VILNIUS UNIVERSITY
MEDICAL FACULTY

The Final thesis

Impact of Non-Alcoholic Fatty Liver Disease, Gut and Intrahepatic Microbiota on the Risk ofMetachronous Colorectal Liver Metastases

Student Sapir **Eliav, VI year, VI group**

Department/ Clinic (where the defense procedure will be taking place) **Institute of Clinical Medicine Clinic of Gastroenterology, Nephrourology and Surgery**

Supervisor Assoc. Prof. Aistė Kielaitė-Gulla

The Head of Department/Clinic Prof. habil. Kęstutis Strupas, MD, PhD

2023

Student's email: sapir.eliave@gmail.com

SUMMARY

This literature review explores the impact of the Ketogenic Diet nutritional strategy, on colorectal liver metastasis (CRLM) factors and CRLM itself. It compares the effects of high-fat ketogenic versus standard diets on non-alcoholic fatty liver disease (NAFLD) and gut microbiome.

Despite progress in cancer diagnosis and treatment, colorectal cancer (CRC) mortality remains high, with liver metastasis being a leading cause of death. The ketogenic diet, traditionally used for pediatric epilepsy, has gained popularity for managing gastrointestinal inflammation, metabolic diseases, and potentially cancer treatment. Recent studies reveal ketone bodies' anti-tumorigenesis effects, though the full benefits of ketosis are uncharted.

Interestingly, the literature suggests that some of the metabolic and anti-tumorigenic effects of the ketogenic diet are age and sex dependent, favoring middle-aged healthy males. However, implementing the ketogenic diet can be challenging, especially for vulnerable advanced cancer patients. Ketone body supplementation is being investigated as an alternative to a restrictive diet. However, there is a lack of experimental data on how to adapt the ketogenic diet to adults, and it is even harder to adapt it to advanced cancer patients. Furthermore, the popularity of the ketogenic diethas led to an abundance and skewed literature. To address these issues, we conducted a literature review using inclusion and exclusion criteria for the studies reviewed.

Informed by the research presented in this study, a manuscript has been crafted and submitted to the "Visceral Medicine" journal.

Keywords: ketogenic diet, CRLM treatment, gut microbiome and CRLM, BHB supplements, NAFLD and CRLM, gut microbiome and KD, NAFLD and KD, metachronous CRLM, and KD adverse effects.

TABLE OF CONTENTS

INTRODUCTION

Colorectal cancer (CRC) is a leading global health challenge, with liver metastases being a critical determinant of disease progression and survival outcomes [1, 2, 4, 14, 17, 19]. The management of colorectal liver metastasis (CRLM) presents a complex challenge, as it is influenced by a multitude of factors, including the tumor microenvironment, liver's pre-metastatic niche, gut and intrahepatic microbiota, and non-alcoholic fatty liver disease (NAFLD), in addition to the extensively studied factors such as epidemiological variants, age, gender, obesity, and other comorbidities [1, 2, 4, 14, 17]. Recent advancements have enabled a deeper understanding of these interactions and the potential for novel diagnostic and therapeutic approaches. Among these, the ketogenic diet (KD) has emerged as a potential intervention for inhibiting cancer growth and proliferation [7, 11, 12, 20]. However, its role in CRLM management and its impact on NAFLD and microbiota remain topics of ongoing debate.

This literature review **aims** to comprehensively investigate the intricate relationships between the tumor microenvironment, liver's pre-metastatic niche, gut and intrahepatic microbiota, NAFLD, and the ketogenic diet, and their implications on CRLM management and treatment. In doing so, it will address **the research hypotheses** stating that the ketogenic diet is a protective factor for either of the two risk factors for CRLM (NAFLD and intrahepatic microbiota) and\or directly protects against CRC.

The **objectives** are:

1. Synthesize the knowledge of current understanding of the pathophysiology and management strategies for CRLM;
2. Examine the impact of the ketogenic diet on CRLM, its mechanisms, efficacy, and safety;
3. Explore the role of gut and intrahepatic microbiota and NAFLD in the context of CRLM;
4. Evaluate the ketogenic diet as a protective factor for CRLM and its potential direct effects on CRC;

The literature review is structured as follows: Chapter 1 will explore the pathophysiology and current management strategies for CRLM, Chapter 2 will delve into the impact of the ketogenic diet on CRLM, its mechanisms, efficacy, and safety, followed by a discussion and conclusions.

