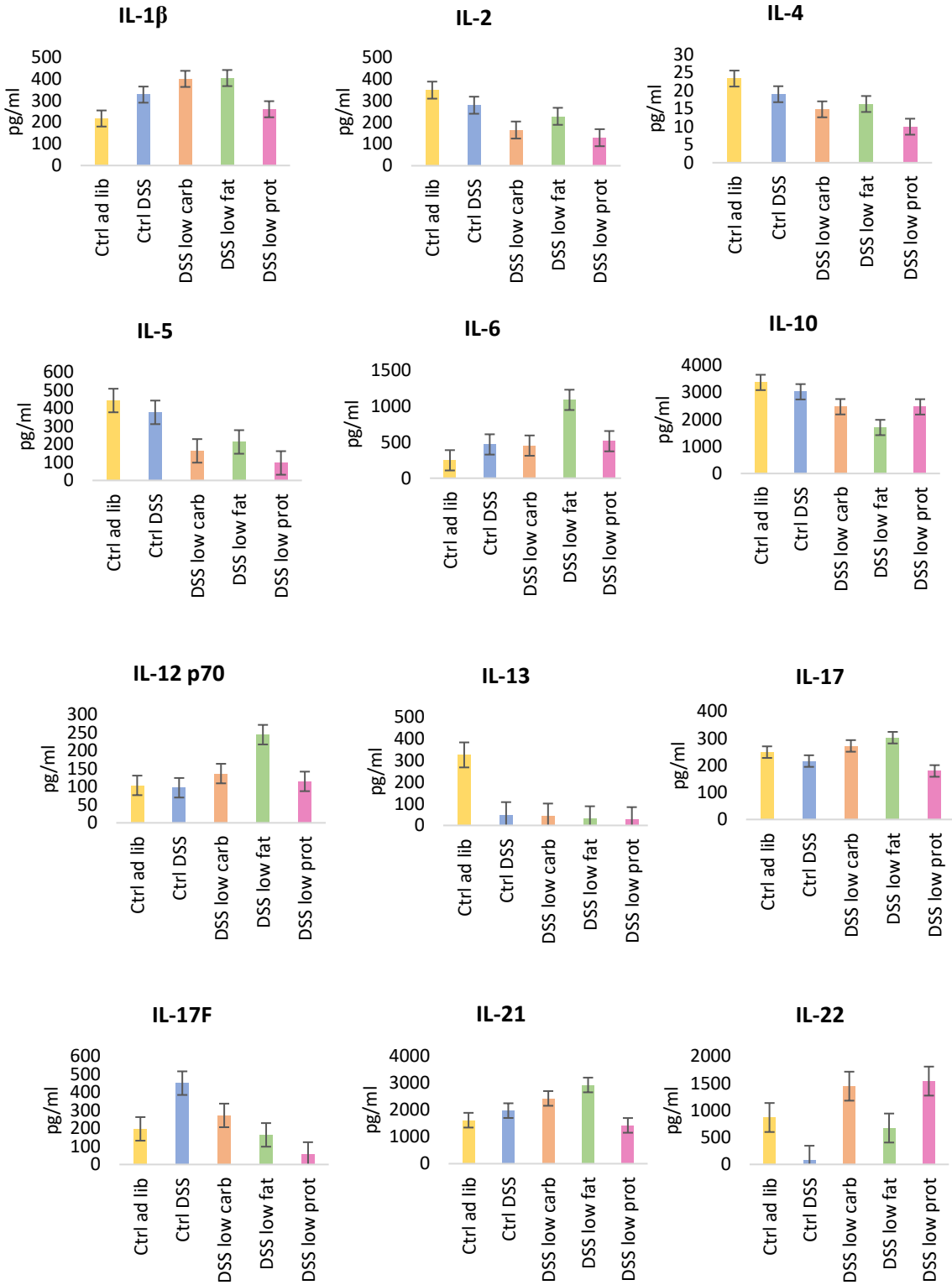
As expected, the DSS-induced control group exhibited a clear and significant increase in cytokine levels within the small intestine samples. This finding supports previous assumptions and suggests the occurrence of an immune response or inflammation in the small intestine due to the DSS treatment. Elevated cytokine levels in this context are often indicative of immune cell activation and the release of pro-inflammatory molecules. In general, when comparing the DSS control group to the low-carbohydrate DSS group, a statistically significant difference was observed. The low-carbohydrate group exhibited reduced cytokine levels compared to the control group.

Similarly, a significant difference was found between the DSS control group and the DSS low-protein group, with lower cytokine levels in the low-protein group. However, no significant difference was observed between the DSS group and the low-fat group in terms of cytokine levels. A statistically significant difference was found for IL-1β between the DSS group and the low-fat group (p=0.0051). The reductions in IL-1β levels were significantly more pronounced in the low-protein group (p=0.000078) and the low-carb group (p=0.00067) compared to the DSS group.

The cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, IL-17, IL-21, IL-23, and TNFα exhibited a statistically significant difference in both the low-carbohydrate and low-protein groups when compared to the DSS control group. When comparing the low-carbohydrate and low-protein diet groups, no statistically significant difference was detected in the reduction of cytokine levels. This non-significant difference suggests that none of the two groups was superior to the other in terms of lowering cytokine levels. The ANOVA test indicated a difference in IL-13 levels among the groups, suggesting some variation in cytokine levels across the experimental conditions. However, when conducting t-tests specifically between the control group with *ad lib diet* and the control group with DSS treatment, a statistically significant difference was observed (p=0,0028345). Conversely, no statistically significant difference was found when comparing IL-13 levels between the low-carb and low-protein groups.

For IL-17F, a statistically significant difference was observed only between the control group with *ad libitum diet* and the control group with DSS treatment (p=0,0189). Although the ANOVA analysis indicated a difference in IL-22, IL-28 and IFNγ levels among the groups, no statistically significant differences were found in subsequent t-tests.

