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Content

Abstract

Ulcerative colitis (UC) is a chronic, inflammatory disease that affects the mucosa and superficial submucosa of the colon. While the exact cause of UC is unknown, several factors including genetics, immunity, environment, and lifestyle factors are believed to play a role in the pathogenesis. Diet has emerged as a potential therapeutic target for UC, and restrictive diets have shown anti-inflammatory potential in other diseases. The comparison of caloric restriction, intermittent fasting, fastening mimicking, keto 1 (only long-chain triglycerides), and keto 2 (long-chain and medium-chain triglycerides) has revealed a positive effect on DSS-induced colitis in C57BL/6NRj mice over the course of a 4-week intervention in the form of attenuated inflammatory markers (eotaxin-1, G-CSF, GM-CSF, IL-7, KC, LIX, MCP-1, MIP-1a, TNFα). These effects seem to be local since the peripheral immune system remained unaffected. Keto 1 has shown the most significant anti-inflammatory capacity that might be caused by the re-programming of macrophages, switching the M1 phenotype into the alternatively activated M2 type, exhibiting anti-inflammatory properties.

Colitis ulcerosa (CU) ist eine chronische, entzündliche Erkrankung, die die Mukosa und die Submukosa des Dickdarms betrifft. Die genaue Ursache von UC ist unbekannt, aber es wird angenommen, dass mehrere Faktoren, einschließlich Genetik, Immunität, Umwelt und Lebensstil, eine Rolle bei der Pathogenese spielen. Die Ernährung hat sich als potenzielles therapeutisches Ziel für CU etabliert, und restriktive Diäten haben ein entzündungshemmendes Potenzial bei anderen Krankheiten gezeigt. Der Vergleich von Kalorienrestriktion, Intermittent Fasting, Fasting Mimicking, Keto 1 (langkettige Triglyceride) und Keto 2 (langkettige und mittelkettige Triglyceride) hat bei C57BL/6NRj-Mäusen eine positive Wirkung auf DSS-induzierte Kolitis gezeigt über einen 4-wöchigen Eingriff in Form reduzierter Entzündungsmarker (Eotaxin-1, G-CSF, GM-CSF, IL-7, KC, LIX, MCP-1, MIP-1a, TNFα). Diese Effekte scheinen lokal zu sein, da das periphere Immunsystem unbeeinflusst blieb. Keto 1 hat die höchste entzündungshemmende Kapazität gezeigt, die durch das Umprogrammieren von Makrophagen zustande kommen könnte, indem der M1-Phänotyp in den alternativ aktivierten M2-Typ umgewandelt wird, der entzündungshemmende Eigenschaften aufweist.

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UC – ulcerative colitis

CD – Chron's disease

IBD – inflammatory bowel disease

ID - indeterminate colitis

IL – interleukin

SNP – single nucleotide polymorphism

TNF – tumor necrosis factor

DSS – dextran sodium sulfate

TNBS - 246-trinitrobenzene sulfonic acid

NFκB – nuclear factor kappa-light-chain-enhancer of activated B cells

MAPK - mitogen-activated protein kinase

TLR – toll-like receptor

HDAC – histone deacetylase

IOIBD – International Organization For the Study of Inflammatory Bowel Disease

STRIDE – Selecting Therapeutic Targets in Inflammatory Bowel Disease

FC – fecal calprotectin

ESR – erythrocyte sedimentation rate

CRP – C-reactive protein

MPO – myeloperoxidase

GSH – glutathione

FODMAP – fermentable oligosaccharides disaccharides monosaccharides and polyols

IF – intermittent fasting

ctrl – control

ad lib – ad libitum

KD – ketogenic/keto diet

FMD – fasting-mimicking diet

CR – caloric restriction

LCT – long chain triglycerides

MCT – medium chain triglycerides

BW – body weight

WAT – white adipose tissue

HTAB – hexadecyltrimethylammonium bromide

DTT – dithiothreitol

HPLC – high-performance liquid chromatography

LCMS – liquid chromatography-mass spectrometry

v/v – volume to volume

BA – bile acid

SEM – standard error of the mean

w/v – weight to volume

TLCA – Taurolithocholic acid

TUDCA – tauroursodeoxycholi acid

TCA – taurocholic acid

TDCA – taurodeoxycholic acid

CA – cholic acid

DCA – deoxycholic acid

CDCA – chenodeoxycholic acid

UDCA – ursodeoxycholic acid

LCA – lithocholic acid

DHCA – CDCA precursor

CT – computed tomography

BLC – B lymphocyte chemoattractant

FAS-L – Fas ligand

G-CSF – granulocyte colony-stimulating factor

GM-CSF – granulocyte-macrophage colony-stimulating factor

ICAM – intercellular adhesion molecule

IFN – interferon

KC – chemokine ligand 1

LIX – C-X-C motif chemokine 5

MCP – monocyte chemoattractant protein

M-CSF – macrophage colony-stimulating factor

MIG – monokine induced by gamma interferon

MIP – macrophage inflammatory protein

PF – platelet factor

RANTES – regulated on activation normal T cell expressed and secreted

TARC – thymus- and activation-regulated chemokine

TCA3 – chemokine (C-C motif) ligand 1

TIMP - tissue inhibitor of metalloproteinases

ZO-1 – tight junction protein 1

AIF1 – allograft inflammatory factor 1

MYD88 – myeloid differentiation primary response 88

TGFβ1 – transforming growth factor beta 1

REG3G – regenerating family member 3 gamma

IRF1 – interferon regulatory factor 1

COX2 – cyclooxygenase-2

NOD2 – nucleotide-binding oligomerization domain-containing protein 2

STAT1 – signal transducer and activator of transcription 1

eEf1a1 – eukaryotic translation elongation factor 1 alpha 1

1. Introduction

Ulcerative colitis (UC), alongside Chron's disease (CD), falls under the umbrella of inflammatory bowel disease (IBD) as a chronic condition characterized by inflammation of the colon mucosa. The most frequent symptoms of UC are rectal urgency, tenesmus, general discomfort in the abdominal area, and bloody diarrhea. Suspicions of UC, based on symptoms must be considered in combination with several factors. A possible infectious cause (viral or bacterial) must be excluded, and lifestyle changes (cessation of smoking, medication use e.g. isotretinoin) need to be accounted for, followed by a final confirmation by proctosigmoidoscopy, colonoscopy, or biopsy. [1] Adding to the diagnostic complexity, clinical presentations can vary depending on the extent of the disease. 30-60% of patients who suffer from proctitis (which is limited to the rectal area) mainly experience urgency and a sensation of incomplete evacuation, while symptoms of left-sided colitis mirror the ones of proctitis with the addition of abdominal pain, cramping, and diarrhea. Pancolitis, also known as extensive colitis, makes up 10-15% of UC cases. These patients additionally experience fever, fatigue, and constipation, as the extent of the inflammation has affected their entire colon. [2] Given the diagnosis's complexity and substantial overlap with CD symptoms, a third type of IBD was proposed, classified as indeterminate colitis (ID). ID has a shared clinical and morphological fingerprint of CD and UC. Often provisional ID cases can be reclassified as UC, or more often CD as additional evidence surrounding the pattern of inflammation is revealed. Still, about 10-15% of IBD cases remain unclassified even after extensive diagnostic tests. Currently, indeterminate colitis is not recognized as an official diagnosis, and patients are classified as "IBD, not yet classified" in the case of uncertainty between a UC and CD diagnosis. [3,4] UC is usually limited to the mucosa and superficial submucosa of the colon, and cyclical in nature, with inactive and active periods characterized by flare-ups, during which symptoms are worsened to the detriment of the life quality of patients. [5] Given this volatile state of the disease, many attempts have been made of classifying UC patients based on disease severity and/or activity, from the Truelove – Witts criteria to modern attempts like UC Disease Activity Index and Mayo Clinic Index. Most often these quantifications are based on stool appearance, frequency, and rectal bleeding, with

additional symptoms like fever, anemia, malnutrition, etc. [1] IBD has a long history, with the first reports of chronic diarrhea with an unexplained cause dating back to the antique. [6] A clinical picture similar in nature to our current understanding of UC was first described by Sir Samuel Wilks in one of his case reports of a 42-year-old woman, dating back to 1859. [7] Chron's disease on the other hand had its first prominent patient in the form of Charles Darwin, who was believed to have suffered from Chagas' disease but in later analysis of his medical records, the symptoms have a better match with CD. [8,9] Matthew Baillie, a British physician, and pathologist was the first to report multiple cases consistent with IBD in his book "Morbid Anatomy of Some of the Most Important Parts of the Human Body" [10] while the term ulcerative colitis was coined by William Hale in 1888, based on several case reports from Europe and the USA. [11] The term, and official diagnosis was introduced in London, at the Royal Society of Medicine in 1909 after the presentation and discussion of 300 potential cases [11], and 40 years later Warren and Sommers published a detailed and pictured pathogenesis of UC. [12] UC is believed to be a multi-factorial condition, but its etiology is not fully understood. One of the leading theories suggests that it might be induced through a faulty immune response to colonic bacteria, triggering an autoimmune reaction, but other factors such as genetics, microbiota composition, environmental and lifestyle factors have been correlated to its onset. [2,13–15] One of the first UC models exploring the genetic background was developed in the 1990s in the form of interleukin-10 (IL-10) knockout mice that spontaneously developed colitis. [16] Later, this model was shown to be dependent on interleukin-22, as IL-10-/- mice failed to develop colitis when IL-22 was deficient, and a double deficiency, reduced fecal IgA and IgG. [17] More recently TRUC mice (T-bet-/- RAG2-/-) have been used as a model, developing juvenile UC and being more susceptible to colorectal cancer. T-bet expression has been observed in innate immune cells, while Rag2 is responsible for T lymphocyte maturation.[18] Neither of the single knockouts T-bet-/- or RAG2-/- develop colitis, which would suggest that the development of UC is linked to a deficiency in both innate, as well as adaptive immunity. [18,19] In humans, coding SNPs make up less than 10% of UC-associated single nucleotide polymorphisms, but their number is far higher than for other complex diseases. 70% of identified loci are

overlapping with other complex diseases, and there is a 5-fold enrichment in loci associated with dysfunctional immunity. [20] 90% of the remaining SNPs are noncoding and can be grouped into three modules that are overexpressed in UC: calcium homeostasis, regulation of stress, and cell motility/adhesion. Additionally, two modules were identified. One contains HLA receptors involved in antigen expression and the other contains mitogen-activated protein kinase (MAPK) or histone deacetylase 7 (HDAC7). [21] MAPKs are pro-inflammatory in UC, inducing the production of TNFα, IL-1, IL-2, and IL-6. As they are ubiquitous, their therapeutic role can be significant, but targeting is challenging.[22] p38 MAPK inhibition with SB 203580 in 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis had a two-sided effect, increasing weight loss and colon weight but improving immune markers. [23] Anemoside B showed promising results in dextran sodium sulfate (DSS) colitis by blocking MD2, which is expressed on the surface of macrophages and connected to the TLR-4/NF-κB/MAPK cascade, when administered at a dose of 6mg/kg, twice a day, for 7 days. [24] HDAC has been shown to exhibit anti-inflammatory properties in multiple inflammation and colitis models. [25] Another prerequisite for the development of colitis seems to be the microbiota, as genetically susceptible models do not develop the disease in a germ-free or *Helicobacter*-free environment, even after re-introduction to specific strains. [15] Environmental factors must be considered as well, diet and activity being the main two. Lifestyle changes following the industrial revolution increased quality of life but lead to significant changes in daily routines with a significant decrease in overall activity and an increase in caloric availability and consumption. A correlation between this rapid change and the incidence of IBD can be observed. [26] This is further supported by the incidence of IBD stabilizing in high-income countries but rapidly increasing in newly industrialized areas. [27] Other dietary factors that could increase the risk of UC are high consumption of ultra-processed foods, emulsifiers (carrageenan, glycerol monolaurate polysorbate 80, carboxymethyl cellulose), dyes (red 40, yellow 6), gluten, lactose, FODMAPs (fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols,), and microparticles. [28] On the other hand, physical activity has a protective role against the onset of IBD, where the protective effect is more pronounced against CD than UC. Most

patients experienced improvements in their quality of life when following an exercise routine but in many cases. While regular activity did not cancel remission, many patients stopped their exercise routines during flare-ups. [29] One of the first treatment options for UC was developed and published by Nanna Svartz in 1948. Sulfasalazine was effectively used as a primary treatment on 124 UC patients but has since taken an alternative role given its poor effectiveness compared to other treatments, mainly mesalamine, as well as several side effects including nausea, diarrhea, and headaches. [30,31] It was later discovered that the active component of sulfasalazine is mesalamine, which makes up one part of the molecule that is bonded to sulfapyridine with an azo bond. While mesalamine is responsible for the beneficial effect, of ameliorating mild to moderate UC symptoms, most of the side effects are linked to sulfapyridine. [32] Given the poorly understood etiology of UC, treatment options are somewhat limited and do not produce consistent results across patients. When it comes to the selection of therapeutic targets the International Organization for the Study of IBD (IOIBD) has launched STRIDE - Selecting Therapeutic Targets in Inflammatory Bowel Disease initiative to summarize available data and present clinicians with a standardized method of determining the effectiveness of treatments. The updated version, STRIDE-II, published in 2020, determined that common clinical symptoms, that are used to form disease activity scores correlate well with endoscopic inflammation in UC. The stance of the IOIBD has not changed with endoscopic healing still being the main target of therapy, while serum and fecal inflammatory biomarkers such as fecal calprotectin (FC), erythrocyte sedimentation rate (ESR), and c-reactive protein (CRP) are not the most reliable. Out of the three, FC, especially when done in regular intervals performs best. On the other hand, histological healing was deemed “not consistent” due to inconsistent grading, as well as many patients in remission who do not display histological improvements. [33] Disease-related factors, mainly disease severity and extent, and patient-related factors must be considered when considering treatment options. For mild to moderate ulcerative colitis the first line of treatment is mesalamine administration in different forms. If patients do not respond, second-line corticosteroids are administered. In moderate to severe cases anti-TNF, anti-integrin, and anti-

interleukin agents are administered in the form of monoclonal antibodies. Additionally, a novel treatment with small molecules, more specifically Janus kinase inhibitors 1 and 3 has emerged with promising results. When it comes to severe acute colitis, where clinical stability is imperative, cyclosporine, steroids, and infliximab (TNFα inhibitor) are administered. As a last resort for severe, chronic cases, unresponsive to treatments, a restorative proctocolectomy with ileal pouch-anal anastomosis is performed in which the entire colon and rectum are removed, and the small intestine is re-routed to the preserved anal sphincter forming a J-, S- or W-pouch that serves as a fecal reservoir. Other treatments with limited evidence are curcumin, probiotics, fecal microbiota transplant, hyperbaric oxygen therapy, and mirikizumab [34] Additional concerns for IBD patients include a shorter life expectancy (6.6-8.1 years for females, 5.0-6.1 years for males), with an even bigger difference in health-adjusted life expectancy when compared to healthy individuals (9.5-13.5 years for females, 2.6-6.7 years for males). [35] IBD patients, especially UC patients have a significantly impaired life quality, with flare-up periods creating an additional burden. [36] While data on the increased risk of colorectal cancer in individuals with IBD are heterogeneous, just like the study population, an increased risk is present, steadily increasing throughout the years and reaching about 18% after 30 years of active disease. [37] IBD had seen a steady increase in 185 countries. Since 1990, the number of IBD patients has increased by 85.1% (3.7mil in 1990 to 6.8mil in 2017), the disease is more prevalent in women, with the highest peak between 60-64 years of age, compared to men where the peak is 70-74. Additionally, a 67% increase in total IBD-related deaths has been observed and the number of years lived with a disability has doubled. [27] With the ever-increasing burden of IBD, coinciding with the radical lifestyle changes following the industrial revolution, dietary factors have been proposed as a major player in the development and progression, as well as potential prevention and treatment of the disease. Ultra highly processed food and food additives, mainly in the form of emulsifiers, have been linked to a higher risk of developing UC and CD, and an overall exacerbation of the conditions. Like other gastrointestinal conditions, elimination/exclusion diets have been proposed as an alternative or addition to pharmacological therapy for the management of symptoms.