Methodology. The materials and methods for this research thesis consist of English-language articles published within the last 5 years, adhering to specific citation and impact factor criteria for selection. To be included in the review, articles must meet the following requirements: i) cited a minimum of three times, ii) if published in a journal, the journal must have an impact factor of at least 2.5, iii) if condition ii is not met, the article should have over 25 citations, and iv) if condition i is not satisfied, the article can still be selected if published in a journal with an impact factor of 2.5 or higher.

An exception (article no. 13) was made due to the limited availability of cross-sectional studies on the adverse effects of the ketogenic diet.

The final selection of studies encompasses 8 overviews, 4 literature reviews, 4 laboratory studies (in vivo and in vitro), 3 clinical trials, 1 cohort study, 1 systematic review, 1 retrospective cross-sectional study, and 1 case series. These studies were selected by examining their full-text content in **PubMed** and **ScienceDirect databases**, while the research keywords were searched within these databases using both abbreviated and non-abbreviated terms.

1. COLORECTAL LIVER METASTASIS: PATHOPHYSIOLOGY TO MANAGEMENT

1.1 Determinants of Synchronous and Metachronous CRLM

Synchronous vs. metachronous CRLM. Synchronous colorectal liver metastasis (CRLM) refers to the simultaneous detection of colorectal cancer (CRC) liver metastases with the primary tumor, while metachronous CRLM refers to the detection of CRC liver metastases after the primary tumor has been treated, even years after [2]. These processes are influenced by various factors, such as alterations in the gut microbiome, which can affect the development and relapse of CRC. Some bacteria have been found to favor the conditions for developing CRC liver metastasis, while others have been shown to restrict it. Fatty liver can facilitate dormant colon disseminated tumor cells (DTCs) and create a favorable microenvironment for them to escape immune surveillance. The role of the gut microbiome in the occurrence of non-alcoholic fatty liver disease (NAFLD) and its relationship with CRC metastases is not yet fully understood, and more research is needed in this area [1, 2, 14, 17, 18, 20].

In CRLM, occurs either synchronously or metachronously in about 30% of cases [2]. Engstrand et al. [2] found that the timing of the diagnosis/operation of the primary CRC is a suitable cutoff point for defining metastasis as synchronous or metachronous. Resectability is a crucial determinant of survival in CRC [1, 2, 14, 17, 18, 20, 23].

The pathophysiology of colorectal liver metastasis (CRLM) is intricate, involving multiple drivers and factors [1, 3, 4, 14, 19]. While a detailed description of the complex pathophysiology of CRLM is beyond the scope of this paper, certain key players will be discussed. In brief, the process involves a small subset of CRC cells that can evade the primary tumor, migrate through the extracellular matrix (ECM), intravasate and extravasate, and ultimately colonize the liver, giving rise to CRLM [4, 14, 17]. (See Figure 1)