5.2.2 Protein array analysis proximal colon



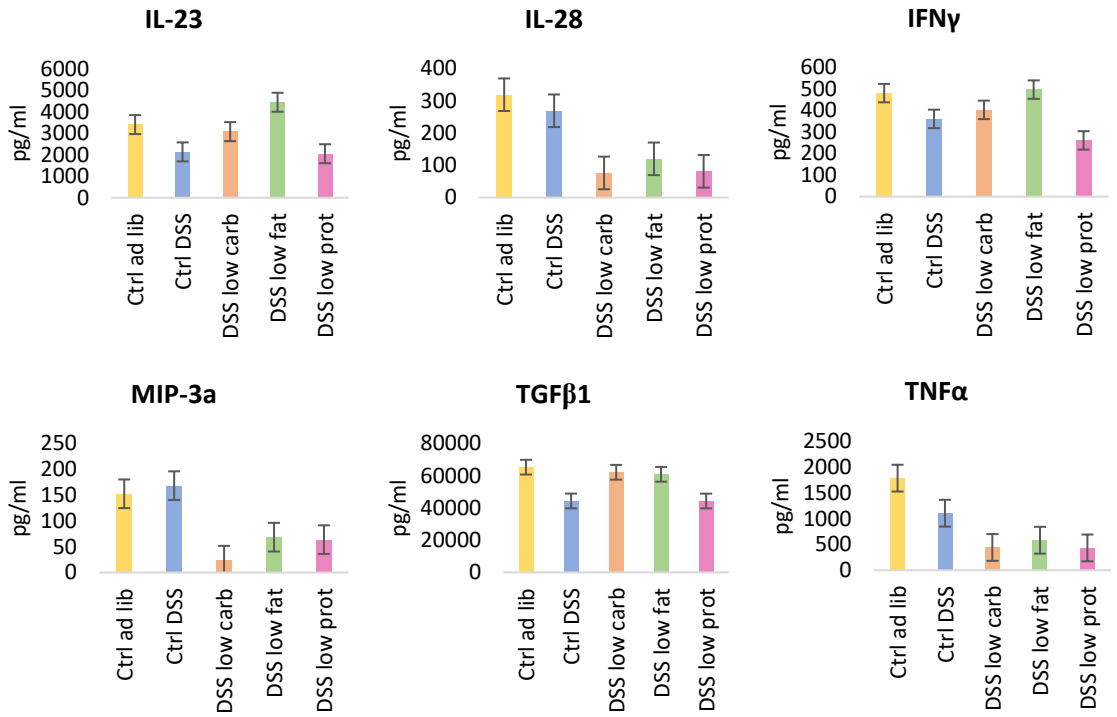


Figure 13 Cytokine expression levels in the proximal colon. Statistical differences examined with ANOVA, followed by T-test analysis: p≤0,01.

The results of ANOVA did not indicate any statistically significant differences between the groups regarding the levels of IL-1β, IL-2 and IL-10. The levels of IL-4, IL-13, IL-17, IFNγ, MIP-3a, and TGFβ1 indicate a statistically significant difference among the groups with ANOVA analysis. However, when conducting t-tests to compare specific pairs of groups, no statistically significant differences were found. According to the t-test analysis, a statistically significant difference was found for IL-5 between the Ctrl DSS group and the Ctrl low-carb group. According to the t-test analysis, there was a statistically significant difference in the levels of IL-6 between the DSS low-fat group and the DSS low-carb group. The IL-6 levels were found to be higher in the DSS low-fat group compared to the DSS low-carb group. The t-test analysis demonstrated a statistically significant difference in IL-12-p70 levels between the low-carb (p=0,0024) and low-fat group, as well as between the low-protein (p=0,0019) and low-fat group. The results indicated that the levels of IL-12-p70 were significantly higher in the low-fat group compared to both the low-carb and low-protein groups. The analysis revealed a statistically significant difference in the levels of IL-17F and IL-21 between the low-carb and low-protein groups. Notably, the cytokine levels were found to be lower in the low-protein group compared to the low-carb group.

A notable finding was the significantly low levels of IL-22 in the Ctrl DSS group. The IL-22 levels in this group showed a statistically significant difference compared to the Ctrl *ad libitum* group, as well as the DSS low-fat and DSS low-protein groups. Both IL-28 and TNFα exhibited statistically significant differences in the ANOVA analysis, as well as in the subsequent t-tests. The t-tests further confirmed significant differences between the groups with low-fat, low-carb and low-protein when compared to the Ctrl DSS group. Remarkably, the levels of IL-28 and TNFα were consistently lower in all three low macronutrient groups in comparison to the Ctrl DSS group.

5.3 Histology results

5.3.1 Small intestine

The histological preparations, stained with hematoxylin and eosin, were examined using an AxioCam and a Zeiss microscope at different magnifications. The focus of interest was on observing morphological changes in the length of villi intestinalis, the depth of crypts and the thickness of the muscularis externa.

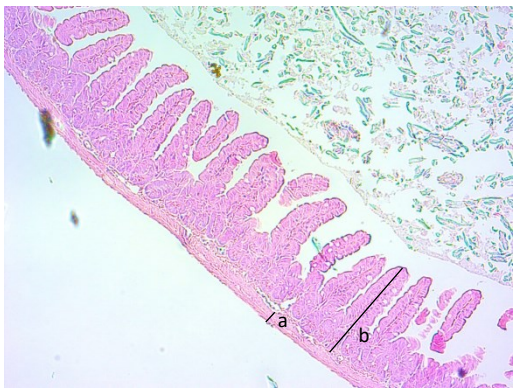


Figure 14 Measurements of thickness of muscularis externa (a) and villi length (b)

The muscularis externa is responsible for segmental contractions and peristaltic movement in the gastrointestinal tract. These muscles ensure that food, along with digestive enzymes, is moved and churned through the gastrointestinal tract. The muscularis externa consists of an inner circular layer and an outer longitudinal muscle layer [31].