Low FODMAP and microparticle diets, elimination of lactose, carrageenan, or gluten have shown varying effectiveness, adding to the theory that IBD has a patient-specific fingerprint, making general recommendations less useful. There have also been multiple attempts at specific dietary interventions such as a Mediterranean diet, specific carbohydrate diet, keto diet, plant-based diet, anti-inflammatory diet, and high fiber diet. Additionally, emerging evidence is seen for reduced sulfur diets, personalized fiber diets, fermented food diets, as well as the first attempts of implementing restrictive diets in the form of intermittent fasting and time-restricted feeding, while traditional caloric restriction has not yet been studied. [28] Generally, 85% of IBD patients believe that an improvement in their dietary pattern can lead to better management of symptoms, and there is a high willingness for such interventions. In addition, most patients (90%) consider proper nutrition as an important factor of IBD. This fact is also mirrored in their expression that educational programs for IBD are important, and that 84% would attend such programs.[38] While a higher dietary adherence to a modified Mediterranean diet was associated with lower disease activity in CD, such a trend was not observed in UC, despite high dietary adherence overall. Disease activity, quality of life, and surgical history were the main factors directly impacting adherence. [39] Even when it comes to other, more radical human interventions such as a low-fat (10E% from fat), high-fiber diet, adherence was around 85% across the intervention period of 4 weeks. [40] Given the prominent role of diet in everyday life, the high willingness of patients to adjust their diets for symptom improvement, coupled with a relatively high adherence in interventions, dietary interventions in UC pose a potent lifestyle factor that could prove to be a valuable tool in managing symptoms, inducing, and maintaining remission. By employing a well-established model of chemically induced colitis, we examined the impact of different restrictive diets on DSS-induced colitis in a susceptible mouse model (C57BL/6NRj). [41] Caloric restriction, intermittent fasting, a fasting-mimicking diet, and three different keto diets, including a keto control diet, were compared to standard chow during the 4-week intervention. Histological analysis of the colon and jejunum was performed. MPO (myeloperoxidase) was measured as a macrophage activity surrogate, while inflammatory, and intestinal barrier markers were

determined using qRT-PCR, Western Blot, and protein arrays. Additionally, bile acids, taurine, GSH, and their conjugates were measured using liquid chromatography, coupled with mass spectrometry to analyze metabolome changes.

2. Materials and methods

2.1 Animal experiments

64, Male, C57BL/6NRj, 9-week-old mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France) and housed in conventional open cages (Tecniplast 1290D EUROSTANDARD TYPE III). The animals were held under specific pathogen-free conditions, in a temperature and humidity-controlled environment, on a 12/12h light/dark cycle. Dextran sodium sulfate - DSS (MP biomedicals #02160110-CF) was administered via drinking water for a total of 6 weeks, at a concentration of 0.02mg/mouse/day. Prior to dietary intervention, the DSS treatment consisted of 23 days of uninterrupted DSS administration and an additional two cycles of 4 days without DSS, followed by 4 days of DSS exposure (31 days in total). An additional 11 days of DSS treatment were conducted in 4 cycles during the dietary intervention: 2 days on + 4 days off, 3 days on + 4 days off, 3 days on + 4 days off, ending with 3 days of DSS treatment followed by sacrifice. Following a one-week acclimatization period, 10-week-old mice were randomly assigned to one of 8 dietary intervention groups: ctrl ad lib, ctrl DSS ad lib, CR DSS, IF DSS, FMD DSS, KD DSS ctrl, KD1 DSS, KD2 DSS. The dietary interventions were conducted over 4 weeks. Ctrl ad lib served as an absolute/healthy control group. These mice were not treated with DSS and were fed a standard chow diet (ssniff Spezialdiäten GmbH) ad libitum. Ctrl DSS ad lib mice (colitis control) were treated with DSS as previously described and were fed the same way as the ctrl ad lib group. CR DSS mice were calorically restricted and received 2.7-2.9g of standard chow per day, equating to ≈70%[42] of their habitual intake. IF DSS underwent an intermittent fasting protocol, alternating between food days, consuming standard chow, and no food days. The FMD DSS dietary intervention consisted of 10 days of a fasting-mimicking diet, split into 3 cycles: 2 days of FMD + 9 days refeed, 4 days FMD + 9 days refeed, ending with 4 days FMD. The FMD composition and protocol were described before. [43] All keto groups

(KD DSS ctrl, KD1 DSS, KD2 DSS) were fed ad libitum. KD DSS ctrl was fed 0.8% long-chain triglycerides (LCT), 16.1% crude protein, 6% sugar, and 51.2% starch (ssniff Spezialdiäten GmbH #S9139-E028). KD1 consisted of 74.6% LCT, 8.1% crude protein, 1.0% sugar, and 0% starch (ssniff Spezialdiäten GmbH #S9139-E025), whereas KD2 was fed 49.6% LCT, 25% medium chain triglycerides (MCT), 8.1% crude protein, 1% sugar, 0% starch. 60% of the MCTs in the keto 2 diet were caprylic acid (C8) and the rest was capric acid (C10) (ssniff Spezialdiäten GmbH #S9139-E032). Mice were euthanized via isoflurane (Dechra Pharmaceuticals plc #021778) overdose, body weight (BW) was measured, and a cardiac puncture was performed to draw blood. Liver, cecum, white adipose tissue (WAT), and spleen weight were recorded. Colon and small intestine lengths were measured, and scrapings were collected. Additionally, lymph nodes and Peyer's patches were extracted. All tissues were snap-frozen and stored at -80◦C.

2.2 Myeloperoxidase (MPO) assay

A 0.5% w/v hexadecyltrimethylammonium bromide - HTAB (Sigma #H6269) solution was prepared with 50mM potassium phosphate buffer (Roth #T875, Roth #3904), pH 6. 20 times the volume of ice-cold HTAB solution was added to approximately 10mg of frozen colon scrapings. Samples were homogenized and spun down for 15min at 15000g, 4°C. Protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific #23225). For the MPO measurement, 12.5µl of sample supernatants were added to a chilled 96-well plate. Each sample was measured in duplicates. 200µl of working solution, consisting of 0.167mg/ml o-Dianisidine dihydrochloride (Sigma #D3252) and 1% H_2O_2 (Sigma #216763) dissolved in potassium phosphate buffer, was added with a multi-channel pipette. Samples were incubated in a plate reader at 37°C and measured in 5min intervals for 30min, including T_0 = 0min. Human leukocyte myeloperoxidase (Sigma #M6908-5UN) served as a standard. Raw values were adjusted to a blank, averaged, and evaluated based on maximal activity between two time points. Values were expressed as MPO units per µg of protein as well as MPO units per mg of tissue.

2.3 qRT-PCR

RNA was extracted from 5-10mg of the jejunum and proximal colon mucosa tissue, Peyer‘s patches, and lymph nodes using the peqGOLD Total RNA kit (VWR Life Science #13-6834-02P). Concentrations were measured using the NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific #ND-ONE-W) and adjusted accordingly. Reverse transcription was performed using the qScript cDNA Synthesis Kit (Quantabio, USA) containing 5x reaction mix (Qunatabio #84006) and reverse transcriptase (Quantabio #84003) according to the user manual. Quantitative real-time PCR was performed with the QuantaStudio 6 Flex system (Thermo Scientific #4485691) using the PerfeCTa SYBR Green PCR Master Mix, ROX (Quantabio #95073-05K). An overview of the used primers is provided in Table 1.

2.4 Western blot

Mucosa tissue from the jejunum and proximal colon (10-20mg) was lysed with 10x the volume of RIPA buffer (EMD Millipore Corp #20-188) containing a protease inhibitor cocktail (Roche #11836153001, Thermo Scientific #36978). Protein concentration was determined using the Pierce™ BCA kit (Thermo Scientific #23225). For ß-actin, a concentration of 1µg/µl and for other antibodies a concentration of 3µg/µl was used. ß-actin, IKB, and occludin were determined for both tissues while TNFα was additionally determined for proximal colon samples. DTT and loading buffer (tris, SDS, glycerol, bromophenol blue, and 2- ß-Mercaptoethanol) was added to the diluted lysate to a total volume of 10µl and the samples were incubated at 95°C, 300RPM for 5min. After incubation samples were briefly put on ice to cool down, spun, and loaded onto the gel with Precision Plus Protein™ Dual Color Standard (BioRad #1610374). Following gel separation, the samples were blotted using the Trans-Blot® Turbo Transfer System (Bio-Rad) onto PVDF membranes (BioRad #1620177). The membranes were briefly dried, the ladder was marked for better visibility, and a Ponceau staining was performed. Membranes were then blocked for 1h with the appropriate blocking solution and incubated with antibodies against ß-actin (Cell Signaling Technology #4970, 1:1000 in 2.5% BSA TBST, overnight at 4°C), IKB (Cell Signaling Technology #9242 1:1000 in 5% BSA

TBST, overnight at 4°C), occludin (Thermo Scientific #33-1500 1:500 in 5% milk powder, 2h at room temperature), and TNFα (Cell Signaling Technology #11948, 1:1000 in 5% BSA TBST, 2h at room temperature). Following another 1h wash the corresponding secondary antibody, anti-mouse or anti-rabbit was applied in a dilution of 1:3000 (Cell Signaling Technology #7076 and #7074). SuperSignal™ West Dura Extended Duration Substrate (Thermo Scientific #34076) was applied to the membranes and the bands were scanned using the ChemiDoc XRS+ (Bio-Rad). The evaluation was performed using ImageJ (version 1.53) in which ß-actin was used as a standard.

2.5 Protein array

Cytokine levels were measured in mucosal tissue of the proximal colon using the Mouse Inflammation Array Q1 (RayBiotech #QAM-INF-1-1) in accordance with the manufacturer's instructions. A total of 40 cytokines were measured: BLC (CXCL13), CD30 Ligand (TNFSF8), Eotaxin-1 (CCL11), Eotaxin-2 (MPIF-2/CCL24), Fas Ligand (TNFSF6), G-CSF, GM-CSF, I-309 (TCA-3/CCL1), ICAM-1 (CD54), IFN-gamma, IL-1 alpha (IL-1 F1), IL-1 beta (IL-1 F2), IL-10, IL-12 p70, IL- 13, IL-15, IL-17A, IL-2, IL-21, IL-3, IL-4, IL-5, IL-6, IL-7, KC (CXCL1), Leptin, LIX, MCP-1 (CCL2), MCP-5, M-CSF, MIG (CXCL9), MIP-1 alpha (CCL3), MIP-1 gamma, Platelet Factor 4 (CXCL4), RANTES (CCL5), TARC (CCL17), TIMP-1, TNFα, TNF RI (TNFRSF1A), TNF RII (TNFRSF1B). Mucosal tissue was lysed in 10x volume of RIPA buffer (EMD Millipore Corp #20-188) containing protease inhibitors (Roche #11836153001, Thermo Scientific #36978). Using the Pierce™ BCA kit (Thermo Scientific #23225) the protein concentration was measured, and the samples were diluted to 0.6mg/ml. The glass slides were tempered at room temperature for 30min, while still sealed after which they were unpacked, and air-dried for an hour. A serial dilution was performed for the reconstituted lyophilized standard (Std1-Std7). After the glass slides were fully dried, they were blocked with 100µl of sample diluent for 30min at room temperature. Sample diluent was decanted, 100µl of standards/samples were applied to each well and incubated overnight at 4°C. Following decanting the wells were washed 5 times (5min each) with 150µl wash buffer I and 2 times (5 min each) with 150µl wash buffer II after which 80µl of the detection antibody was added and incubated for 2h. The washing steps were repeated, and 80µl of dye-conjugated streptavidin was added to

each well and incubated for 1h. Washing was repeated with wash buffer I after which the glass slides were disassembled, removing the gasket, and freeing the glass slides which were transferred in a specialized slide washer tube and fully covered with wash buffer I for 15min during which they were gently rocked. This step was repeated with buffer II for 5min. Water droplets were removed, and the glass slides were fully dried. They were scanned with a green channel Cy3 wavelength, the raw data was normalized based on the positive controls, and the concentrations in pg/ml were determined using a standard curve. KD DSS ctrl was omitted from this measurement.

2.6 Histology

Pieces of jejunum and colon were collected in tissue cassettes during dissection and placed into 10% phosphate-buffered formalin for 24h. Following a 15min wash under cold, running tap water they were incubated in 75% and 85% ethanol for an hour each before overnight incubation in 95% ethanol and 5% methanol. The following day tissue cassettes were transferred into 100% ethanol. This step was repeated 3 times, for a total of 3 hours, each time with fresh ethanol. Two 45min steps in xylol were followed by three 30min paraffin steps before the samples were embedded. Samples were cut to a thickness of 4µm using the Leica RM2245 Microtome, dewaxed using xylol (2x10min), rehydrated using a descending ethanol series (100%, 95%, 85%, 70%, 30%, 10min each), stained with hematoxylin for 70 seconds, followed by a 30-second rinse in tap and 10min wash in double distilled water. After dehydrogenation with 70% ethanol for 8min, they were counterstained in eosin for 2 seconds, dehydrated in an ascending ethanol series (95%, 100% for 10 seconds each), and cleaned twice with xylol (2x10min). A mounting medium was applied to the samples, followed by a cover glass after which they were dried under deduction for 24h. Axiolab 5 Digital Lab Microscope microscope (Zeiss #AL-1D), Axiocam ERc 5s camera (Zeiss #51343), and the ZEN (Zeiss Efficient Navigation) software were used to acquire images which were analyzed using ImageJ (version 1.53).