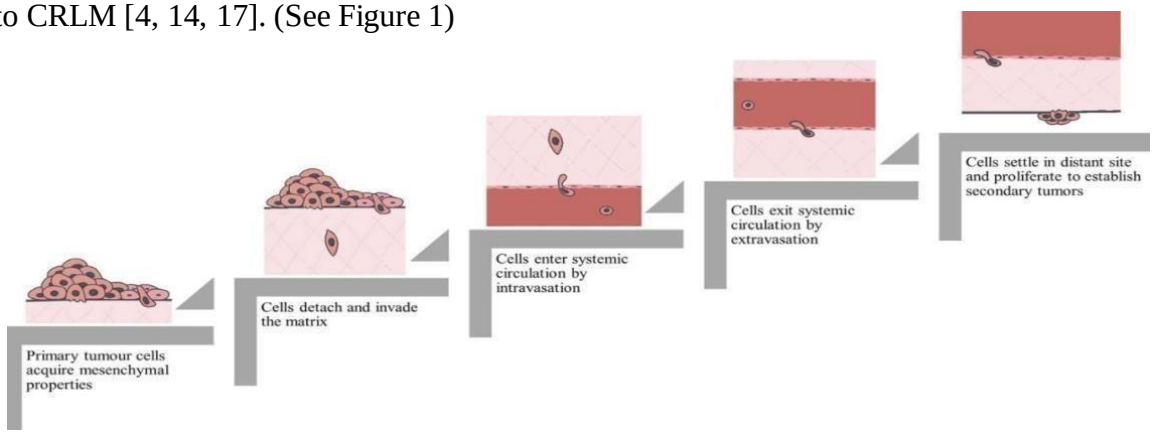

Figure 1. Simplified Process of Metastasis. (Patel et al., 2022[4])

According to the "seed and soil theory," tumor cells metastasize to a supportive environment, such as the liver [1, 4, 14, 17]. The liver's immune microenvironment plays an important role, with

Kupffer cells (KC) and T regulatory cells (Treg) being essential in immune regulation [5, 14, 17]. Targeting chemokines CCL2/CCR2 and inhibiting Treg can reduce metastasis [14]. Neutrophil extracellular traps (NETs) contribute to the formation and development of CRLM by secreting high mobility group box 1 (HMGB1) inflammatory proteins, promoting the migration and invasion of CRC cells, and participating in a positive feedback loop with interleukin 8 (IL-8) [14]. This loop promotes the development of CRLM, suggesting the role of NETs in post-resection recurrence of the disease [14].

Cytokines, chemokines, exosomes, and key signaling pathways play significant roles in the progression of CRLM and cancer development. Interleukin-6 (IL-6) and Interleukin-33 (IL-33) are known for their proinflammatory and procarcinogenic properties [1, 14, 19]. Dysregulated chemokines contribute to liver metastasis and angiogenesis (e.g., CCL9-CCL15-CCR1, CCL2-CCR2, CXCL8-CXCR2 signaling, and CXCL1-CXCR2 signaling) [14]. Exosomes in CRC patients promote a premetastatic niche by altering immunosuppression, vascular leakage, and inflammation [14]. Key signaling pathways implicated in CRLM include Hepatocyte growth factor/c-Met (HGF/c-Met), Phosphatase of regenerating liver (PRL3), Transforming Growth Factor β (TGFβ), Notch, and several other pathways [14]. Genetic factors, such as mutations in BRAF, KRAS, PI3KCA, NRAS, SMAD, and NOTCH, also influence CRLM [11, 14, 17, 23]. Additionally, demographic factors like sex and race impact the likelihood of developing CRC and liver metastasis [2, 14].

The microenvironment can affect immune cells and genetic mutations, such as SMAD4, which is present in 62% of CRC patients and enhances tumor cell migration and invasion [14, 17]. Lgr5, a reserve stem cell reservoir, is in the intestinal crypts and replenishes all epithelial cells. When epithelial integrity is at risk, Lgr5- will convert to Lgr5+. Lgr5 is considered the initial “seed” for both CRC and CRLM [7, 11, 12, 14], playing a crucial role in the initiation of CRC and CRC metastasis, replenishing all epithelial cells [1, 7, 11, 12, 14]. Disseminated tumor cells/circulating tumor cells (DTC/CTCs) possess prognostic value in colorectal liver metastases (CRLM) and recruit cancer stem cells, contributing to metastasis [1, 4, 11, 14, 16, 17]. Targeting Lgr5 and DNA methylation may offer potential therapeutic approaches [7, 11, 12, 14]. DTC/CTCs exhibit prognostic value in colorectal liver metastases (CRLM) and contribute to metastasis through recruiting CSCs and polarized Kupffer cells, which secrete interleukin-6 and activate the JAK2/STAT3 pathway [1, 4, 11, 14, 16, 17]. The fatty liver's vulnerability and associated inflammation may support the pre-metastatic niche (PMN), CRC cell dormancy, and colonization of circulating cells onto the liver in a metachronous setting [1]. The multifactorial nature of NAFLD development and its connections to metabolic disorders, gut microbiota, obesity, inflammation, immune cells, DTCs, and CSCs contribute to CRC and CRLM establishment.