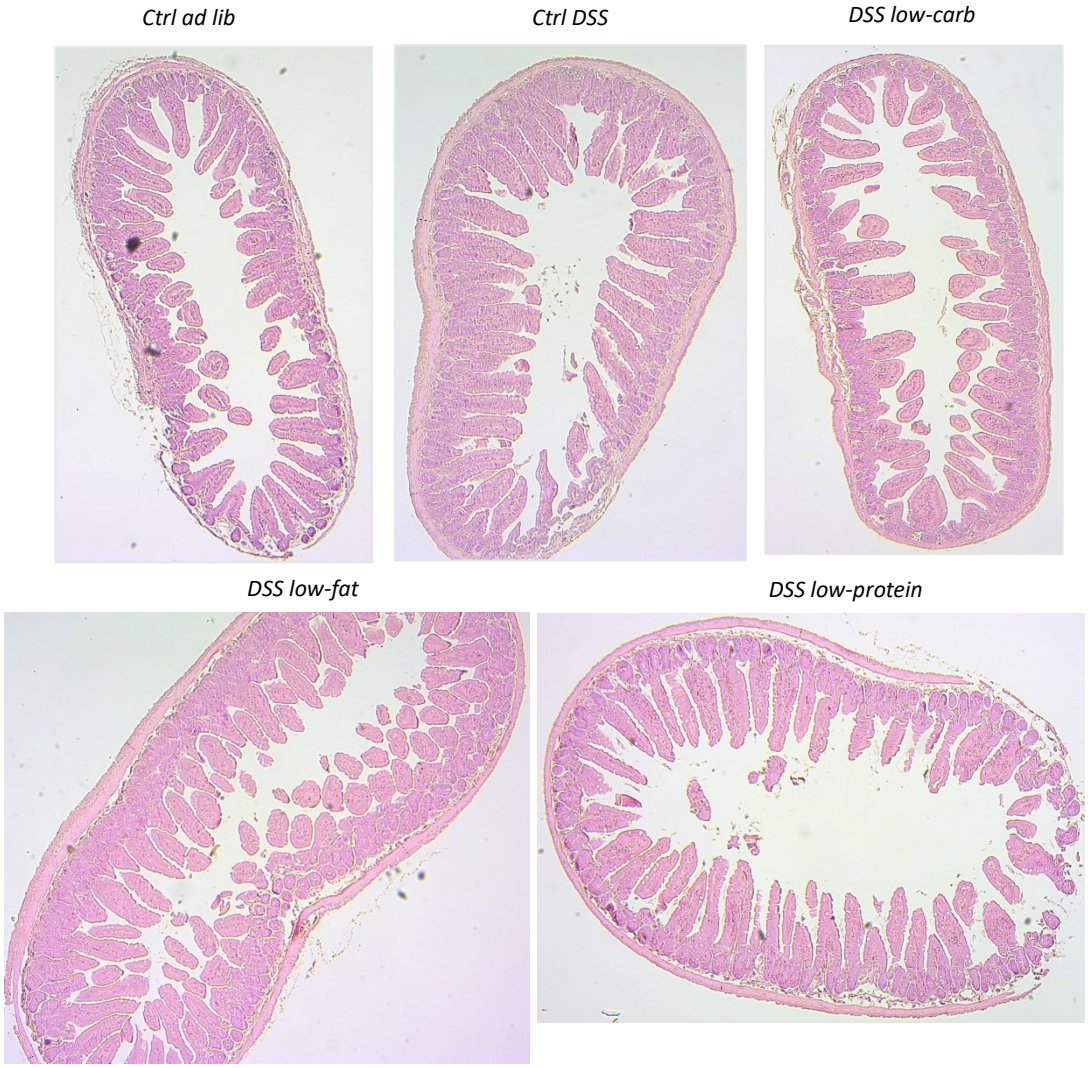


Figure 15 Microscopic images of small intestinal tissues: tissue sections were prepared from paraffin blocks, with sections measuring 4 µm in thickness. The images were captured using a Zeiss microscope equipped with an AxioCam camera at 5x magnification; tissue sections were stained using the H&E staining method.

In terms of thickness, ANOVA analysis detected a significant difference among the groups in the small intestine samples. However, subsequent t-tests did not reveal a significant difference between the *ad libitum* control group and the DSS control group. Significant differences were observed between the DSS control group and both the low-carb group and the low-protein group.

Notably, the low-carb and low-protein groups exhibited a reduction in thickness of the muscularis externa compared to the DSS control group. These findings suggest that both a low-carb diet and a low-protein diet can impact the thickness of the muscularis externa in the small intestine.

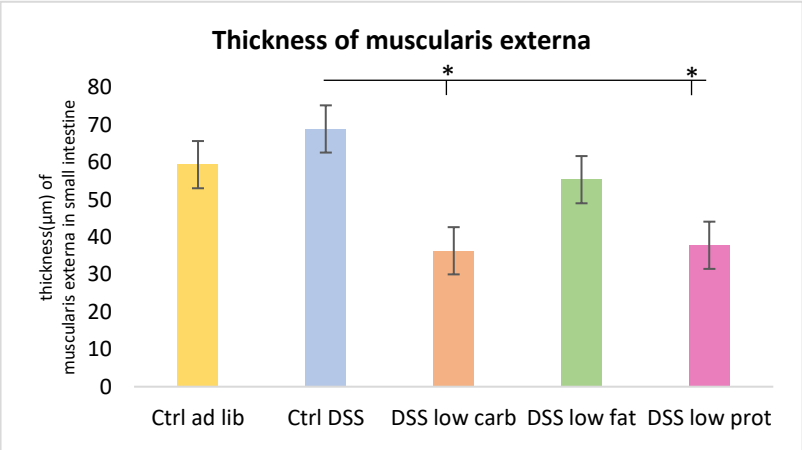


*Figure 16 Thickness of musclaris externa in the small intestine: One-way ANOVA was used to examine the statistical significance, followed by t-test analysis: * means p ≤0.01.*

Intestinal villi, formed by the epithelial cells of the small intestine, are finger-like folds that significantly amplify the surface area for absorption. They enhance absorption capacity by 8 to 10 times. Through rhythmic contractions occurring 3 to 6 times per minute, the villi facilitate contact between the intestinal mucosa and contents, promoting the transport of absorbed fluids and substances. This pumping and stirring function of the villi further enhances absorption capacity. [32]

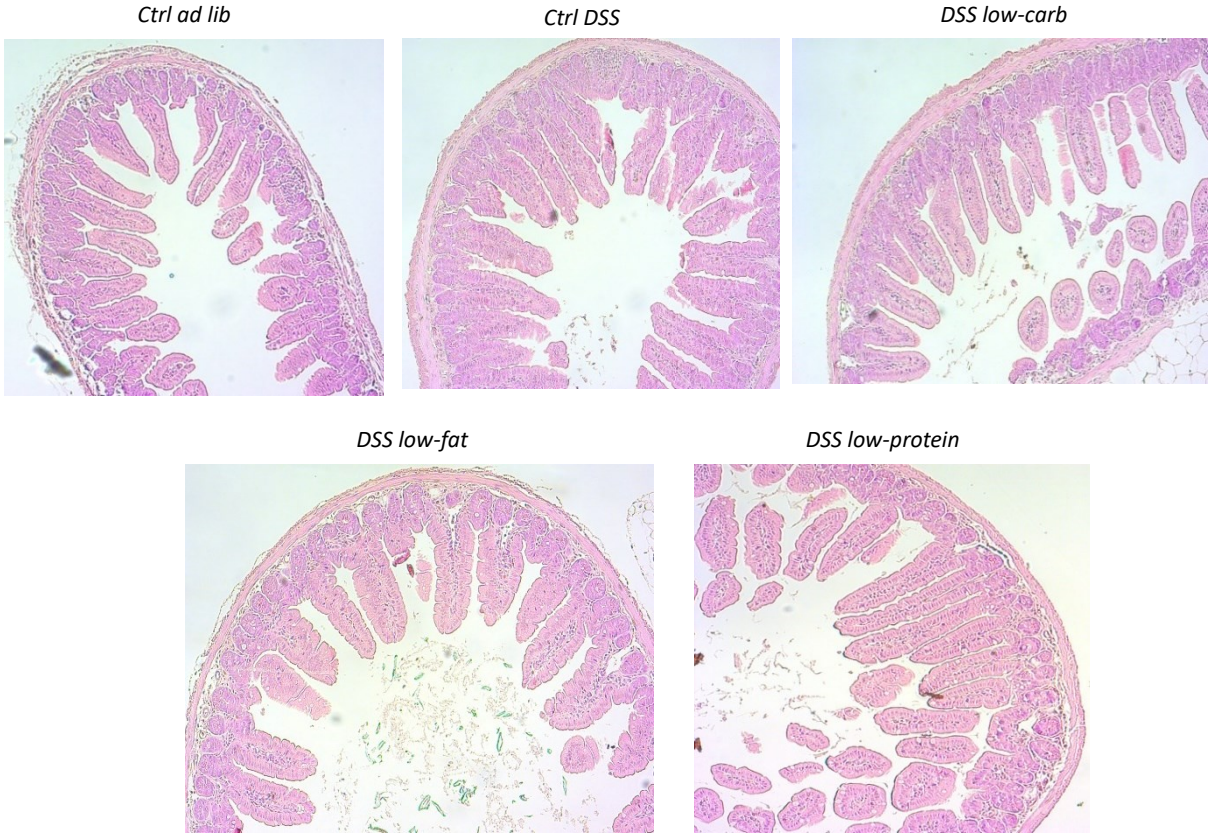


Figure 17 Small Intestine microscopic images were captured using a Zeiss microscope and AxioCam camera at 10x magnification.