2.7 Bile acids measurement

Measurements were performed with a modified method by Wegner et al.[44]. 7-10mg of frozen ileum mucosa samples were cut and transferred into Precellys homogenizing

tubes with 1.4 mm ceramic beads and nine times the volume of ice-cold (-20°C) absolute ethanol. Prepared samples were homogenized using the Precellys 24 Tissue Homogenizer (Bertin Instruments, #P000669-PR240-A) for two 30s steps at 4000RPM, centrifuged at 3500g for 5min at 4°C. Supernatants were transferred to new tubes and centrifugation was repeated at 13000g to remove any potential debris, after which the supernatants were transferred into HPLC vials. Samples were analyzed using the LCMS-8040 Liquid Chromatograph Mass Spectrometer (Schimadzu Corporation #C146-E188E) in positive modus, using the Atlantis T3 3µm column 2.1 x 150 (Waters #186003719), with a column temperature of 30°C. Mobile phase A consisted of water, while eluent contained acetonitrile and methanol in a 3/1 v/v ratio. 0.1% formic acid and 20mM ammonium acetate were added to both phases. A gradient was maintained from an initial 30% B for 5min to 100% at 25min and kept for 20min, followed by a return to the initial radio within 2min and 10min re-equilibration. The area under the curve for each molecule: TLCA (Taurolithocholic acid) TUDCA (Tauroursodeoxycholi acid) TCA (Taurocholic acid) TDCA (Taurodeoxycholic acid) CA (Cholic acid) DCA (Deoxycholic acid) CDCA (Chenodeoxycholic acid) UDCA (Ursodeoxycholic acid) LCA (Lithocholic acid), DHCA were measured and compared. All chemicals needed for solvents (HPLC water, acetonitrile, methanol, formic acid, ammonium acetate) were purchased from Carl Roth GmbH & Co. KG, Karlsruhe, Germany, while all standards were prepared from raw chemicals bought from Sigma Aldrich CHEMIE GmbH, Steinheim, Germany.

2.8 Taurine and GSH measurement

Sample preparation was performed analog to the bile acid measurement. Samples were analyzed using the LCMS-8040 Liquid Chromatograph Mass Spectrometer (Schimadzu Corporation #C146-E188E) in negative modus, using the Atlantis T3 3µm column 2.1 x 150 (Waters #186003719), with a column temperature of 0°C. Phase A consisted of 0.1% formic acid in water and phase B 0.1% formic acid in acetonitrile. The initial gradient of 5% B at 2.5min was increased to 20% at 8min, returning to 5% B at 9min and held for one minute. The area under the curve for each molecule was evaluated and compared. All chemicals needed for solvents (HPLC water, acetonitrile, methanol, formic acid, ammonium acetate) were purchased from Carl Roth GmbH & Co. KG, Karlsruhe,

Germany, while all standards were prepared from raw chemicals bought from Sigma Aldrich CHEMIE GmbH, Steinheim, Germany

2.9 Statistics

Differences between groups were determined using one-way ANOVA and post hoc analysis was performed with paired t-tests using the Bonferroni correction where $p < 0.05$ was considered significant. The healthy control group (ctrl ad lib) was only compared to the ctrl DSS ad lib group, while all other groups (CR DSS, IF DSS, FMD DSS, KD DSS ctrl, KD1 DSS, KD2 DSS ctrl) were compared to the ctrl DSS ad lib group to establish if the animals were influenced by the interventions when compared to untreated colitis. Data is presented as mean ± SEM.

3. Results

3.1 Restrictive diets modulated several anthropometric factors

Ctrl ad lib DSS, CR DSS, and KD2 DSS groups had a 100% survival rate. 7 out of 8 mice survived in the ctrl ad lib, IF DSS, KD ctrl DSS, and KD1 DSS groups, while the FMD DSS group had the lowest survival rate of 75% (n=6/8). Table 2 provides an overview of the anthropometric data. Significant BW differences were observed. Healthy controls (ctrl ad lib) had a higher average weight when compared to their colitis counterparts (ctrl DSS ad lib). CR DSS, FMD DSS, and KD1 DSS mice had a significantly lower BW when compared to the ctrl DSS ad lib group, while the BW of IF DSS, KD DSS ctrl, and KD2 DSS was not significantly changed. KD DSS ctrl had a higher weight compared to the ctrl DSS ad lib group. Given the significant BW differences, the tissue/organ weights were adjusted as %BW (percent of body weight). Liver %BW was higher for the colitis group (ctrl DSS ad lib) when compared to the ctrl ad lib group, but the difference was not significant, yet all intervention groups had significantly lower liver %BW. The same trend was observed for spleen %BW, except for the KD DSS ctrl group which was similar to the colitis control. Colitis controls had less BW-adjusted white adipose tissue compared to healthy controls, while intermittent fasting, keto control, and the keto 2 diet not only preserved WAT but increased it beyond the level of healthy mice. The colitis control groups had heavier cecum contents compared to healthy mice, which are comparable

Name	Forward	Reverse 5'-3'
Occludin	CCTCCAATGGCAAAGTGAAT	CTCCCCACCTGTCGTGTAGT
Zo-1	CCACCTCTGTCCAGCTCTTC	CACCGGAGTGATGGTTTTCT
Stat1	GCTGAGTGCTGTTCCCATCT	AAGTCCTTCAGAGTAACAG
MCP1	TGATCCCAATGAGTAGGCTGGAG	ATGTCTGGACCCATTCCTTCTTG
IL1ß	TCCTGTGTAATGAAAGACGGC	GGTGCTGATGTACCAGTTGGG
Tnfα	CCACAGGGCTGCAATTTTCC	CCACAGGGCTGCAATTTTCC
Irf1	CCCAGCTCTTGCTTTCGGA	AAGCCCAGTAGTTCACGACC
Tlr3	GTATTGCCTGGTTTGTTAATTGG	AAGAGTTCAAAGGGGGCACT
Tgfß1	CCACAGGGCTGCAATTTTCC	CCACAGGGCTGCAATTTTCC
Ifnγ	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
IL33	TGAGACTCCGTTCTGGCCTC	CTCTTCATGCTTGGTACCCGAT
Nod2	GGCAACAGTGTAGGTGATAAGGG	TAGTGACTTGTTCTTCTCCAGCATC
Reg3γ	CTCCCCACCTGTCGTGTAGT	CTCCCCACCTGTCGTGTAGT
MyD88	GCACCTGTGTCTGGTCCATT	TGTTGGACACCTGGAGACAG
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL7	TCTGCTGCCTGTCACATCATC	GGACATTGAATTCTTCACTGATATTCA
COX2	TTACAGGAGAGAAGGAAATGG	TAGCATCTGGACGAGGTT
AIF1	GGAGACGTTCAGCTACTCTGAC	CATCCACCTCCAATCAGGGC
eEf1a1	CCTGGCAAGCCCATGTGT	TCATGTCACGAACAGCAAAGC

Table 1. List of primers (5' 3') used for qRT-PCR. Name, forward, and reverse sequence

Correlations		
	BW-Liver	0,85
	BW-WAT	0,56
	BW-Cecum	0,24
	BW-Spleen	0,76
	BW-SI	-0,16
	BW-Colon	0,59
	Liver-Spleen	0,92
	Liver %BW-Spleen %BW	0,86

Table 3. Correlations between the means of anthropometric measurements between all groups for bodyweight - BW (g), white adipose tissue – WAT (g), cecum (g), spleen (g), small intestine – SI (cm), colon (cm). Additionally, the correlation for liver:spleen was calculated with bodyweight adjusted weights, expressed as %BW. n = 6-8

	Bodyweight (g)	Liver (g)	Liver %BW	WAT (g)	WAT %BW	Cecum (g)	Cecum %BW	Small intestine [cm]	Colon [cm]	Spleen (g)	Spleen %BW
Ctrl ad lib	30.76 ± 0.68	1.65 ± 0.06	5.37 ± 0.15	0.54 ± 0.04	1.75 ± 0.14	0.68 ± 0.03	2.22 ± 0.08	31.97 ± 0.79	7.37 ± 0.11	0.086 ± 0.005	0.280 ± 0.011
Ctrl ad lib DSS	27.85 ± 0.50$^{\#}$	1.73 ± 0.04	6.22 ± 0.12$^{\#}$	0.29 ± 0.02$^{\#}$	1.05 ± 0.07$^{\#}$	0.85 ± 0.05	3.04 ± 0.14$^{\#}$	32.19 ± 0.85	6.75 ± 0.31	0.118 ± 0.009	0.423 ± 0.032$^{\#}$
CR DSS	23.14 ± 0.23*	1.11 ± 0.03*	4.77 ± 0.11*	0.30 ± 0.01	1.29 ± 0.06	0.72 ± 0.03	3.11 ± 0.16	31.83 ± 0.85	6.20 ± 0.19	0.054 ± 0.002*	0.234 ± 0.009*
IF DSS	25.89 ± 0.35	1.18 ± 0.03*	4.57 ± 0.07*	0.44 ± 0.03*	1.70 ± 0.11*	0.77 ± 0.03	2.98 ± 0.11	34.06 ± 0.88	7.06 ± 0.13	0.057 ± 0.004*	0.219 ± 0.014*
FMD DSS	22.97 ± 0.43*	0.90 ± 0.02*	3.94 ± 0.11*	0.26 ± 0.04	1.11 ± 0.17	0.46 ± 0.06*	2.01 ± 0.28*	29.67 ± 1.22	5.60 ± 0.17	0.050 ± 0.003*	0.219 ± 0.011*
KD ctrl DSS	28.70 ± 0.47	1.39 ± 0.04*	4.86 ± 0.08*	0.61 ± 0.06*	2.13 ± 0.21*	0.31 ± 0.03*	1.06 ± 0.09*	27.71 ± 0.39*	5.69 ± 0.28	0.097 ± 0.010	0.315 ± 0.033
KD1 DSS	22.34 ± 1.30*	1.05 ± 0.09*	4.65 ± 0.18*	0.45 ± 0.07	1.89 ± 0.28	0.17 ± 0.03*	0.73 ± 0.10*	32.57 ± 1.14	5.06 ± 0.24*	0.055 ± 0.004*	0.245 ± 0.011*
KD2 DSS	27.08 ± 0.63	1.20 ± 0.05*	4.42 ± 0.14*	0.65 ± 0.06*	2.43 ± 0.24*	0.25 ± 0.01*	0.92 ± 0.04*	31.23 ± 0.60	5.53 ± 0.17*	0.075 ± 0.005*	0.278 ± 0.015*

Table 2. Summary of anthropometric data gathered during dissection. Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

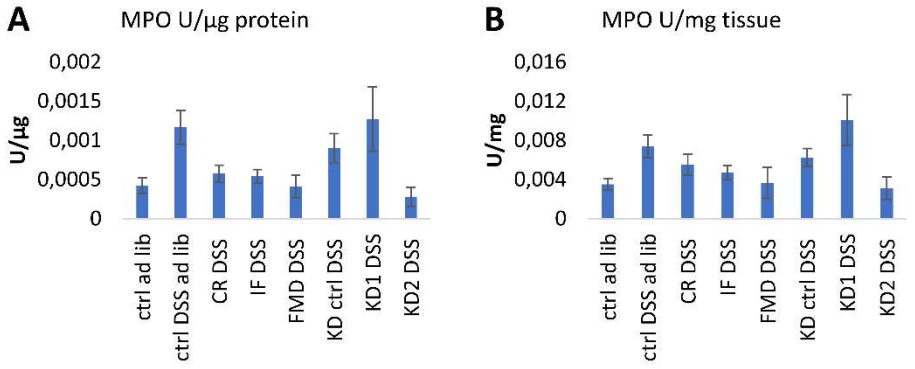


Figure 1. The expression of mucosal tissue myeloperoxidase (MPO) in the proximal colon when standardized to the amount of protein (A) or amount of tissue (B). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

to the CR DSS and IF DSS group, while the FMD DSS, KD ctrl DSS, KD1 DSS, and KD2 DSS mice had significantly lower cecum weights after adjusting them to BW. Small intestine and colon lengths were very similar. Healthy controls and colitis controls show no differences. KD ctrl DSS mice had significantly shorter small intestines at 27.7±1.0cm when compared to the ctrl ad lib DSS group 32.2±2.4cm. KD1 DSS and KD2 DSS had the

shortest colons. This difference was significant compared to colitis control mice. Strong correlations were observed for BW-liver, BW-spleen, liver-spleen and liver %BW-spleen %BW (Table 3).

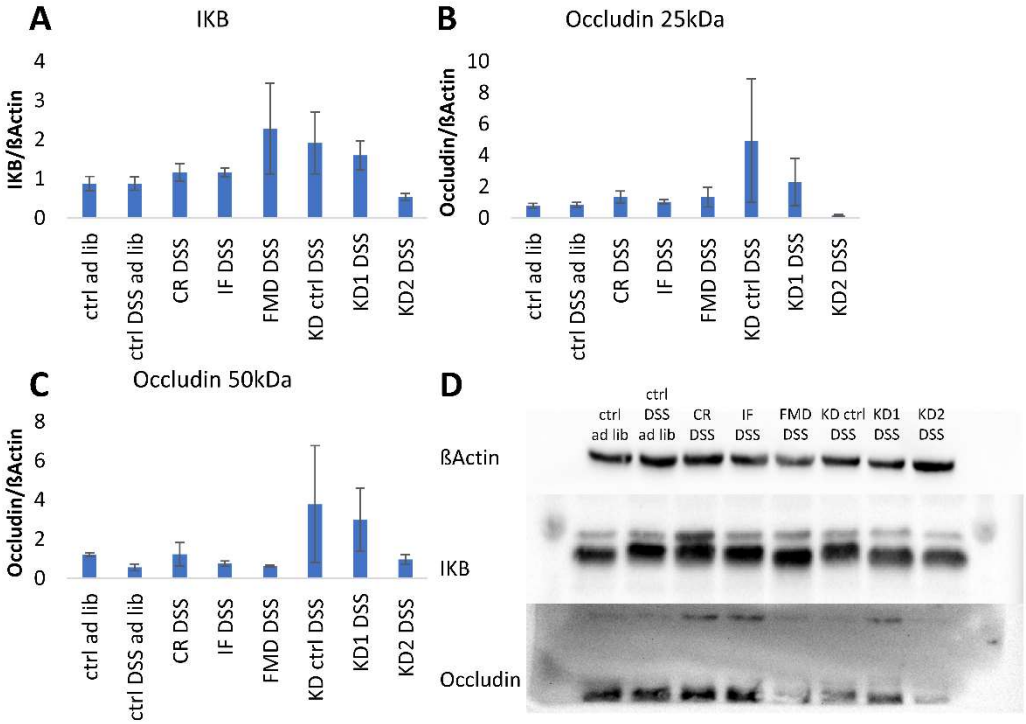


Figure 2. Protein expression in mucosal tissue of the jejunum measured by Western Blot. Relative expression of IKB (A), occludin 25kDa (B) and occludin 50kDa (C), when standardized with βAactin (D). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

3.2 Myeloperoxidase levels remained the same regardless of intervention in colon mucosa

One-way ANOVA has shown differences between groups in both units/µg of protein and units/mg of tissue, but these differences were not observed in the post hoc analysis. The trends were very similar. In both cases, the levels of MPO were highest in the colitis control and KD1 DSS groups, while the lowest levels were observed in the KD2 DSS group (Figure 1A, B).