Angiogenesis is a critical step in cancer initiation and metastasis, mediated by factors like VEGF, FGF, TNF-α, and TGF-β (vascular endothelial growth factor, fibroblast growth factor, tumor necrosis factor alpha and transforming growth factor beta) [1, 4, 8, 10, 12, 14, 17-19]. Metachronous CRLM diagnosis includes cancer marker screening, genetic mapping, and imaging modalities. RAS, BRAF, and MSI/MMR (microsatellite instability\ DNA mismatch repair) testing have prognostic and treatment-guided values [14, 17, 23]. Imaging techniques like liver ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography-CT (PET-CT) are used for surveillance and diagnosis [12, 17, 23]. The "cancer immunogram" combines techniques like immunoscore, tumor genomics analysis, and liquid biopsy for personalized therapy and prognosis estimation [17].

1.2 Current treatments for CRLM

The prognosis for CRLM patients has significantly improved over the past few decades, leading some experts to consider CRLM a chronic disease rather than an acute relapsing condition [17]. Current treatments for CRLM include lifestyle and wellness guidelines, surgical intervention, adjuvant approaches, liver transplantation, radiation therapy, ablation techniques, systemic management, and experimental modalities [1, 4, 6, 14, 17-20, 23]. Furthermore, RAS mutations, such as KRAS and NRAS, serve as negative prognostic indicators and may influence treatment tolerance [23].

National Comprehensive Cancer Network (NCCN) guidelines for CRLM patients suggest regular screenings, healthy weight, moderate exercise, daily aspirin, plant-based diet, avoiding alcohol and smoking, and consistent doctor visits [23].

Surgical intervention remains the primary curative option, with patient suitability for surgery or systemic therapy determined by various factors such as oncological circumstances, future liver remnant (FLR) preservation, resection margins(R0), and surgical feasibility [14, 17, 23]. Techniques such as portal vein embolization (PVE) and portal vein ligation (PVL) can be used to enhance FLR [17, 23].

Alternative approaches like liver transplantation face challenges like donor availability and high recurrence rates [17, 23]. Radiation therapy and ablation techniques may offer favorable outcomes when safe surgical resection is unattainable or as adjuncts to surgery [14, 17, 23].

Systemic management involves the complex selection of chemotherapy and other therapies, with commonly used agents including FOLFOX (Folinic acid, fluorouracil[5-FU] and oxaliplatin), FOLFIRI ((Folinic acid, 5-FU, irinotecan), XELOX (capecitabine plus oxaliplatin), infusional 5-FU/LV, capecitabine, and FOLFOXIRI (fluorouracil, oxaliplatin, and irinotecan) [14, 17, 23]. Antiangiogenic therapy with anti-EGFR antibodies (bevacizumab, panitumumab, and cetuximab) can

improve resectability but may face resistance [14, 17, 23]. Chemotherapy and systemic modalities can cause adverse effects, including immune suppression and chemotherapy-induced liver injury (CLI) [1, 4, 6, 14, 17-20, 23].

Immunotherapy can be combined with other modalities, involving immune checkpoint antibodies targeting T cell inhibitory receptors like PD1 and CTLA-4 [1, 4, 12, 14, 16, 17, 19]. A multidisciplinary team is essential for individualized decision-making when combining therapies [14, 17, 19, 20, 23].

Experimental modalities under investigation include irreversible electroporation (IRE), isolated hepatic perfusion (IHP), stereotactic body radiotherapy (SBRT), yttrium-90 (Y-90), COX inhibitors, renin-angiotensin system (anti-RAS) with bevacizumab, photodynamic therapy (PDT), and nanotechnology [14, 17, 23].

1.3 Gut Microbiota, NAFLD, CRLM, and KD Effects

The gut microbiota has gained considerable attention in recent years due to its significant influence on CRC development, inflammation, immune system modulation, epigenetic regulation, and treatment responses [1, 3, 4, 8, 9, 14] (see Figure 2, which demonstrates the involvement and role of gut microbiota in the development of CRLM). Factors such as genetics, geographical location, and obesity shape the highly individualized gut microbiome [3, 4, 8, 9]. Dysbiosis, an imbalance in the gut microbiota, has been associated with CRLM development [1, 3, 4, 14]. Dysbiosis can manifest as a disruption in the region-specific healthy microbiome population (beta diversity) [8] or a reduction in the overall richness of strains (alpha diversity) [1, 3, 4, 8, 9, 14]. Further research is needed to determine the full extent of dysbiosis' impact on CRLM and the specific roles of various microbiota.