Based on the ANOVA test, a significant difference was observed. However, when conducting t-tests comparing the Ctrl DSS group with the low macronutrient groups, no significant differences were found. Significantly, the low-protein group displayed a remarkable and statistically significant increase in villi length compared to both the low-fat and low-carb groups.

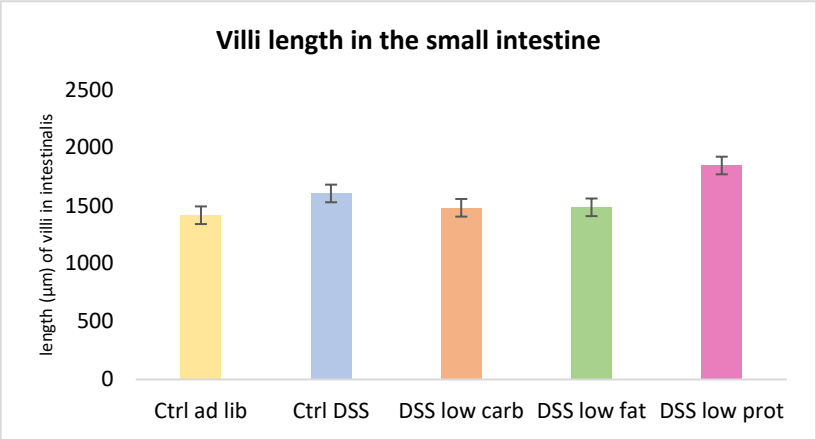


Figure 18 Villi length small intestine: Statistically significant differences were determined using one-way ANOVA analysis, followed by a post hoc T-test to assess the distinctions between the groups.

5.3.2 Proximal colon

In the proximal colon, a statistically significant difference was initially observed in the t-test between the *ad libitum* and Ctrl DSS group. A significant difference was found between the Ctrl DSS group and all three low macronutrient groups, with these groups showing an increased thickness of the muscularis externa compared to the DSS-treated group.

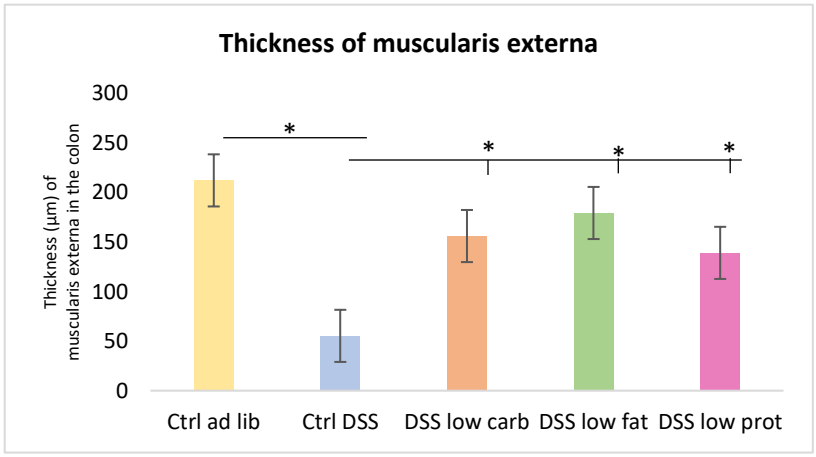


Figure 19 Thickness of muscularis externa in the proximal colon: To identify statistically significant differences, a one-way ANOVA test was initially conducted, followed by a t-test analysis with a significance level set at $p \leq 0.01$.

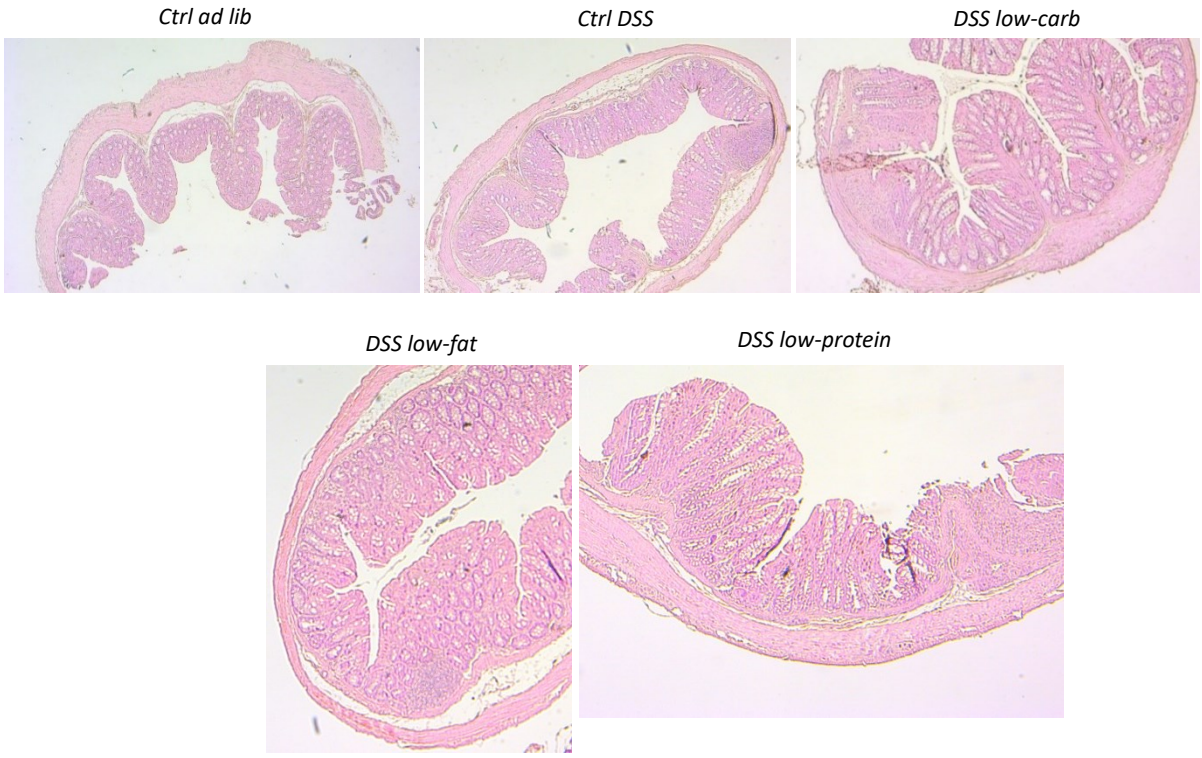


Figure 20 Microscopic images of the proximal colon were taken to identify crypt depth and thickness of the muscularis externa. Magnifications used were 5x and 10x. H&E staining was applied for visualization. Images were captured using a Zeiss microscope and AxioCam.