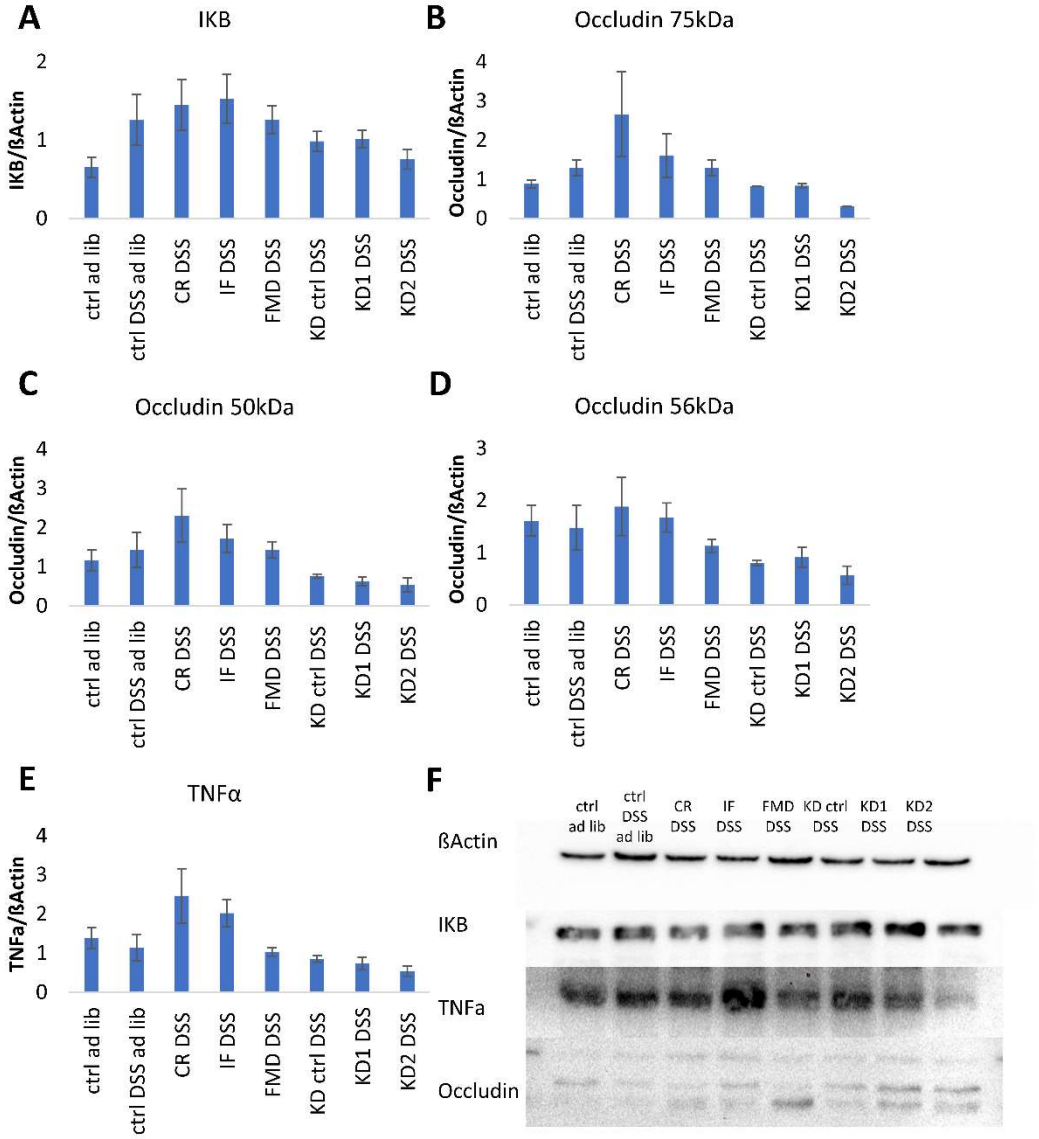
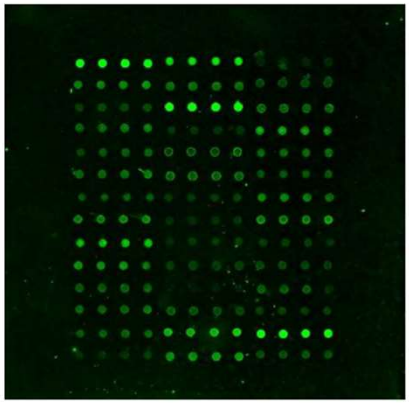


Figure 3. Western Blot analysis of proximal colon mucosal tissue. Relative expression of IKB (A), occludin 75kDa (B), occludin 50kDa (C), occludin 56kDa (D), and TNFα (E) when standardized with βAactin (F). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

3.3 Protein expression remained unchanged in the jejunum with minor changes in the proximal colon

In both tissues, multiple bands were observed for occludin. In jejunum samples, bands were observed at 25kDa and 50kDa (Figure 2D) and for the proximal colon, distinct bands were observed at 50kDa, 56kDa, and 75kDa (Figure 3F). Similar levels of IKB and occludin were observed for jejunum samples for all groups (Figure 2A-D). No differences have been identified between groups for IKB and occludin 75kDa expression in the

proximal colon (Figure 3A, B). Occludin 50kDa, occludin 56kDa, and TNFα on the other hand were significantly different in one-way ANOVA, but these differences were not visible in the post hoc t-tests (Figure 3C-F). Caloric restriction and intermittent fasting slightly increased levels of occludin and TNFα in the colon, but the increase was not significant (Figure 3B-F).



	1	2	3	4	1	2	3	4	1	2	3	4
A	Positive control 1				Positive control 2				BLC (CXCL13)			
B	CD30 Ligand (TNFSF8)				Eotaxin-1 (CCL11)				Eotaxin-2 (MPIF-2/CCL24)			
C	Fas Ligand (TNFSF6)				G-CSF				GM-CSF			
D	ICAM-1 (CD54)				IFN-gamma				IL-1 alpha (IL-1 F1)			
E	IL-1 beta (IL-1 F2)				IL-2				IL-3			
F	IL-4				IL-5				IL-6			
G	IL-7				IL-10				IL-12 p70			
H	IL-13				IL-15				IL-17A			
I	IL-21				KC (CXCL1)				Leptin			
J	LIX				MCP-1 (CCL2)				MCP-5			
K	M-CSF				MIG (CXCL9)				MIP-1 alpha (CCL3)			
L	MIP-1 gamma				Platelet Factor 4 (CSCL4)				RANTES (CCL5)			
M	TARC (CCL17)				I-309 (TCA-3/CCL1)				TIMP-1			
N	TNF alpha				TNF RI (TNFRSF1A)				TNF RII (TNFRSF1B)			

Figure 4. Visual representation of „raw" protein array data for one sample measured with green channel Cy3 wavelength. Each cytokine (listed in the adjacent table) was measured in quadruplicates.

3.4 Several pro-inflammatory factors which were upregulated by colitis were attenuated by dietary interventions, especially the keto diets

Each cytokine was determined in quadruplicate, followed by intra- and inter-slide normalization based on the two positive controls (Figure 4). The DSS treatment increased the inflammatory response when compared to untreated controls. CR, IF, and FMD seemed to be outperformed by the keto diets, especially the KD1 DSS group (Figure 5). Differences between groups for BLC, IL-3, IL-12 p70, IL-15, Leptin, M-CSF, MIG, RANTES, and TIMP-1 have been identified by one-way ANOVA, but t-tests did not show significant impacts of interventions when compared to the colitis control. It should be noted that in the case of BLC, IL-3, IL-12 p70, and leptin the keto groups (KD1 DSS and KD2 DSS) have shown lower levels compared to other interventions. In the case of IL-15 and RANTES, lower levels were only observed in the KD1 DSS mice. Although not significant in subsequent t-tests, due to high interindividual variation, TIMP-1 levels were decreased by all interventions (Figure 6A). For CD30 Ligand, Eotaxin-2, Fas Ligand, ICAM-1, IL-1 alpha, IL-1 beta, IL-10, IL-21, MCP-5, MIP-1 gamma, TARC, TCA-3, TNF RI, TNF RII one-way ANOVA has revealed significant differences between groups, but post

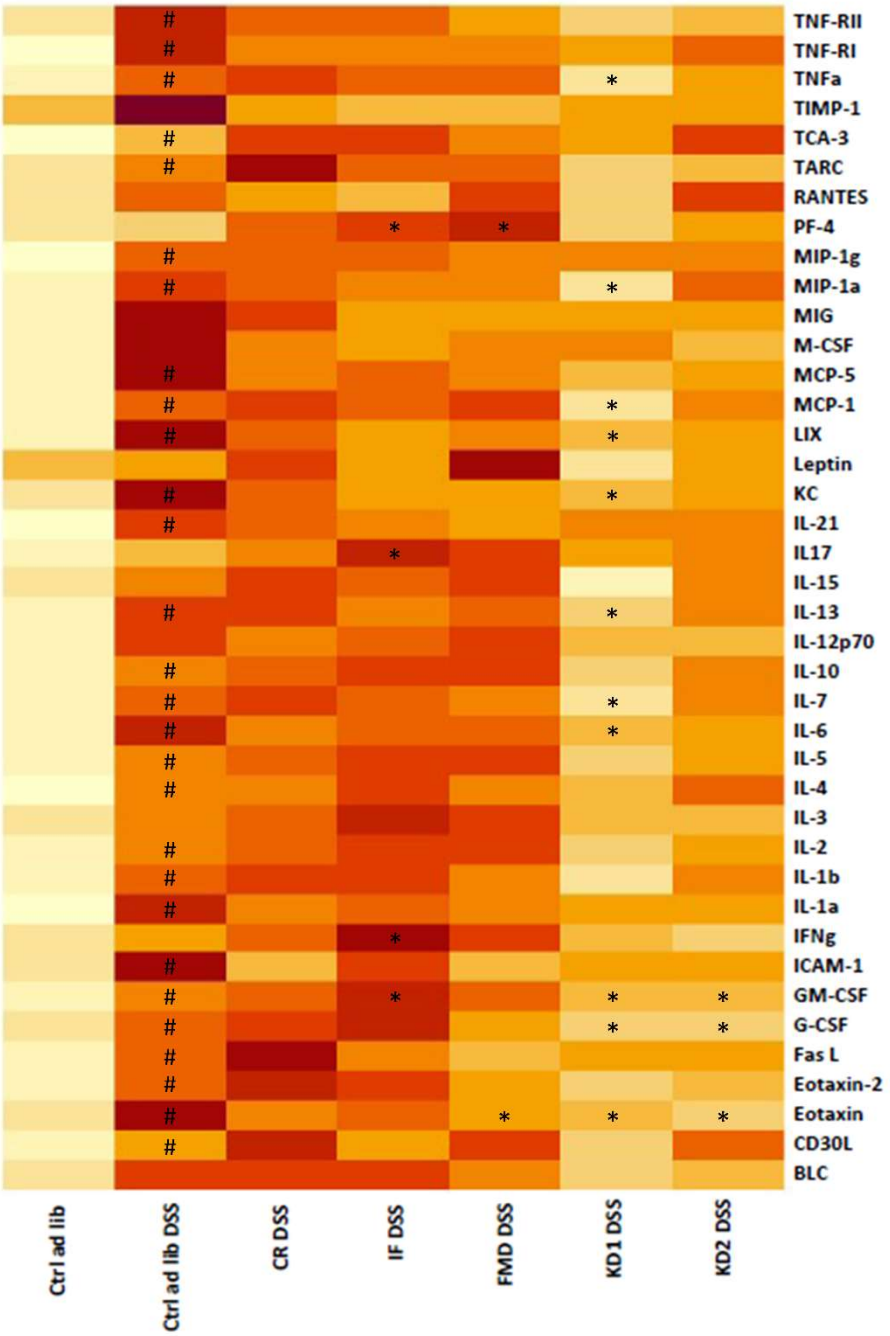


Figure 5. 40 inflammation-related cytokines were determined in proximal colon mucosa tissue via protein array. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

hoc analysis has only shown significant differences between the ctrl ad lib and DSS ctrl ad lib mice. In the case of these cytokines, colitis and/or DSS were observed to have an effect while the dietary interventions did not have a significant impact on cytokine levels (Figure 5). Significant differences between groups were seen for IL-6, IL-7, IL-13, KC, LIX, MCP-1, MIP-1a, and TNFα. Post hoc analysis has shown significantly higher levels in the