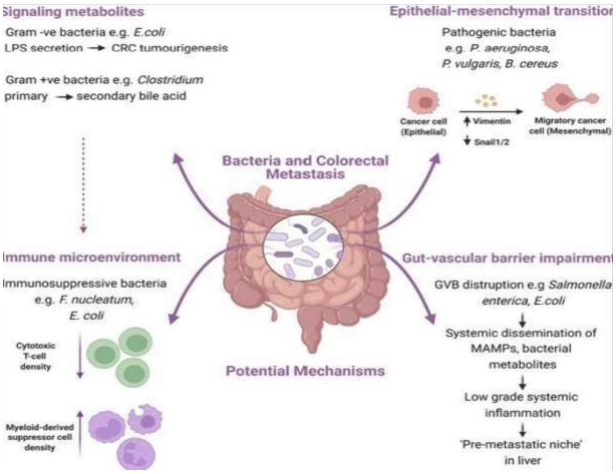

Figure 2. Potential Mechanisms of CRLM Involving Gut Microbiota (Patel et al., 2022)

NAFLD is a recognized risk factor for CRC and CRLM [1, 2, 14, 17, 18, 20]. The composition of the gut microbiota and its metabolites can influence the occurrence of NAFLD and its relationship with CRC metastases [1, 2, 14, 17, 18, 20]. For example, short-chain fatty acids (SCFAs) possess

anti-inflammatory properties that may counteract the CRC-promoting environment [1, 4, 8, 9, 15]. Conversely, ammonia, a byproduct of amino acid fermentation, is thought to be pro-carcinogenic due to the generation of reactive oxygen species (ROS) and subsequent inflammatory responses [4]. This evidence supports the recommendation of a plant-based diet to reduce meat consumption [23].

Considering the intricate interplay between gut microbiota, NAFLD, and CRLM, further investigation into the potential impact of the ketogenic diet (KD) on these factors is warranted. Gaining a better understanding of how KD may modulate the gut microbiome, help prevent or manage NAFLD, and influence CRLM development could offer valuable insights for CRC prevention and treatment strategies [1, 3, 4, 8, 9, 14].

SCFAs, anti-inflammatory metabolites derived from dietary fiber fermentation, exhibit potential antitumorigenic properties [1, 4, 8, 9]. SCFAs, such as propionate, acetate, and butyrate, can inhibit histone deacetylase (HDAC), which may contribute to reducing CRC burden [1, 7, 11, 12, 19].

Disruption of the gut vascular barrier (GVB) can lead to low-grade inflammation and the formation of pre-metastatic niches [4]. Elevated plasmalemma vesicle-associated protein (PV-1) levels are linked to increased pathogenic bacterial growth in metachronous CRLM patients. Dysbiosis, specific bacteria, and various CRC and CRLM treatment modalities can negatively affect GVB integrity [4]. Improved understanding of the GVB may help develop strategies to protect against undesired gut microbiota influences.

The role of gut microbiota in cancer treatment efficacy and adverse effects is an area of growing interest [4, 8]. A study involving 2,283 CRC patients from multiple countries investigated the impact of probiotics on CRC chemotherapies, but due to individual variability, no optimal bacterial composition was identified [4, 8]. The literature presents conflicting views on specific phyla considered beneficial or harmful, and the effects of three selected bacteria are presented in Table 3.

An experimental trial by Yuan et al. [3] conducted an in vivo and in vitro study using 60 male mice to examine the effects of bacterial strains from the Firmicutes and Bacteroides phyla on CRLM. The results demonstrated that Bacteroides vulgatus (B. vulgatus) levels were inversely related to CRLM nodules ($P=0.041$), while Proteus mirabilis (P. mirabilis) populations positively correlated with liver nodules ($p<0.001$) and negatively with Kupffer cell (KC) expression ($P=0.028$) (see Figure 3).