The crypt depth was also analyzed using the ImageJ software. The results of the ANOVA test indicated a statistically significant difference. This observation was further confirmed in the subsequent t-tests, which revealed a significant difference between the *ad libitum* and DSS-treated group. Notably, the Ctrl DSS group exhibited a significantly reduced crypt depth.

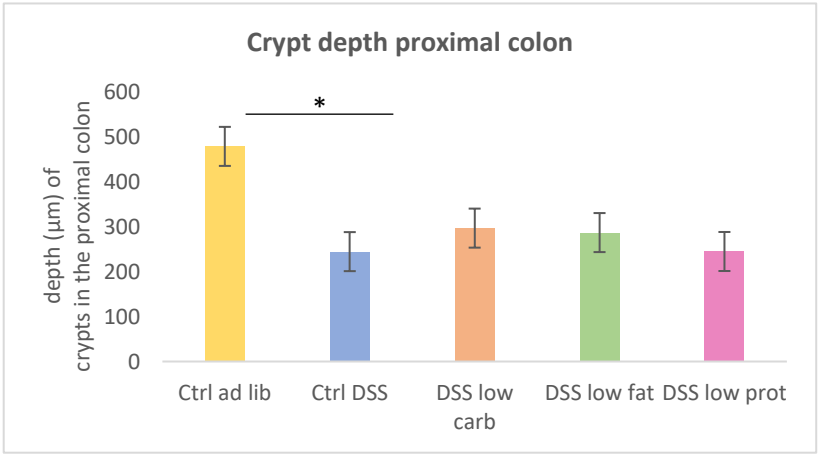


Figure 21 Crypt depth in the proximal colon: Statistical analysis using ANOVA and post hoc t-tests: implies a statistically significant difference between Ctrl ad lib and Ctrl DSS: p≤0,01.*

5.4 MPO results

The results of the myeloperoxidase assay indicate a potential difference between the tested groups according to ANOVA. However, this could not be implicitly confirmed in the t-tests. The DSS-treated group, compared to the low macronutrient groups, exhibited an increased MPO activity without showing a significant difference.

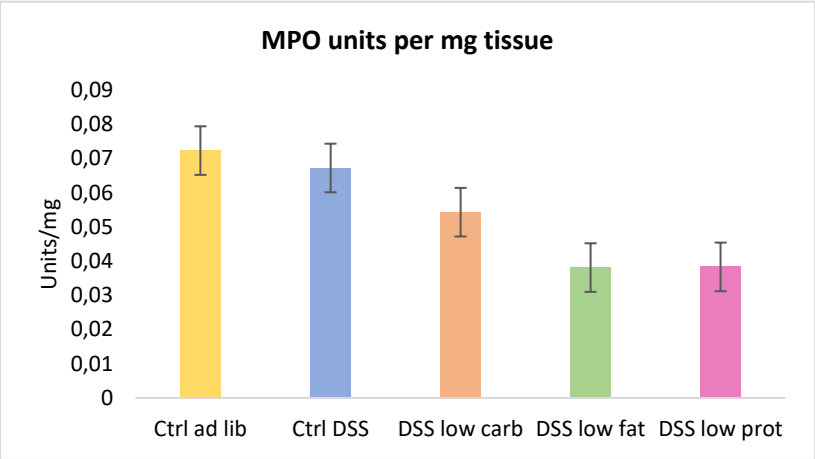


*Figure 22 The results of measuring MPO activity in the small intestine: Statistical analysis involved ANOVA followed by a T-test analysis, where * indicates a statistically significant difference at $p \leq 0.01$.*

6 Discussion

In the analysis of ulcerative colitis etiology, diet emerges as a significant factor. Nevertheless, research primarily reveals associations rather than definitively establishing a direct causal link [33]. Numerous studies have highlighted the pro-inflammatory effects of a Western diet high in sugar and saturated fat on gut inflammation. Despite this a direct causal correlation between the reduction of these macronutrients and the subsequent decrease in inflammation has proven to be challenging [34]. In light of these studies, it becomes evident that the foremost consideration for dietary recommendations in the context of inflammatory bowel diseases should prioritize the quality of macronutrients, particularly focusing on the types of simple carbohydrates and animal fats consumed, rather than solely emphasizing their quantity [33].

6.1 Gene expression and inflammation

An elevated IL-1β level was observed in the small intestine tissues of the low-protein and low-fat group. IL-1β possesses proinflammatory effects on inflammatory processes. Thus, an increased IL-1β level indicates an enhanced inflammatory response and associated heightened immune reaction [35]. The results indicate that the low-carb group had the highest levels of Irf1 compared to the other groups. Conversely, very low levels were observed in the low-fat and low-protein group. It is important to note that this gene does not have a proinflammatory effect but plays a crucial role in the immune response against pathogens. A study in mice showed that a deficiency in this gene induced a pathological condition similar to ulcerative colitis in these animals. Furthermore, it was found that a reduced level of this gene is correlated with a predisposition to inflammatory bowel diseases. These findings suggest that a decreased Irf1 level may favor the risk of ulcerative colitis and the development of colorectal cancer [36]. MyD88 is an essential adapter protein in the immune system that mediates the signal transduction of TLRs and IL-1 receptors to trigger immune responses against infections and inflammatory processes [37]. In a study, MyD88 gene-deficient mice were compared to wild-type mice. The primary focus was on analyzing how these two groups of mice responded to a treatment involving dextran sodium sulfate and identifying differences in the development of intestinal inflammation. Notably, MyD88 gene-deficient mice displayed severe intestinal inflammation when exposed to DSS, highlighting the potential protective role of the MyD88 gene in regulating gut inflammation [38].

The highest expression of the MyD88 gene in the small intestine was observed in the low-carb treatment group and this difference compared to the Ctrl DSS group was statistically significant. STAT1, a protein integral to cellular signaling pathways, is activated through tyrosine phosphorylation in response to interferon (IFN) signaling. It serves as a pivotal regulator of gene expression, immune responses and inflammatory processes. In the context of colitis and inflammatory bowel diseases, the upregulation of STAT1 suggests its significant role in disease pathology. Indeed, studies conducted in murine models have provided evidence that STAT1 actively promotes colitis [39]. The results did not indicate elevated STAT1 expression in the DSS control group. On the contrary, the expression in this group was lower compared to the other groups. Despite the reduced availability of macronutrients, the expected downregulation did not occur, which had been the intended outcome. Although no direct association between ulcerative colitis and TGFβ1 was identified, it is evident that TGFβ1 plays a role in maintaining intestinal homeostasis by modulating the functions of immune cells, the epithelium and the luminal microbiota, all of which are associated with the pathogenesis of IBD. Studies involving samples from IBD patients have indicated elevated TGFβ1 levels in those with active disease [40]. In the analysis of the TGFβ1 gene, a slight increase in TGFβ1 levels was found in the Ctrl DSS group and this difference was statistically significant compared to the Ctrl *ad libitum* group. However, the low macronutrient diet interventions did not contribute to the reduction. IL-33 has recently been found to be increased in people with IBD. In ulcerative colitis, IL-33 cells often formed shield-like' clusters beneath ulcerated areas and some IBD patients also had IL-33 cells within the muscularis layer. This upregulation of IL-33 was observed in the inflamed mucosa of IBD patients with active disease compared to non-involved patient biopsies or colon tissue from those without IBD [41]. These findings could not be confirmed in the results. Instead, they indicated a decreased IL-33 expression in the Ctrl DSS group. The expectation of higher IL-7 levels in ulcerative colitis was not confirmed, as the results from lymph node samples did not reveal any upregulated levels of IL-7 [42]. Overall, the conducted diets did not lead to any discernible alterations in mRNA gene expression within the lymph nodes and Peyer's patches.