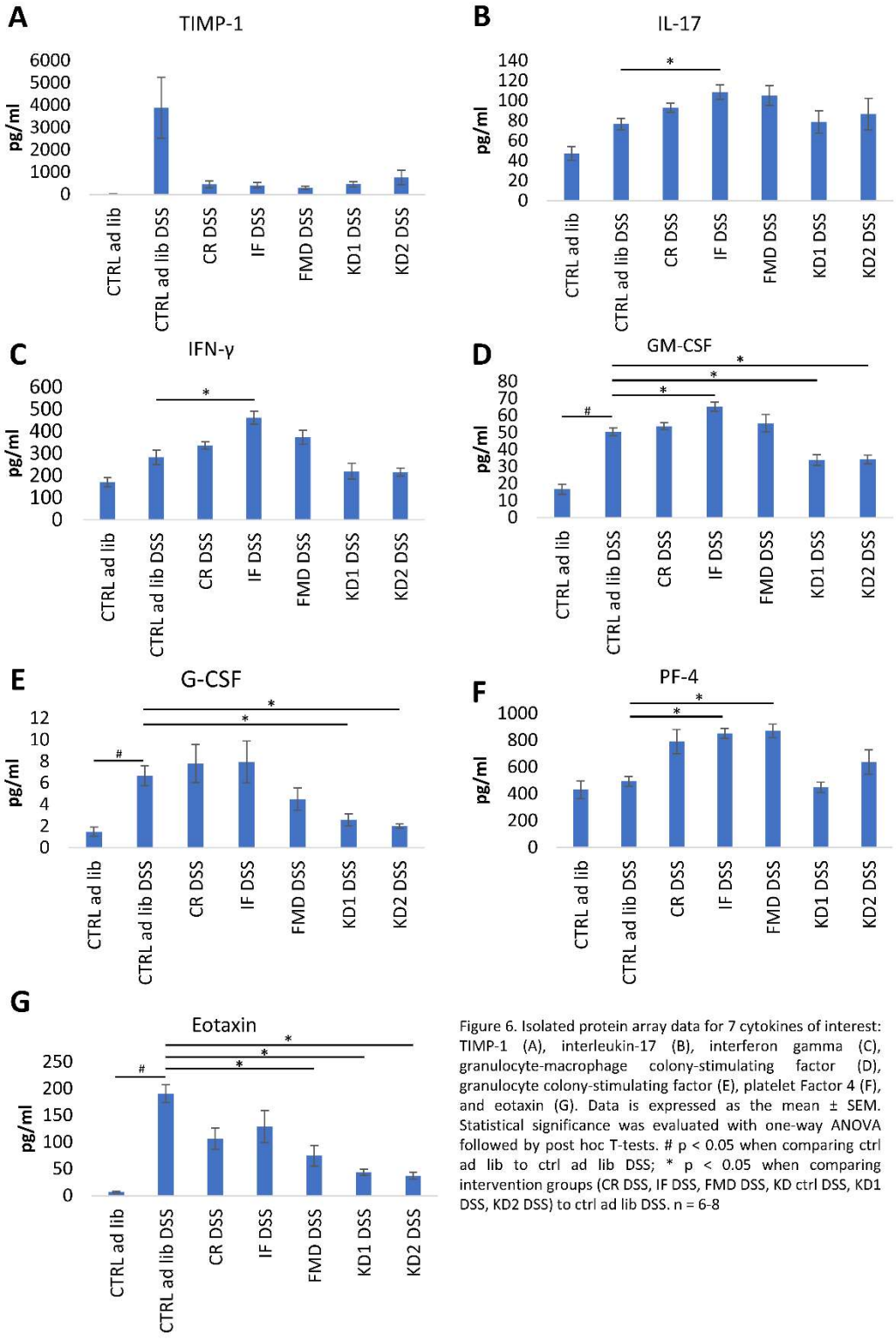


Figure 6. Isolated protein array data for 7 cytokines of interest: TIMP-1 (A), interleukin-17 (B), interferon gamma (C), granulocyte-macrophage colony-stimulating factor (D), granulocyte colony-stimulating factor (E), platelet Factor 4 (F), and eotaxin (G). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

ctrl ad DSS mice compared to healthy, untreated controls. KD1 DSS was the only group with significantly lower levels of these cytokines compared to the colitis controls, and in the case of IL-7, MCP-1, MIP-1a, and TNFα, the levels were similar to healthy controls (Figure 5). One-way ANOVA revealed differences between the groups for IL-17 and IFN-

γ. Further analysis with post hoc t-test has shown that intermittent fasting has significantly increased levels of interleukin 17 and interferon-gamma in comparison to the ctrl DSS ad lib group (Figure 6B, C). G-CSF and GM-CSF had significant differences between groups and these differences were further analyzed. Higher levels were measured in the colitis control compared to healthy mice, and GM-CSF was highest in the IF DSS group. This increase was significant in comparison to the ad lib colitis mice (Figure 6D). Both keto interventions (KD1 DSS and KD2 DSS) significantly attenuated G-CSF and GM-CSF (Figure 6D, E). Differences between all groups were observed for PF-4 and post hoc t-tests revealed that the fasting-mimicking diet and intermittent fasting led to a significant increase (Figure 6F). Eotaxin-1 levels were drastically different between groups. While healthy mice had negligible amounts (7.40±1.37pg/ml), the colitis control had levels that were close to 30x higher (190.62±16.42pg/ml). The fasting-mimicking diet significantly lowered eotaxin-1, but it was outperformed by both keto diets which had the lowest levels (Figure 6G).

3.5 Restrictive diets restore DSS-induced villus shortening but colon thickness was not affected

DSS treatment significantly reduced the villus length when compared to healthy animals. While healthy mice had an average villus length of 267 ± 9µm, the ctrl DSS ad lib group had significantly shorter villi 222 ± 7µm. CR, IF, FMD, and KD ctrl all restored villi to a healthy length, whereas KD1 and KD2 increased the villus length beyond the baseline to 415 ± 26µm and 431 ± 26µm respectively (Figure 7A, B). Colon muscle thickness was increased by DSS and remained mostly unaffected by the diets. Only KD2 significantly reduced the thickness of the outermost muscle layer (Figure 8A, B).

3.6 Alterations in mRNA expression were mainly observed in the jejunum and proximal colon while Peyer's patches and lymph nodes remained stable independent of DSS and/or diet

In mucosal tissue from the jejunum, no differences were observed for MCP-1, TGFβ1, COX2, MYD88, NOD2, IL-7, and IL-1β (Figure 9A, C, E, H, I, N, O). TNFα was significantly lower in the KD2 group when compared to the ctrl DSS ad lib mice (Figure 9D). A similar

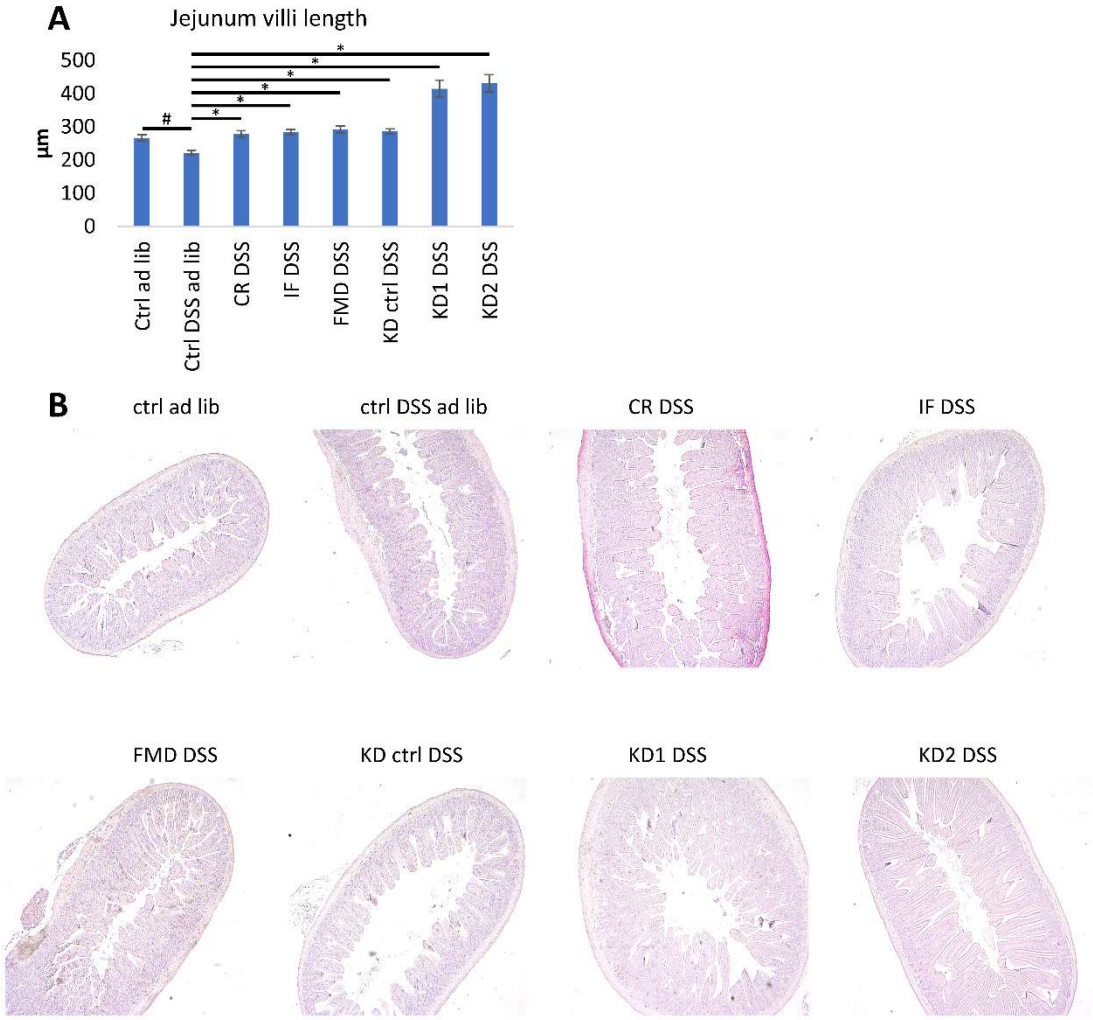


Figure 7. Villus length in the jejunum (A) measured after hematoxylin and eosin staining (B). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

observation was made for ZO-1 which was stable across all interventions but had lower levels in the FMD mice when compared to the DSS control (Figure 9B). Occludin levels were significantly lowered by the fasting-mimicking diet and both keto 1 and 2 (Figure 9G). Similarly, AIF1 has seen a reduction in FMD and KD1 mice (Figure 9F), IFN-γ g and IRF1 levels were lower in KD1 and KD2 mice (Figure 9J, M), while REG3G had decreased levels in all keto groups, including the keto control, lowering the levels well below the baseline of healthy animals (Figure 9L). A significant increase of IL-33 occurred in the caloric restriction and intermittent fasting groups (Figure 9K). Proximal colon mucosal tissue had stable levels of IL-7, IL-1b, TLR-3, ZO-1, occludin, MCP-1, IFN-γ, COX2, and REG3G across all groups (Figure 10A-C, E, G, J, L, M, O). Stat1 expression was increased

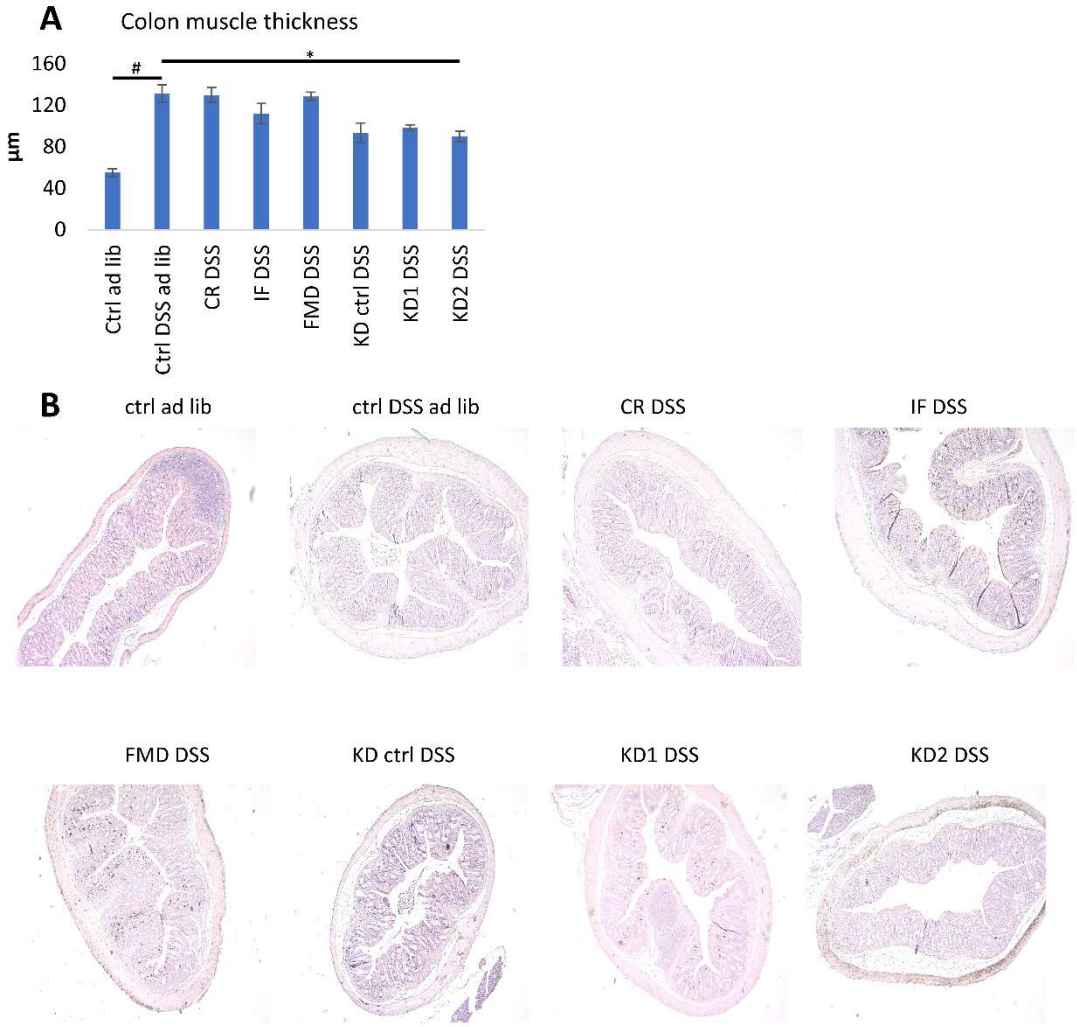


Figure 8. *Muscularis externa* thickness in the colon (A) measured after hematoxylin and eosin staining (B). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

by DSS when compared to untreated mice but the levels were not significant. All restrictive diets effectively lowered stat1 expression by a significant margin (Figure 10D). TNFα increased significantly with DSS treatment, and all interventions, except the fasting-mimicking diet, lowered the expression by a significant amount, returning close to baseline or even under the baseline of healthy animals, which was the case in the IF DSS group (Figure 10F). The ctrl DSS ad lib group has seen a significant increase in AIF1, which was reversed by CR, IF, and FMD (Figure 10I). An effective reduction of TGFβ1 was achieved by all intervention diets except CR (Figure 10H). The only significant difference for MYD88 was seen in the FMD group, which has seen a significant decrease compared to the DSS control mice (Figure 10K). Intermittent fasting, keto 1 and 2, effectively