These findings emphasize the involvement of (KCs and other mediators, such as interleukin-17 (IL-17), in CRLM pathophysiology. The relationship between gut microbiota, CRLM, and KCs suggests that immunological factors play a vital role in mitigating the impact of CRLM. Further research is needed to better comprehend and utilize these relationships for developing more effective prevention and treatment strategies for CRC and CRLM.

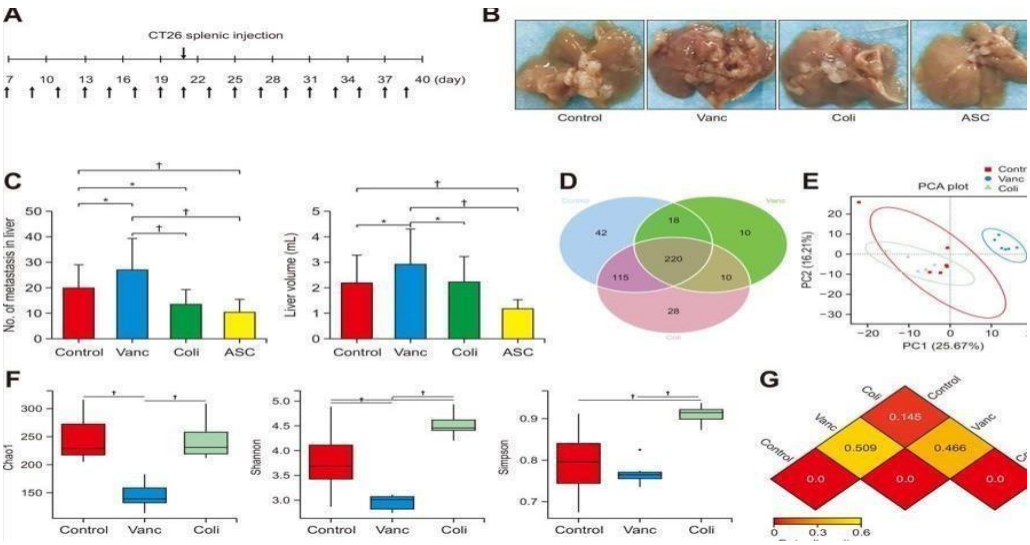

Figure 3. The Effect of Either Colistin or Vancomycin on Gut Microbiota. (Yuan et al., 2022[3])

1.4 The Interconnected Pathophysiology of NAFLD to CRLM

NAFLD is correlated with CRC and other metabolic conditions, including obesity, diabetes, and hyperlipidemia [1, 4, 5, 7, 9, 11, 19, 20]. NAFLD can progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), increasing susceptibility to CRC [1, 5, 15]. The pathophysiology of NAFLD development and its progression to CRLM remains complex and not entirely understood.

Obesity, inflammation, immune cells, endoplasmic reticulum (ER) stress, DTCs, and CSCs contribute to NAFLD and CRLM development, with the Western diet and obesity linking NAFLD and cancer [1, 5, 9, 12, 13, 15-17, 18-22]. Obesity-related factors, including increased fat deposition, decreased lipolysis, and enhanced lipogenesis, also affect NAFLD pathophysiology [1, 4, 5].

Inflammation is a critical factor linking metabolic disorders and cancers, including CRC and CRLM. Chronic, low-grade inflammation characterizes both obesity and NAFLD [1, 5], leading to insulin resistance [1, 5, 9] and promoting oncogenesis [1, 4, 6, 8, 11, 12, 15-20]. Elevated levels of inflammatory markers like IL-6, C-reactive protein (CRP), and TNFα are observed in NAFLD patients [1, 5]. Inhibiting these inflammatory markers, signaling mediators, or cells is crucial for ameliorating NAFLD and impeding CRC and CRLM prognosis.

DTCs and cancer stem cells CSCs play a significant role in CRLM and recurrence [1]. The vulnerable fatty liver and accompanying inflammatory state are believed to support the pre-metastatic niche (PMN), CRC cell dormancy, and colonization of circulating cells onto the liver in a metachronous setting [1].