6.2 Histological differences

In histological experiments involving the small intestine, it was observed that the thickness of the muscularis externa was reduced in both the low-carb and low-protein diet groups when compared to the DSS control group. This suggests that low-carb and low-protein diets have the effect of reducing the thickness of the muscularis externa in the small intestine. In contrast, increased thickness of the muscularis externa was observed in the DSS control group, which was related to the presence of colitis. Scientific literature has also reported an increase in the thickness of the muscularis externa in connection with ulcerative colitis [43]. In a distinct study, it was observed that the implementation of restrictive diets resulted in a notable decrease in intestinal thickness. And this again underlines the fact that dietary factors have an impact on this layer [44]. Interestingly, in the proximal colon, it was noted that the thickness of the muscularis externa increased in the low macronutrient groups, whereas in the DSS control group, a reduced thickness was observed quite unexpectedly. Indeed, typically, an increased thickness would be expected in the presence of colitis [44]. The villi length showed a minor increase in the low-protein group, though no significant difference was emphasized compared to the other groups. This contrasts with previous studies, which would confirm an increase in villi length with calorie restriction or a ketogenic diet [44]. In the control group exposed to DSS, a reduction in crypt depth was observed in microscopic examinations. During active intestinal inflammation, alterations such as crypt shortening and distortions are commonly identified [45]. Moreover, it has been well-documented that dietary intake can significantly affect crypt depth. Consuming food leads to an increase in crypt depth, while the withdrawal or restriction of nutrients tends to result in reduced crypt depth [44, 46]. These associations have also been substantiated through conducted experiments, where restrictive diets involving low-carbohydrate, low-fat and low-protein macronutrient groups were found to exhibit decreased crypt depth.

6.3 Myeloperoxidase activity

In ulcerative colitis, neutrophils accumulate in the inflamed intestinal mucosa. These neutrophils release enzymes like myeloperoxidase, which fight bacteria but can also harm tissue. Myeloperoxidase activity correlates with neutrophil numbers. Patients with ulcerative colitis have higher myeloperoxidase levels in stool, indicating increased neutrophil activity [47].

It would have been expected to observe higher MPO activity in the Ctrl DSS group compared to the *ad libitum* group, however, this difference was not apparent in the conducted assay. A reduced MPO activity was observed in the macronutrient-reduced groups, although this difference was not statistically significant. Overall, the three low-macronutrient groups did not exhibit a substantial difference in MPO activity reduction; however, some effect of the dietary factor on MPO can be discerned.

6.4 Cytokine expression and immune response

The protein array results revealed a noticeable trend towards decreased levels of most cytokines in the low-carb and low-protein group, underscoring their potential role in inhibiting inflammation. The reduction in pro-inflammatory cytokine levels, as previously observed during calorie restriction, was confirmed in the results, emphasizing the influence of a low-protein and low-carb diet on these pro-inflammatory cytokines, including TNF-α and IL-6 [48]. In the context of experimental colitis, it becomes more evident that IL-6 plays a pivotal role during the chronic phase, a proposition supported by extensive mouse studies conducted. In several mouse models, IL-6 is of central importance, with both T-cells and macrophages contributing to its production. However, T-cells are of particular significance as IL-6 exerts a strong anti-apoptotic effect on them. Notably, the use of antibodies against IL-6 receptors resulted in a significant reduction in colitis in mice [49].

Within the inflammation cascade, antigen-presenting cells like macrophages and dendritic cells induce the activation and differentiation of CD4+ T lymphocytes. These T cells differentiate into T helper (Th)-1 and Th-2 cells, with Th-1 cells releasing IFNγ, IL-2, IL-12 and IL-18, while Th-2 cells release IL-4, IL-5, IL-6, IL-10 and IL-13. IFNγ, produced in response to Th-1 activation, stimulates macrophages and monocytes to generate TNFα [50].
Ulcerative colitis (UC) is characterized by an atypical cytokine expression pattern, with a dominant humoral Th-2 immune response and a relatively diminished Th-1 response. UC exhibits an atypical Th-2 response marked by CD4 T cells that express intracellular IL-13 and bear a natural-killing T- cell marker. In patients with ulcerative colitis, significantly higher levels of IL-1β, IL-4, IL-5, IL-8, IL-12p40, IFNγ and TNFα were observed in comparison to the control group [50]. This could also be confirmed in the results of this study. In this context, the group with DSS-induced colitis exhibited elevated cytokine levels.

Interestingly, adopting a low-macronutrient diet, particularly one that is low in carbohydrates and proteins, resulted in a significant reduction in these cytokines. TNFα is a proinflammatory modifier which plays a central role in the pathogenesis of IBD. It is produced by various cells and leads to inflammation. Soluble TNFα binds to receptors, activates macrophages, enhances the response of T-cells and promotes inflammation. TNFα also activates NF-κB, which contributes to mucosal breakdown. Increased TNFα production and NF-κB activity is found in UC patients [50].

7 Conclusion

In summary, this master thesis unquestionably lays a robust foundation for future research in this field, with the aim of consolidating the promising findings. It convincingly highlights a clear and compelling trend demonstrating the anti-inflammatory effects of macronutrients in the context of colitis.

The results of the protein array analysis significantly contributed to our understanding of the anti-inflammatory effects of low-macronutrient diets in the treated mice. However, the mRNA expression results of cytokines did not exhibit a clear pattern, primarily because the administration of DSS did not induce excessive colitis in the treated mice, resulting in lower levels of measured inflammation parameters. In this context, a significant reduction in colitis-associated cytokines such as IL-6, TNFα and IL-1β was clearly observed when implementing a low-carbohydrate and low-protein diet. Particularly, the low-carbohydrate group, characterized by the restriction of carbohydrates, including processed sugars, appeared to have a distinct impact. This effect was further complemented by the low-protein group, especially in reducing cytokine levels, indicating a synergistic relationship with the low-carbohydrate group. Furthermore, microscopic analysis of histological sections revealed observable effects on various aspects such as villi length and the thickness of the muscularis externa. While ulcerative colitis resulted in shortening, distortion and abnormalities in these parameters, a definite positive influence on histology was achieved through low-macronutrient diets.