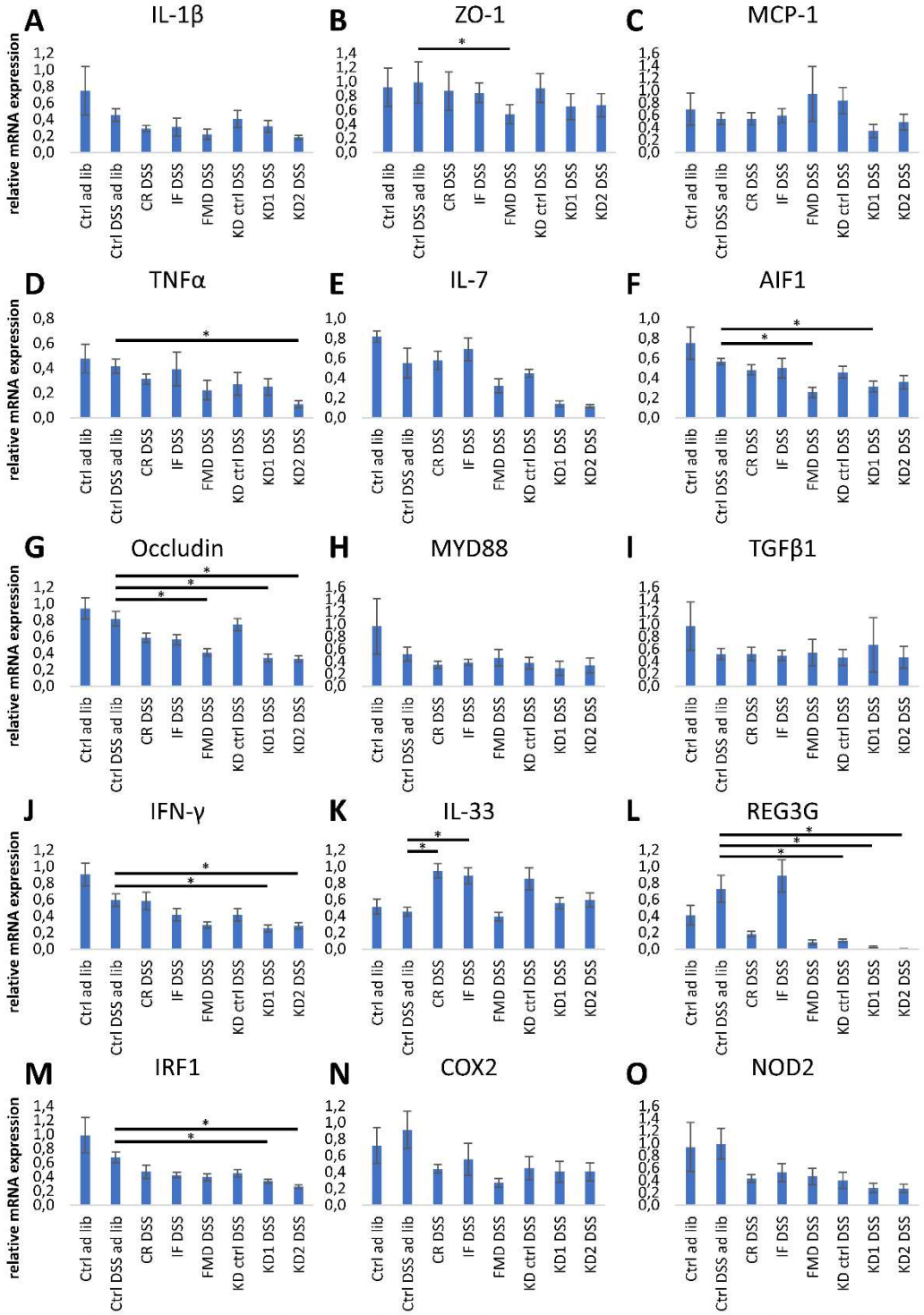


Figure 9. Relative mRNA expression of interleukin-1β (A), ZO-1 (B), monocyte chemoattractant protein-1 (C), TNFα (D), interleukin-7 (E), allograft inflammatory factor 1 (F), occludin (G), MYD88 (H), TGFβ1 (I), IFN-γ (J), interleukin-33 (K), REG3G (L), IRF1 (M), cyclooxygenase-2 (N), and nucleotide-binding oligomerization domain-containing protein 2 (O), measured by qRT-PCR in mucosal tissue of the jejunum and standardized to elongation factor 1-alpha 1 (eEF1a1). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

reduced the expression of IL-33 in the colon (Figure 10Q). IRF1 levels were similar except for the keto control which reduced its levels (Figure 10N). A decrease in NOD2 was seen

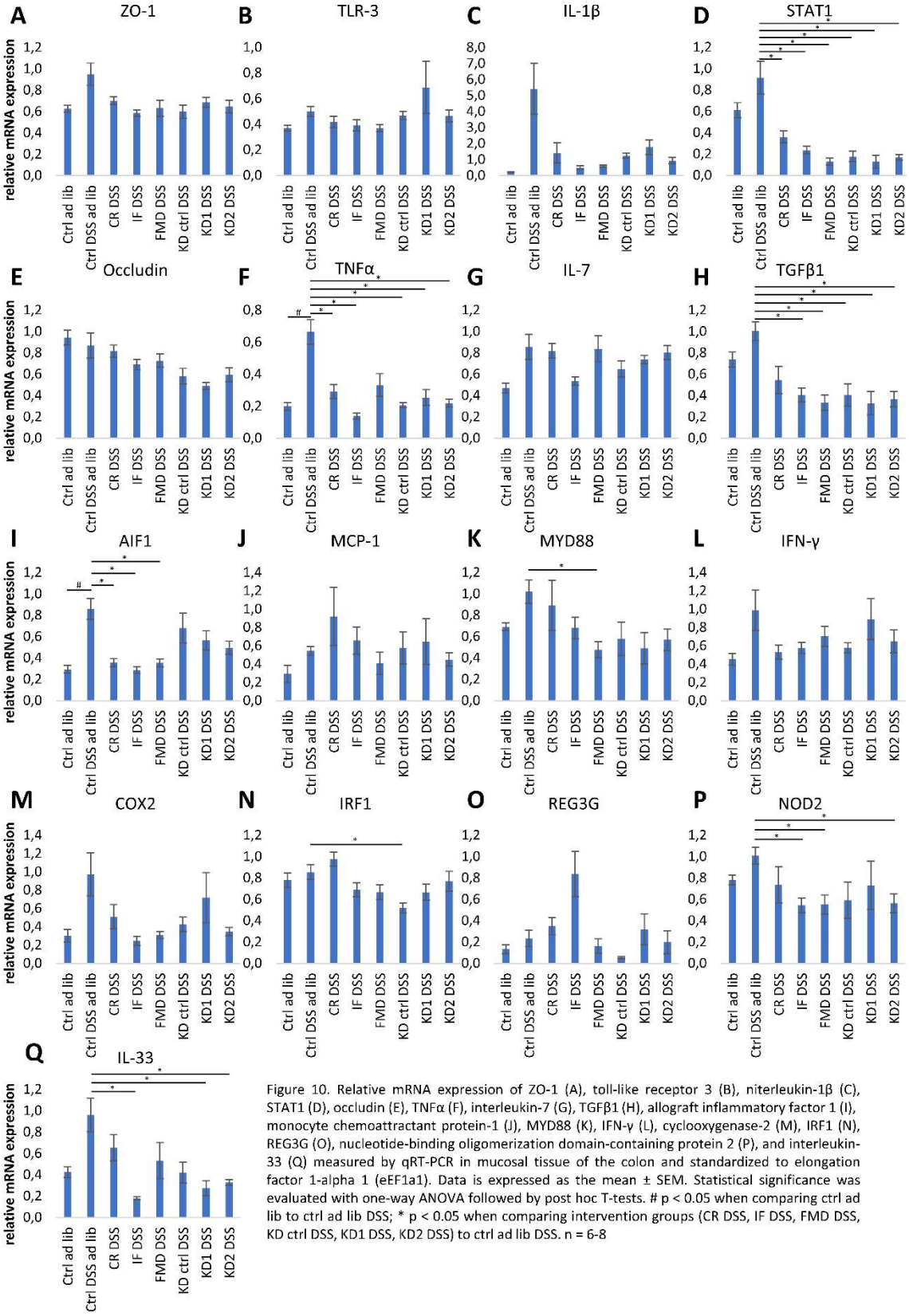


Figure 10. Relative mRNA expression of ZO-1 (A), toll-like receptor 3 (B), niterleukin-1β (C), STAT1 (D), occludin (E), TNFα (F), interleukin-7 (G), TGFβ1 (H), allograft inflammatory factor 1 (I), monocyte chemoattractant protein-1 (J), MYD88 (K), IFN-γ (L), cyclooxygenase-2 (M), IRF1 (N), REG3G (O), nucleotide-binding oligomerization domain-containing protein 2 (P), and interleukin-33 (Q) measured by qRT-PCR in mucosal tissue of the colon and standardized to elongation factor 1-alpha 1 (eEF1a1). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

in the two fasting groups, as well as the keto 2 diet (Figure 10P). For lymph nodes, the only change was observed for TLR-3, which had significantly lowered levels in the ctrl

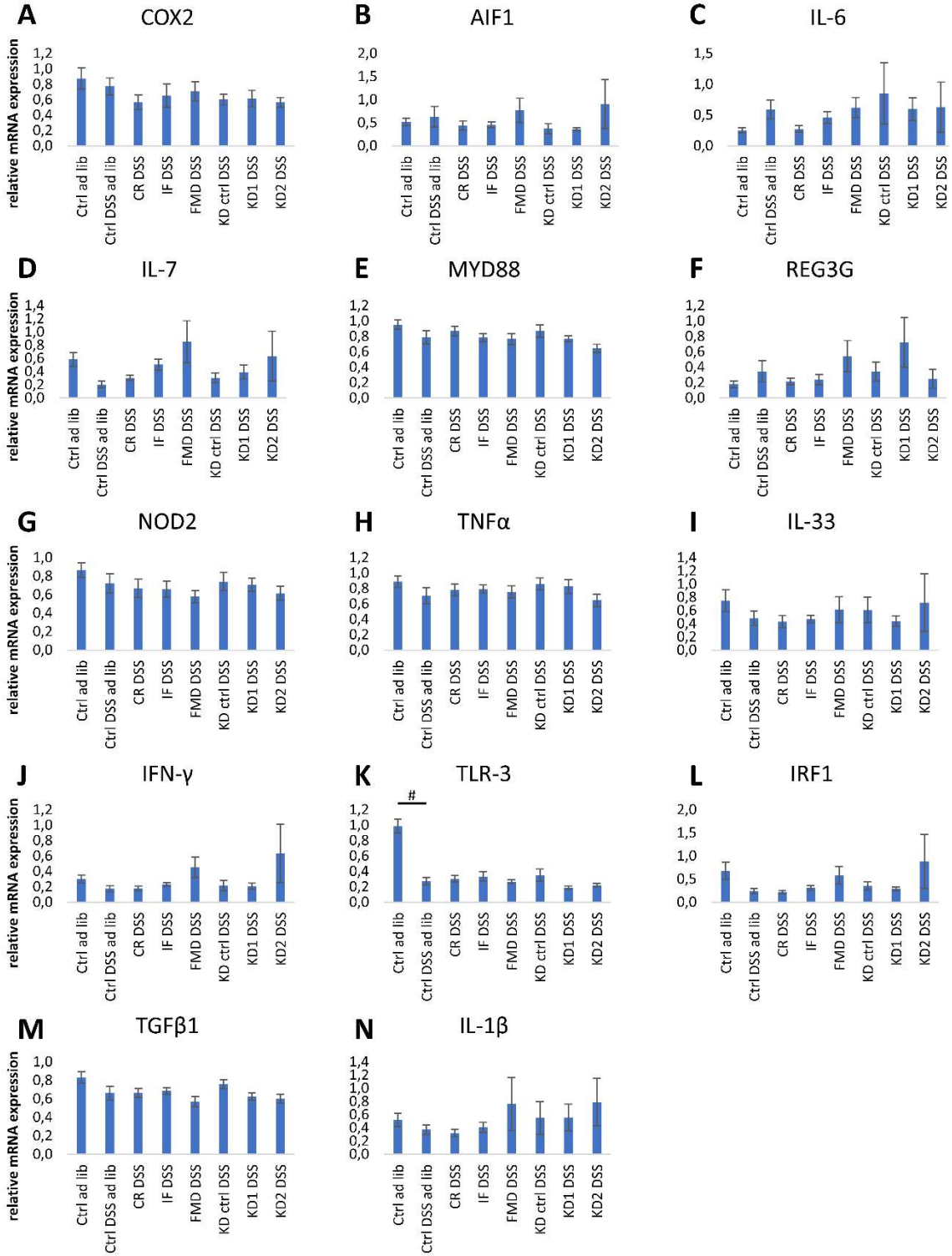


Figure 11. Lymph nodes were analyzed using qRT-PCR for COX2 (A), AIF1 (B), IL-6 (C), IL-7 (D), MYD88 (E), REG3G (F), NOD2 (G), TNFα (H), IL-33 (I), IFN-γ (J), TLR-3 (K), IRF1 (L),), TGFβ1 (M); and IL-1β (N). eEF1a1 was used as a housekeeping gene for standardization. Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

DSS ad lib group in comparison to ctrl ad lib (Figure 11). Similar to lymph nodes, Peyer's patches remained mostly stable when it comes to mRNA expression. NOD2 was