In conclusion, the interconnected pathophysiology of NAFLD to CRLM involves complex relationships between obesity, inflammation, immune cells, DTCs, and CSCs. Understanding these

interactions is essential to develop more effective prevention and treatment strategies for CRC and CRLM.

2. THE KETOGENIC DIET AND ITS IMPACT ON COLORECTAL LIVER METASTASIS

2.1 KD, Variants, Ketone Production, and Ketosis Definition

While no specific diet regimen has been conclusively proven to effectively treat or prevent colorectal cancer (CRC) or colorectal liver metastases (CRLM) to date, the ketogenic diet is an emerging area of research with potential implications for CRC and CRLM management and treatment. Conditions resembling starvation, such as those experienced during the KD, have been shown to inhibit glycolysis and deprive cancer cells of glucose, thereby opposing the Warburg effect—a phenomenon in which cancer cells rely predominantly on glycolysis for energy production, even in the presence of oxygen [6, 8-11, 13, 16, 20-22].

This dietary strategy, first introduced by Dr. Russel in 1922 [6, 8-11, 13, 16, 20-22], induces a metabolic state known as ketosis, wherein ketone bodies are produced via the oxidation of fatty acids. As the body's glucose reservoir becomes depleted, gluconeogenesis occurs, converting glucose into triacylglycerols (TAG) or utilizing existing TAG if abundant, as observed in individuals adhering to a Western diet.

The literature presents some controversy surrounding the onset of the ketosis state. While certain authors base the onset on carbohydrate restriction (e.g., consuming less than 40-50 grams/day of carbohydrates) [6, 8, 9, 18], others rely on direct measurements of ketone bodies in the blood using a device like a glucometer employed by diabetic patients [8, 10, 18, 20]. The threshold levels of ketone bodies indicative of ketosis are not universally agreed upon, though it is generally estimated that levels of ≥ 0.5 mmol/L suggest ketosis [7, 10, 11].

The ketogenic diet (KD) is known to mimic starvation conditions and has gained popularity in recent years for managing various metabolic disorders. This can be attributed to its ability to regulate glucose and insulin levels, as well as the rapid weight loss observed within days of initiating the regimen [6, 8-11, 13, 16, 20-22]. Due to the inability of fatty acids to cross the blood-brain barrier (BBB), ketone bodies serve as the primary energy source for the brain under such conditions [6, 7, 11, 12, 21].

In addition to being a more efficient energy source than glucose, ketone bodies exhibit a range of other functions, such as antioxidant and anti-inflammatory properties [6, 7, 11, 12, 18, 20]. They also play a role in immune signaling, mitochondrial support, and have recently been implicated in the

emerging field of anti-tumorigenic and anti-metastatic properties, particularly in relation to beta-hydroxybutyrate (βHB) [6, 7, 10-12, 18-20].

Ketone bodies, particularly acetoacetate and β-hydroxybutyrate (βHB), are generated in the liver's mitochondria when glucose is scarce. They function not only as an alternative fuel source but also as agents in anti-inflammatory and anti-oxidative processes, and gene expression. The process begins with beta oxidation generating acetyl-CoA (AcCoA). If the citric acid cycle is low in oxaloacetate, AcCoA stimulates the production of ketone bodies [6, 8-11, 13, 16, 20-22], which is mediated by 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) [8, 19].

The enzyme succinyl-CoA:3-ketoacid CoA transferase (SCOT) facilitates the use and breakdown of ketone bodies, which are absent in liver cells, enabling ketone bodies to leave the liver and supply energy to the rest of the body [19]. Once outside the liver, ketone bodies can revert to AcCoA for energy production. Moreover, ketone bodies can interchange via the βHB dehydrogenase enzyme and the reduction of nicotinamide adeninedinucleotide (NAD+) to NAD+H (NADH). The NADH produced can then be used in other cellular processes, such as the electron transport chain, where it is oxidized back to NAD+ as illustrated in Figure 4.