To conclude, this work presents a fundamental milestone for further investigation into the potential of macronutrient restriction in relieving inflammation associated with colitis. While it would be premature to claim a significant effect based on the available data alone, the influence of calorie restriction on inflammation is evident and the indications pointing towards the positive impact of macronutrient restriction should not be overlooked. This work not only sets the stage for future research, but also highlights the promising potential of macronutrient modulation in the treatment of colitis-related inflammation. The insights gained from this study will continue to shape our understanding and ultimately contribute to more effective approaches for the treatment of colitis-associated inflammation as this field continues to advance.

8 References

1. Pak, H.H., Haws, S.A., Green, C.L., Koller, M., Lavarias, M.T., Richardson, N.E., Yang, S.E., Dumas, S.N., Sonsalla, M., Bray, L., Johnson, M., Barnes, S., Darley-Usmar, V., Zhang, J., Yen, C.-L.E., Denu, J.M., Lamming, D.W.: Fasting drives the metabolic, molecular and geroprotective effects of a calorie-restricted diet in mice. Nature metabolism **3**(10), 1327–1341 (2021). doi: 10.1038/s42255-021-00466-9
2. Mattson, M.P., Wan, R.: Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. The Journal of Nutritional Biochemistry **16**(3), 129–137 (2005). doi: 10.1016/j.jnutbio.2004.12.007
3. Hofer, S.J., Carmona-Gutierrez, D., Mueller, M.I., Madeo, F.: The ups and downs of caloric restriction and fasting: from molecular effects to clinical application. EMBO Molecular Medicine **14**(1), e14418 (2022)
4. Venn, B.J.: Macronutrients and Human Health for the 21st Century. Nutrients **12**(8) (2020). doi: 10.3390/nu12082363
5. Felixberger, J.K.: Proteine – essenzieller Bestandteil unserer Ernährung. In: Ghadiri, A., Vilgis, T., Bosbach, T. (eds.) Wissen schmeckt: Die Magie der Wissenschaften beim Kochen erklärt – mit 16 Rezepten, pp. 169–195. Springer Fachmedien Wiesbaden, Wiesbaden (2018)
6. Iacone, R., Scanzano, C., Santarpia, L., Cioffi, I., Contaldo, F., Pasanisi, F.: Macronutrients in Parenteral Nutrition: Amino Acids. Nutrients **12**(3) (2020). doi: 10.3390/nu12030772
7. Hoffman, J.R., Falvo, M.J.: Protein - Which is Best? Journal of sports science & medicine **3**(3), 118–130 (2004)
8. Pezeshki, A., Chelikani, P.K.: Low Protein Diets and Energy Balance: Mechanisms of Action on Energy Intake and Expenditure. Frontiers in nutrition **8**, 655833 (2021). doi: 10.3389/fnut.2021.655833
9. Leitzmann, C.: Ernährung in Prävention und Therapie: ein Lehrbuch; 165 Tabellen. Georg Thieme Verlag (2003)
10. Papadopoulou, S.K., Nikolaidis, P.T.: Low-Carbohydrate Diet and Human Health. Nutrients **15**(8) (2023). doi: 10.3390/nu15082004
11. Oh, R., Gilani, B., Uppaluri, K.R.: Low Carbohydrate Diet. StatPearls Publishing, Treasure Island (FL) (2022)
12. Kakodkar, S., Mutlu, E.A.: Diet as a Therapeutic Option for Adult Inflammatory Bowel Disease. Gastroenterology clinics of North America **46**(4), 745–767 (2017). doi: 10.1016/j.gtc.2017.08.016
13. The Functions of Fats in the Body (2023)

14. Bajželj, B., Laguzzi, F., Röös, E.: The role of fats in the transition to sustainable diets. The Lancet. Planetary health **5**(9), e644-e653 (2021). doi: 10.1016/S2542-5196(21)00194-7
15. Rohr, M.W., Narasimhulu, C.A., Rudeski-Rohr, T.A., Parthasarathy, S.: Negative Effects of a High-Fat Diet on Intestinal Permeability: A Review. Adv Nutr **11**(1), 77–91 (2020). doi: 10.1093/advances/nmz061
16. Kim, J.Y.: Optimal Diet Strategies for Weight Loss and Weight Loss Maintenance. Journal of obesity & metabolic syndrome **30**(1), 20–31 (2021). doi: 10.7570/jomes20065
17. Gajendran, M., Loganathan, P., Jimenez, G., Catinella, A.P., Ng, N., Umapathy, C., Ziade, N., Hashash, J.G.: A comprehensive review and update on ulcerative colitis. Disease-a-Month **65**(12), 100851 (2019). doi: 10.1016/j.disamonth.2019.02.004
18. Ordás, I., Eckmann, L., Talamini, M., Baumgart, D.C., Sandborn, W.J.: Ulcerative colitis. Lancet (London, England) **380**(9853), 1606–1619 (2012). doi: 10.1016/S0140-6736(12)60150-0
19. Kaur, A., Goggolidou, P.: Ulcerative colitis: understanding its cellular pathology could provide insights into novel therapies. Journal of Inflammation **17**(1), 15 (2020). doi: 10.1186/s12950-020-00246-4
20. Adams, S.M., Bornemann, P.H.: Ulcerative colitis. American family physician **87**(10), 699–705 (2013)
21. Ungaro, R., Mehandru, S., Allen, P.B., Peyrin-Biroulet, L., Colombel, J.-F.: Ulcerative colitis. Lancet (London, England) **389**(10080), 1756–1770 (2017). doi: 10.1016/S0140-6736(16)32126-2
22. Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S.C., Ferrante, M., Shen, B., Bernstein, C.N., Danese, S., Peyrin-Biroulet, L., Hibi, T.: Ulcerative colitis. Nature Reviews Disease Primers **6**(1), 74 (2020). doi: 10.1038/s41572-020-0205-x
23. Xu, C.-T., Meng, S.-Y., Pan, B.-R.: Drug therapy for ulcerative colitis. World journal of gastroenterology **10**(16), 2311–2317 (2004). doi: 10.3748/wjg.v10.i16.2311
24. Segal, J.P., LeBlanc, J.-F., Hart, A.L.: Ulcerative colitis: an update. Clinical medicine (London, England) **21**(2), 135–139 (2021). doi: 10.7861/clinmed.2021-0080
25. Google Docs: Projektvorschlag Macronutrients.docx (2023)
26. Hedrich, H.J.: The Laboratory Mouse, 2nd edn. Elsevier Science & Technology Books, Jordan Hill (2012)
27. RayBiotech. 2019. Quantibody Mouse TH17 Array 1. quantitative measurement RayBiotech. 2019. Quantibody Mouse TH17 Array 1. quantitative measurement of 18 mouse TH1-TH2-TH17 cytokines. https://doc.raybiotech.com/pdf/Manual/ QAM-TH17-1.pdf