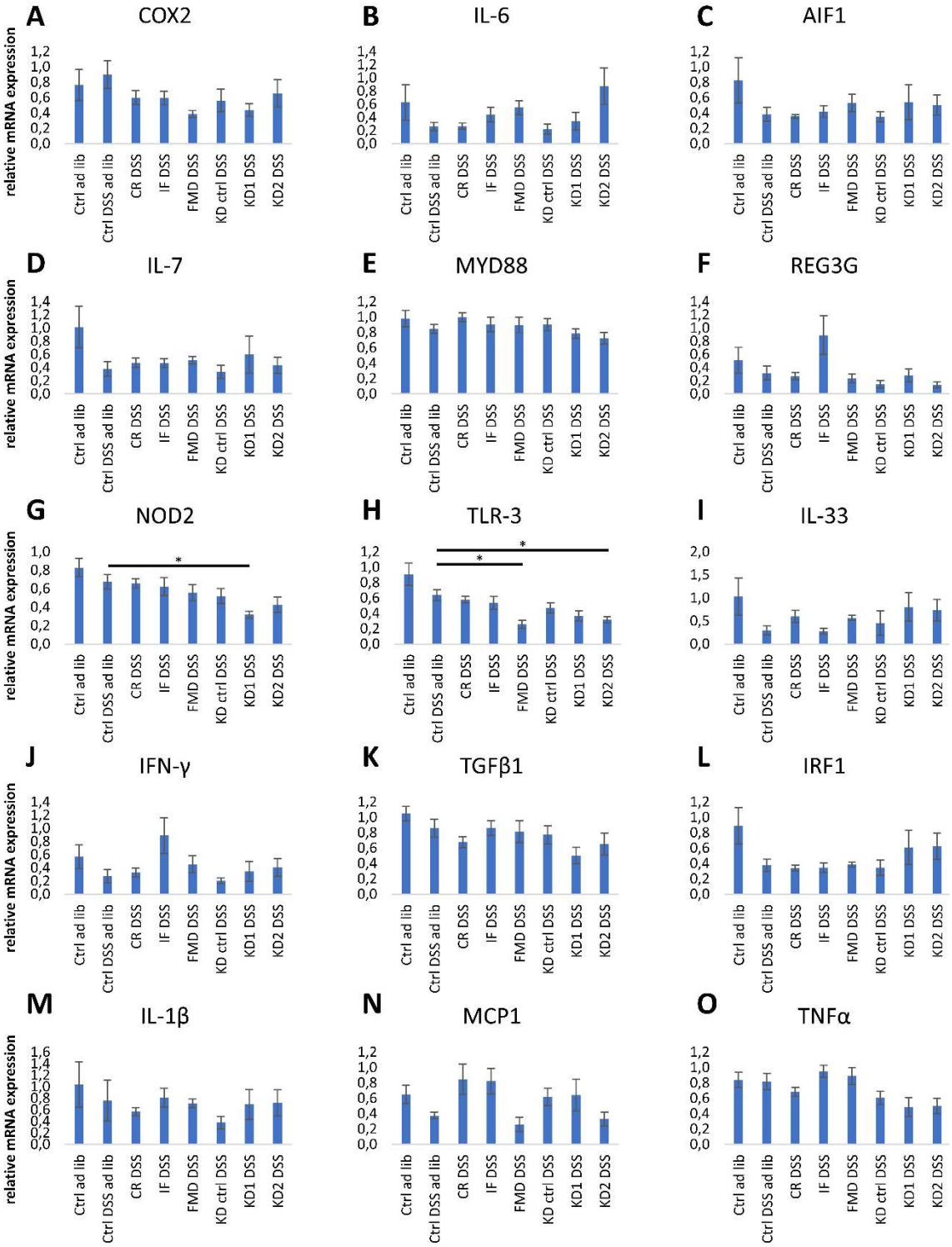


Figure 12. mRNA expression was measured in Peyer's patches for COX2 (A), IL-6 (B); AIF1 (C), IL-7 (D), MYD88 (E), REG3G (F), NOD2 (G), TLR-3 (H), IL-33 (I), IFN-γ (J), TGFβ1 (K), IRF1 (L), IL-1β (M), MCP1 (N) and TNFα (O). All genes were standardized to eEF1a1. Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

significantly lowered by keto 1, while TLR-3 was decreased in the FMD and KD2 groups (Figure 12).

3.7 Bile acid levels remained stable despite significant differences in diet composition

One-way ANOVA revealed significant differences between groups for TLCA, TUDCA, TCA, and DHCA but these differences were not within the scope of our comparison (ctrl DSS ad lib vs restrictive diet DSS groups), which was shown in the posthoc analysis. A trend of marginally increased levels of bile acids was observed for the mice that underwent caloric restriction, FMD, and the KD ctrl mice (Figure 13A, B). Keto 1 and keto 2 cluster in close proximity across both PLS 1 and PLS 2, which is similar to the ctrl DSS ad lib group. All the other groups do not seem to form defined clusters across either axis (Figure 13C). Even though it is not significant, caloric restriction seems to promote the formation of TLCA, TDCA, and TCA, while an increasing trend of LCA, DCA, CDCA, UDCA, and CA can be seen in FMD mice. Both keto 1 and keto 2 have a similar pattern which is very similar to the control DSS mice that did not undergo a restrictive diet intervention. This is in stark contrast to the keto control group which is a lot more similar to the CR and FMD pattern (Figure 13D).

3.8 Taurine, GSH, and their conjugates are mainly affected by fasting and caloric restriction

DSS alone does not have an impact on taurine, GSH, GT, or any other conjugate in the ileum as seen in the comparison of the ctrl ad lib and ctrl DSS ad lib groups. Taurine levels were increased by IF, FMD, and the keto-control diet. GSH remained unchanged (14A-D), as did the conjugates with a mass-to-charge ratio of 162>83, 299>124, and 404>143. CR, IF, FMD, and KD ctrl groups had significantly higher levels of 159>80, 484>124, and 520>124 conjugates. 170>124, 198>124, 249>124, 270>124, 308>124, and 372>124 were higher in the mice that underwent intermittent fasting, a fasting-mimicking diet, and the control keto diet. FMD exhibited higher levels of two conjugates (304>124, 342>124) in comparison to the DSS control group. GT was only increased in the KD1 group, while all diets except CR increased the amount of the conjugated with a mass-to-charge ratio of 291>124. 362>124 has shown higher levels in FMD and KD ctrl mice. All diets except KD1 significantly increase the amount of 388>124 conjugate but only the FMD, KD ctrl and KD1 mice experienced increases in the 429>124 conjugate.

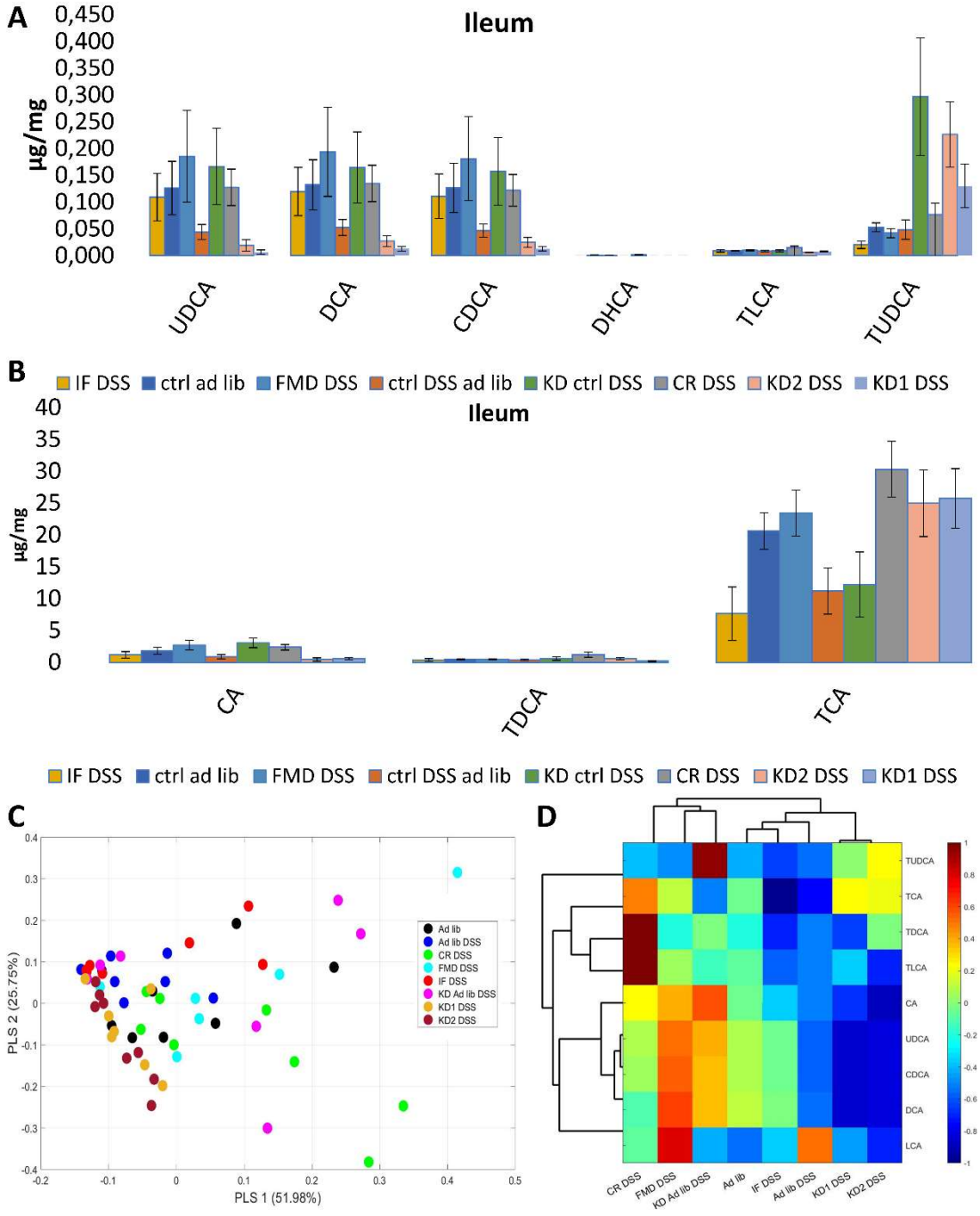


Figure 13. Primary and secondary bile acids were measured using LC-MS in mucosal samples of the ileum (A,B), a partial least squares models was performed (C) and a heatmap generated (D). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

Fasting mimicking and keto control exhibit a similar pattern which is contrasting with the patterns on the ctrl ad lib and ctrl DSS ad lib mice. Keto 1, keto 2, and CR are similar with the most notable difference being in the form of 429>124 and 299>124 conjugates

(Figure 14D). Distinct clustering can be observed for all groups across the PLS 1 axis (Figure 14E).

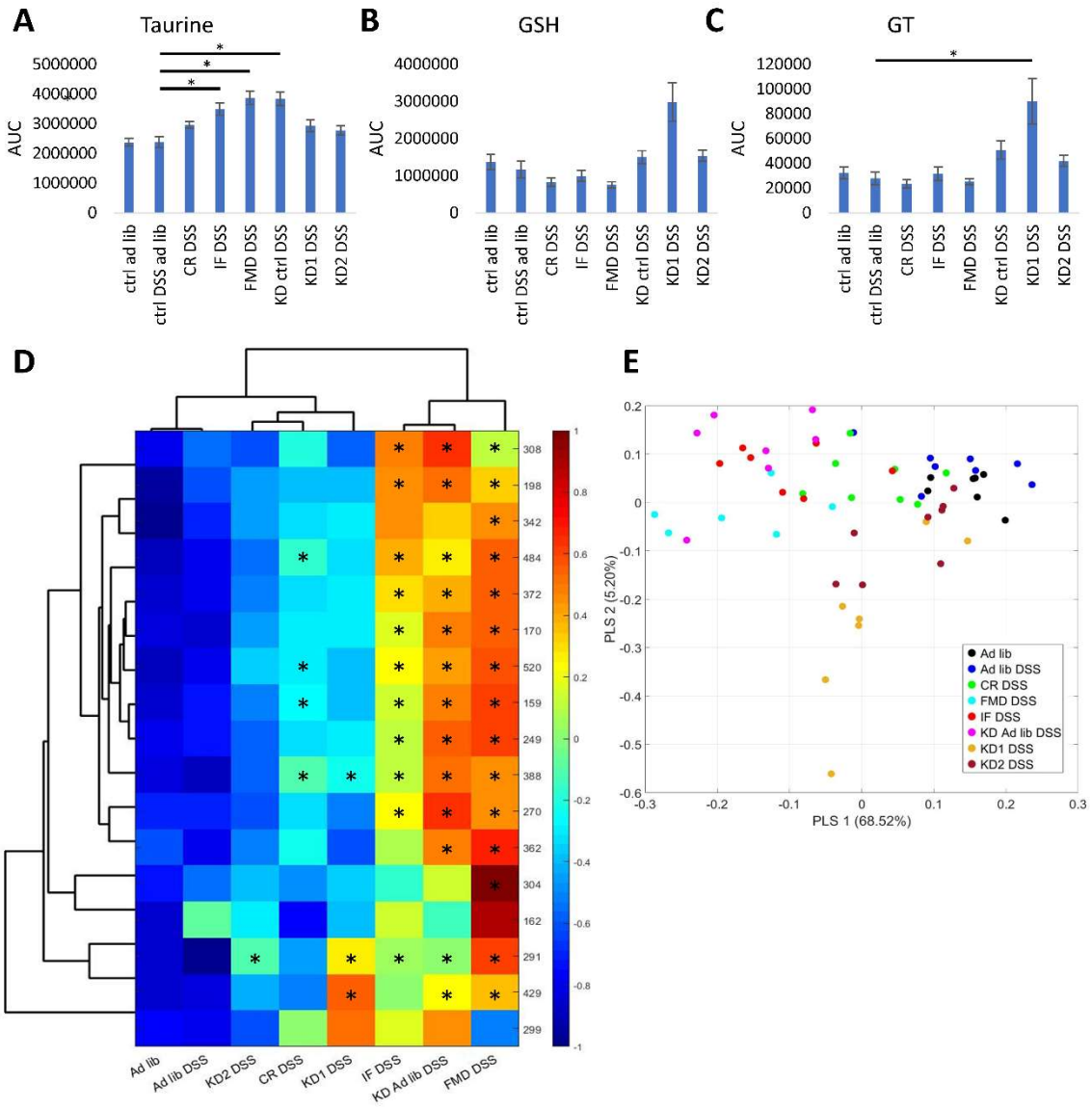


Figure 14. Taurine (A), GSH (B) and GT (C) were measured in mucosal tissue collected from the ileum. The partial least squares models shows group clustering (E) and the data is summarized in the heatmap (D). Data is expressed as the mean ± SEM. Statistical significance was evaluated with one-way ANOVA followed by post hoc T-tests. # $p < 0.05$ when comparing ctrl ad lib to ctrl ad lib DSS; * $p < 0.05$ when comparing intervention groups (CR DSS, IF DSS, FMD DSS, KD ctrl DSS, KD1 DSS, KD2 DSS) to ctrl ad lib DSS. n = 6-8

4. Discussion

Bodyweight loss has been established as one of the main markers for the monitoring and assessment of DSS-induced colitis and has shown a high correlation with disease activity in severity in most studies. 45 We have observed a significant reduction of BW when DSS was administered to mice that did not undergo a dietary intervention, proving

an effective induction of chemical colitis. Intermittent fasting, KD ctrl, and KD2 mice had similar body weight at sacrifice to the DSS control group, while CR, FMD, and KD1 have seen a significant reduction in their body weight. Caloric restriction was the only intervention where the caloric intake was strictly controlled and limited, which would explain the additional bodyweight loss beyond the effect of DSS. FMD has been reported to have temporary weight loss due to the nature of the protocol, but it was not associated with a reduced health outcome possibly due to the overcompensation during re-feed days as well as the maintenance of beneficial body composition, retaining lean body mass and modulating fat tissue towards a reduction of visceral fat. [46] Stable bodyweight prior to DSS administration has been reported for keto diets with conflicting data is seen after induction of colitis. [47,48] It should be noted that the KD1 group had the lowest average weight but also the highest variability between animals. In humans, a reduction of BW in UC patients seems to stem mainly from loss of skeletal muscle mass when body composition changes are analyzed using CT scans. Loss of muscle mass correlates with disease activity as well as malnourishment with patients being more likely to lose more muscle when they experience a more severe and/or active form of UC. [49] While muscle mass was not analyzed, KD1, CR, and FMD were the only interventions that did not increase the BW-adjusted amount of WAT. Cecum contents of KD1 and FMD mice were also significantly lighter than those of ctrl ad lib DSS mice. This would at least in part explain the significant reduction in BW, which would normally correlate with a far worse outcome than the one that was observed in our experiment. Considering the significant difference in body weight, an adjustment of other tissues in accordance with BW was necessary for comparison. Hepatobiliary manifestations are not uncommon in UC, with pharmacological treatments, namely sulfasalazine, increasing the risk of steatosis-induced hepatomegaly. [50–53] In a cohort of 158 individuals from Sothern Italy it was determined that 66% of enrolled IBD patients had hepatic steatosis. [54] With a median duration of about 5 years, roughly 30% of IBD patients will develop NAFLD. [55,56] All dietary interventions effectively reduced the BW-adjusted liver weight. This would suggest that any intervention in the form of a restrictive diet would be superior to a “normal” diet. Despite most mice being fed ad libitum, including the

DSS control, only restrictive interventions have seen a positive effect on liver weight suggesting that the composition, rather than the quantity of a diet might be the determining factor when it comes to the prevention of hepatic IBD comorbidities. Similar to hepatomegaly, splenomegaly, an enlargement of the spleen was observed, with DSS close to doubling the BW-adjusted spleen weight when compared to healthy mice. All dietary interventions, except KD ctrl managed to attenuate the DSS effect and maintained a normal spleen size. Enlarged spleens, by volume, have been observed in both types of IBD but only remained significant in CD when adjusted for body weight. The authors proposed that the proximity of the affected organs of IBD might exhibit an effect on other neighboring organs, inducing a pro-inflammatory effect and pathological changes. [57] Mice undergoing DSS treatment exhibit an enlarged spleen in addition to other morphological changes. In murine models, splenomegaly shows a positive correlation with the extent of inflammation and anemia.[43] Considering the proximity of the colon to the spleen and in-vivo predictions, this data would suggest that restrictive diets might be able to prevent multiple UC-related comorbidities of organs in close proximity to the colon. Colon shortening is a hallmark of DSS-induced colitis, and can also be observed in severe cases of human pancolitis. [43,58] While DSS did shorten the colon, compared to healthy mice, these differences were not significant. Contrary, the two keto diets, KD1 and KD2 had a significant impact on colon length, reducing its length significantly. This falls in line with a recent report. [48] Time-restricted fasting and intermittent energy restriction have been shown to rescue colon length in DSS colitis in addition to improving inflammatory markers. [59] We additionally examined the length of the small intestine, despite UC mainly affecting the colon. As expected, SI length remained largely stable, even between the DSS control and healthy mice. Interestingly, the intestine of the keto control mice was around 5cm shorter compared to the DSS control group. This difference is statistically significant but histological changes in the SI have not been regularly reported despite changes in permeability, absorption, and motility being observed in UC patients, especially in chronic and extensive cases. [60] Macrophages are antigen-presenting cells and one of the main MPO sources in the body. They play a crucial role in the pathology of IBD due to their ability to differentiate into

one of two phenotypical states. M1 macrophages are classically activated by IFN-γ or lipopolysaccharides through TLRs, and its downstream signaling involves MYD88. They promote the production of pro-inflammatory cytokines such as IL-1, IL-6, IL-12, and TNFα. As such they induce differentiation of inflammatory T cells, and phosphorylation of IKB, leading to the activation of NF-κB and its downstream signaling. Alternatively, activate, M2 macrophages differentiate after exposure to IL-4, IL-10, or IL-13. After activation, they enhance the production of IL-10 and IL-13 and play a crucial role in tissue repair. If intestinal homeostasis is disturbed, a shift toward pro-inflammatory (M1) macrophages is induced and they migrate toward the intestinal mucosa where they accumulate. [61–64] By measuring MPO levels in the proximal colon we indirectly assessed macrophage activity. Despite an increasing trend, there were no significant changes in DSS mice, compared to the ctrl ad lib group in units per µg of protein or units per mg of processed tissue. KD1 containing only LCTs, demonstrated similar levels to ctrl ad lib DSS mice, while KD2 with 25% MCTs had the lowest levels overall. MCTs have been reported to reduce the inflammatory response in 2,4,6-Trinitrobenzenesulfonic acid colitis by directly inhibiting the production of inflammatory cytokines from macrophages. [65] A decreased infiltration of macrophages into the crypt base was observed for time-restricted feeding, which could explain comparably low levels in the IF group.[66] While tight junctions play an important role in intestinal barrier function, the role of occludin is often contradicting. [67–70] On a translational level, occludin expression remained stable in the colon and jejunum across all interventions. No significant changes were observed between healthy and DSS-treated animals, with a trend toward higher levels of caloric restriction, specifically in the 50kDa and 56kDa variants. The expression of mRNA on the other hand has shown differences in the jejunum for FMD, KD1, and KD2 which had lower levels, while the expression remained stable in the colon for all groups. Additionally, the keto diets demonstrate generally lower protein levels which failed to reach significance but this observation would be in line with the limited, available data. [48] Cytokines are small proteins, usually 10–30 kDa in size, secreted by activated immune cells.[71] Analysis of the mucosal tissue collected from the proximal colon revealed that DSS induced an inflammatory phenotype with 28 out of 40 analyzed cytokines being

significantly increased. KD1, containing only LCTs significantly reduced the levels of 11/28 cytokines, outperforming other restrictive diets. Most notably KD1 reduced eotaxin-1, G-CSF, GM-CSF, as well as IL-6. Eotaxin-1 was first examined in 2002 in the venous blood of 35 UC patients. UC patients had significantly higher levels compared to healthy controls and oral corticosteroids, a second-line treatment for mild to moderate UC increased the levels even further.[72] Eotaxin-1, also known as CCL11 has been associated with non-beneficial long-term dietary factors in IBD patients such as high alcohol and coffee intake. [73] It has outperformed CRP, and fecal calprotectin as predictive markers on its own, while in combination with serum amyloid A (SAA) IL-6, IL-8, IL-17A, and TNF-α it demonstrated an optimism-adjusted area under the ROC curve of 0.84. [74] CCL11 deletion ameliorates DSS colitis by reducing body weight loss and histological injury, while a Mendelian randomization analysis of 159 UC patients has revealed a causal involvement of CCL11 dysregulation in the pathogenesis of ulcerative colitis. [75,76] Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are responsible for the differentiation of hematopoietic precursors, namely neutrophils, granulocytes, and macrophages. [77] GM-CSF has been revealed to preferentially induce pro-inflammatory, M1 macrophage differentiation. [78] This GM-CSF lowering, in combination with, lower mRNA expression of REG3G and the observed non-significant increase of MPO in the KD1 group might indicate a shift towards repair-oriented M2 macrophages, in return reducing systemic inflammation. Interleukin 6 consists of 4 α-helical bundles and three receptor binding sites. There are fundamental differences between human and murine IL-6, yet both species share two signaling pathways. Trans-signaling activation via the IL-6/soluble-IL-6R complex is present in most cells since it is dependent on gp130 which is expressed ubiquitously, while classic signaling is present only in a few cell types including macrophages, neutrophils, and hepatocytes, through the membrane-bound IL-6 receptor that is specific to those cell types. [79] Additionally, IL-6 has a dual function, acting as an anti-inflammatory cytokine in acute inflammation, and shifting its role to a pro-inflammatory modulator in chronic conditions such as UC where it primarily acts through trans-signaling since soluble-IL-6R mainly stems from macrophage surface shedding. The

shift towards a pro-inflammatory phenotype in chronic conditions could be explained through the anti-apoptotic effect of IL-6 on T-cells, which is eliminated by blocking trans-signaling. This observation is in line with T-cell apoptosis resistance that is seen in IBD patients. [79–82] Considering the turn-table function of IL-6 in UC, a reduced expression can have several downstream effects on other inflammatory markers which in sum lead to significant anti-inflammatory response. Contrary to KD1, intermittent fasting was the only dietary intervention to significantly impact the levels of IFN-γ, increasing them compared to DSS mice that did not undergo a dietary intervention, which contrasts with previous reports. [59,83] GM-CSF and IL-17 were also significantly increased, with IF producing the only significant change in IL-17 levels across all comparisons, despite IF being associated with a lowering effect in neuroprotective studies.[84] Platelet factor 4, a recently piloted IBD-activity biomarker has also seen an increase in IF mice, as well as FMD, potentially linking irregular eating patterns to a worse UC outcome but confounding factors such as repeated stress due to cessation of food availability in must be considered. [85] Despite not reaching significant differences, mainly due to extreme variability, TIMP1 is a point of interest considering its involvement with cell proliferation, growth, migration, cycle, and the role it plays in the development of colorectal cancer, as it is consistently overexpressed in UC-associated colorectal cancer. [86] Reduced epithelial recovery, through impaired repair in TNF-driven intestinal inflammation such as UC, has been well documented. [87] While DSS significantly reduced the villi length of the jejunum, all restrictive diets restored it, with KD1 and KD2 increasing the length well above the levels of healthy animals. These findings of restrictive diets having such effects are in line with previous experiments, all be it without the induction of inflammation. [88] While colon thickening cannot be used as a stand-alone diagnostic, it has it can be utilized in addition to other findings, especially in more progressive forms of the disease. [89] In humans, colon thickening is often non-specific and mostly explained through infection and diverticulitis, rather than UC. [90] A significant reduction of colon thickness was only seen in KD2, with a similar observation being made previously. [88] Still, this only strengthens the point that colon thickness is not a strong indicator of an overall inflammatory state given the fact other diets, that did not have decreased colon

thickness had far better inflammatory markers. mRNA expression of genes associated with inflammatory response has remained largely stable across jejunum mucosal tissue, lymph nodes, and Peyer's patches with a select few changes being mainly observed for KD1 and KD2. As UC is mainly affecting the colon, this would suggest that the positive effects of the dietary interventions on the transcriptome are local and that all benefits are directly related to local immune modulation, with the peripheral immune system in the form of lymph nodes and Peyer's patches remaining close to completely unaffected. Locally in the colon, signal transducer and activator of transcription 1 (STAT1) is linked to UC, with higher expression and activation being observed in patients compared to healthy controls. [91] All dietary interventions effectively downregulated STAT1, beyond the levels of healthy mice. While the protein array only revealed a significant decrease for KD1, mRNA expression levels were lower for all groups except FMD in the case of TNFα. The role of TNFα in inflammation has always been prominent, and ulcerative colitis is no exception. It has been one of the first therapeutic targets and remains a focus of therapy to this day with TNF-α blockers being effective in reducing serious adverse effects, managing symptoms, inducing remission short-term, and long-term remission, and healing the mucosa. [92] TGFβ1 is poorly understood in the context of UC with multiple interventions focusing on the activation of its pathways, and elevated levels being associated with active IBD, suggesting a defective regulation in patients of this anti-inflammatory factor. [93] While DSS did not increase the expression of transforming growth factor beta in comparison to ctrl ad lib mice, caloric restriction was the only intervention that failed to decrease mRNA expression measured with qRT-PCR, posing the question if CR could cause dysfunction of signaling. We analyzed bile acids in the ileum, the primary site of re-absorption using LCMS. Levels remained stable across all dietary interventions, and DSS by itself did not have a significant impact either. Analysis of concentration in feces would likely reveal similar patterns since only a small amount of bile acids reach the colon and ultimately exit the enterohepatic circulation. Bile acids have recently been postulated as an important factor in IBD, with upregulation of primary bile acids and downregulation of secondary bile acids being observed alongside regulatory effects on the mucosa, regulation of macrophages, dendritic cells,

and cellular processes such as apoptosis and autophagy. [94] Despite promising preliminary results, translation into clinical applications could prove to be difficult given mice have a significantly different bile acid pool compared to humans. [95] Data on taurine in the context of restrictive diets is very limited with only a handful of studies looking at CR, KD, and fasting, in mice, humans, and canines respectively, seeing increased levels. [96–98] On the contrary, interventions with taurine via drinking water or novel nanoparticles have improved inflammatory and injury markers across different models of chemically induced colitis. [99–102] FMD outperformed most diets but was closely followed by KD ctrl DSS and IF, all of which clustered in the upper left quadrant of the PLS regression, demonstrating similar patterns across all conjugates. CR had a lesser impact than expected while DSS controls and healthy mice had close to identical patterns, indicating dietary patterns, mainly in the form of temporal variation, rather than chemical colitis are the main driving factors in the modulation of taurine levels.

5. Conclusion

This is the first attempt at analyzing and comparing multiple restrictive diets in a mouse DSS colitis model. Overall, restrictive diets have a predominantly positive effect on chemical colitis when looking at inflammatory cytokines, genes, and histopathological changes. All changes seem to be limited to local effectors, with the peripheral immune system in the form of lymph nodes and Peyer's patches remaining unaffected. Keto diets, mainly KD1, exclusively containing long-chain triglycerides, seemed to outperform others by effectively downregulating pro-inflammatory markers such as eotaxin-1, G-CSF, GM-CSF, IL-6, IL-7, KC, LIX, MCP-1, MIP-1a, and TNFα. Considering markers for intestinal permeability, pathogen recognition, and their downstream signals remained stable, while expression of macrophage-related factors indicated a shift towards alternatively activated, anti-inflammatory (M2) macrophages, the positive changes could be associated with a local change in macrophage composition. Given the incomplete understanding of UC and limited data on the effects of dietary interventions in IBD patients, further research is needed to fully understand the underlying mechanism of the reported effects and their translation into clinical use.

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