28. Davies, M.J., Hawkins, C.L.: The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. Antioxidants & Redox Signaling **32**(13), 957–981 (2020). doi: 10.1089/ars.2020.8030
29. Lockhart, J.S., Sumagin, R.: Non-Canonical Functions of Myeloperoxidase in Immune Regulation, Tissue Inflammation and Cancer. International journal of molecular sciences **23**(20) (2022). doi: 10.3390/ijms232012250
30. Pulli, B., Ali, M., Forghani, R., Schob, S., Hsieh, K.L.C., Wojtkiewicz, G., Linnoila, J.J., Chen, J.W.: Measuring myeloperoxidase activity in biological samples. PloS one **8**(7), e67976 (2013). doi: 10.1371/journal.pone.0067976
31. 22.5C: Muscularis. Libretexts, 22 July 2018. https://med.libretexts.org/Bookshelves/Anatomy_and_Physiology/Anatomy_and_Physiology_(Boundless)/22%3A_Digestive_System/22.05%3A_Layers_of_the_Alimentary_Canal/22.5C%3A_Muscularis. Accessed 15 July 2023
32. Darmzotten (2018)
33. Castro, M.M. de, Pascoal, L.B., Steigleder, K.M., Siqueira, B.P., Corona, L.P., Ayrizono, Maria de Lourdes Setsuko, Milanski, M., Leal, R.F.: Role of diet and nutrition in inflammatory bowel disease. World journal of experimental medicine **11**(1), 1–16 (2021). doi: 10.5493/wjem.v11.i1.1
34. Bertani, L., Ribaldone, D.G., Bellini, M., Mumolo, M.G., Costa, F.: Inflammatory Bowel Diseases: Is There a Role for Nutritional Suggestions? Nutrients **13**(4) (2021). doi: 10.3390/nu13041387
35. Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., Masumoto, J.: The role of interleukin-1 in general pathology. Inflammation and regeneration **39**, 12 (2019). doi: 10.1186/s41232-019-0101-5
36. Jeyakumar, T., Fodil, N., van der Kraak, L., Meunier, C., Cayrol, R., McGregor, K., Langlais, D., Greenwood, C.M.T., Beauchemin, N., Gros, P.: Inactivation of Interferon Regulatory Factor 1 Causes Susceptibility to Colitis-Associated Colorectal Cancer. Scientific reports **9**(1), 18897 (2019). doi: 10.1038/s41598-019-55378-2
37. Lutz, C., Weder, B., Hünerwadel, A., Fagagnini, S., Lang, B., Beerenwinkel, N., Rossel, J.B., Rogler, G., Misselwitz, B., Hausmann, M.: Myeloid differentiation primary response gene (MyD) 88 signalling is not essential for intestinal fibrosis development. Scientific reports **7**(1), 17678 (2017). doi: 10.1038/s41598-017-17755-7
38. Araki, A., Kanai, T., Ishikura, T., Makita, S., Uraushihara, K., Iiyama, R., Totsuka, T., Takeda, K., Akira, S., Watanabe, M.: MyD88-deficient mice develop severe intestinal inflammation in

dextran sodium sulfate colitis. Journal of gastroenterology **40**(1), 16–23 (2005). doi: 10.1007/s00535-004-1492-9

39. Crnčec, I., Modak, M., Gordziel, C., Svinka, J., Scharf, I., Moritsch, S., Pathria, P., Schlederer, M., Kenner, L., Timelthaler, G., Müller, M., Strobl, B., Casanova, E., Bayer, E., Mohr, T., Stöckl, J., Friedrich, K., Eferl, R.: STAT1 is a sex-specific tumor suppressor in colitis-associated colorectal cancer. Molecular oncology **12**(4), 514–528 (2018). doi: 10.1002/1878-0261.12178
40. Ihara, S., Hirata, Y., Koike, K.: TGF-β in inflammatory bowel disease: a key regulator of immune cells, epithelium, and the intestinal microbiota. Journal of gastroenterology **52**(7), 777–787 (2017). doi: 10.1007/s00535-017-1350-1
41. Mamdouh A K Sedhom, Mélanie Pichery, Jenna R Murdoch, Benoit Foligné, Nathalie Ortega, Sylvain Normand, Kirsten Mertz, Devika Sanmugalingam, Lea Brault, Teddy Grandjean, Emma Lefrancais, Padraic G Fallon, Valérie Quesniaux, Laurent Peyrin-Biroulet, Gieri Cathomas, Tobias Junt, Mathias Chamaillard, Jean-Philippe Girard, Bernhard Ryffel: Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. Gut **62**(12), 1714 (2013). doi: 10.1136/gutjnl-2011-301785
42. Barata, J.T., Durum, S.K., Seddon, B.: Flip the coin: IL-7 and IL-7R in health and disease. Nature Immunology **20**(12), 1584–1593 (2019). doi: 10.1038/s41590-019-0479-x
43. Krugliak Cleveland, N., Torres, J., Rubin, D.T.: What Does Disease Progression Look Like in Ulcerative Colitis, and How Might It Be Prevented? Gastroenterology **162**(5), 1396–1408 (2022). doi: 10.1053/j.gastro.2022.01.023
44. Gregor, A., Huber, L., Auernigg-Haselmaier, S., Sternberg, F., Billerhart, M., Dunkel, A., Somoza, V., Ogris, M., Kofler, B., Longo, V.D., König, J., Duszka, K.: A Comparison of the Impact of Restrictive Diets on the Gastrointestinal Tract of Mice. Nutrients **14**(15) (2022). doi: 10.3390/nu14153120
45. Villanacci, V., Antonelli, E., Geboes, K., Casella, G., Bassotti, G.: Histological healing in inflammatory bowel disease: a still unfulfilled promise, United States (2013 Feb 21)
46. Blackmore, K., Zhou, W., Dailey, M.J.: LKB1-AMPK modulates nutrient-induced changes in the mode of division of intestinal epithelial crypt cells in mice. Experimental biology and medicine (Maywood, N.J.) **242**(15), 1490–1498 (2017). doi: 10.1177/1535370217724427
47. Masoodi, I., Tijjani, B.M., Wani, H., Hassan, N.S., Khan, A.B., Hussain, S.: Biomarkers in the management of ulcerative colitis: a brief review. German medical science : GMS e-journal **9**, Doc03 (2011). doi: 10.3205/000126
48. Montefusco, L., D'Addio, F., Loretelli, C., Ben Nasr, M., Garziano, M., Rossi, A., Pastore, I., Plebani, L., Lunati, M.E., Bolla, A.M., Porta, M.D., Piuri, G., Rocchio, F., Abdelsalam, A., Assi, E., Barichella, M., Maestroni, A., Usuelli, V., Loreggian, L., Muzio, F., Zuccotti, G.V., Cazzola, R.,

Fiorina, P.: Anti-inflammatory effects of diet and caloric restriction in metabolic syndrome. Journal of Endocrinological Investigation **44**(11), 2407–2415 (2021). doi: 10.1007/s40618-021-01547-y

49. Mudter, J., Neurath, M.F.: Il-6 signaling in inflammatory bowel disease: Pathophysiological role and clinical relevance. Inflamm Bowel Dis **13**(8), 1016–1023 (2007). doi: 10.1002/ibd.20148
50. Sands, B.E., Kaplan, G.G.: The Role of TNFα in Ulcerative Colitis. The Journal of Clinical Pharmacology **47**(8), 930–941 (2007). doi: 10.1177/0091270007301623