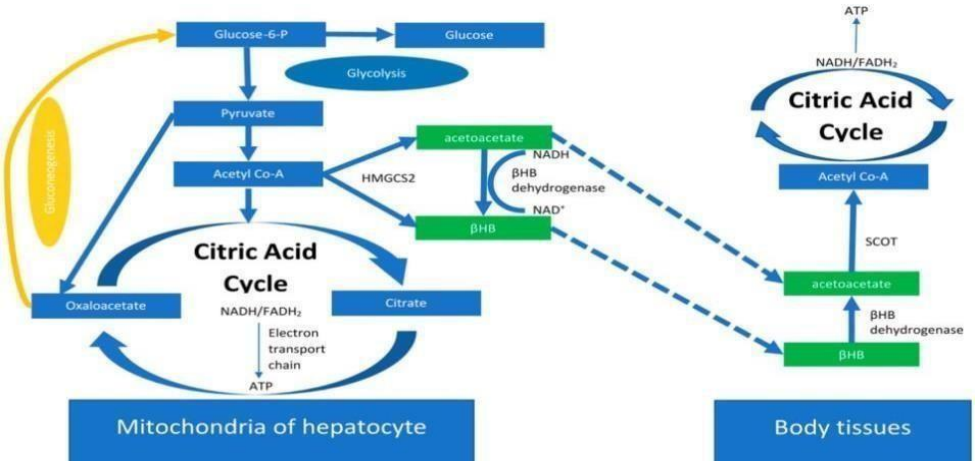

Figure 4. Production of Ketone bodies (Tamraz et al., 2023[19])

The newly formed βHB yields more adenosine triphosphate (ATP) per molecule than its alternative, pyruvate [6]. Cancer cells, however, are unable to utilize ketone bodies due to the absence of either β-hydroxybutyrate dehydrogenase (β-OHBDH) or SCOT enzymes [11, 18, 19]. Consequently, a metabolism based on ketone bodies and a lack of glucose can deprive cancer cells of the energy necessary for growth and proliferation. Therefore, ketosis has the potential to prevent both primary cancer and the subsequent metastatic process, as well as to enable the immune system to reverse cancer progression [7, 11, 12].

Variants and compositions. Adapting to the KD presents significant challenges, particularly for vulnerable cancer patients [6, 13, 15, 16, 19, 20]. Consequently, numerous efforts have been made to develop a therapeutic yet practical dietary regimen. KD variants exhibit diversity in their fat-to-

nonfat ratios (i.e., the ketogenic ratio), duration, and intermittent patterns. Although this paper will focus on specialized KD regimens, the classic KD, which has been employed for epilepsy treatment for over a century, features a 4:1 ketogenic ratio.

This means that 70-80% of caloric intake is derived from fats, while the remaining 20-30% is primarily allocated to proteins (15%-20%) and, to a lesser extent, carbohydrates (5%-10%), preferably from dietary fibers instead of unhealthy carbohydrate sources. However, alternative ratios such as 3:1 and 2.5:1 have also been documented [6, 8-11, 13, 16, 20-22].

The very-low-calorie KD (VLCKD) is a short-term intervention designed to initiate weight loss, with a daily caloric intake of only 500-800 kcal [8, 9]. Additionally, some researchers emphasize the potential benefits of incorporating intermittent fasting or simple caloric restriction into KD regimens [7, 11, 12, 18, 21].

An intriguing variant of the ketogenic diet (KD) regime was proposed by Hagihara et al. [20], in which 55 stage IV cancer patients, including those with CRLM, underwent a gradual glucose restriction and adjusted ketogenic ratio. The patients initially received a 2:1 ketogenic ratio in the first week (induction phase), followed by 1.5:1 for three months, and finally 1:1 from the third month onwards (maintenance phase). Concomitantly, daily carbohydrate intake gradually increased from 10g/day to 20g/day, and ultimately 30g/day. Participants were also provided with medium-chain triglyceride (MCT) and supplementation and a ketogenic formula (Ketonformula® 817-B).

During the trial, patients' ketone bodies were measured, and results indicated effective ketosis, which intensified as carbohydrate restriction eased. By the end of the 6-month period, some patients were no longer in therapeutic ketosis. Glucose and insulin levels initially declined sharply but increased with higher carbohydrate consumption, remaining below baseline measurements. However, by the third month, 29.7% of patients (11 out of 37) demonstrated low or no ketosis by the sixth month of the trial.

Regarding survival rates, the control group had an approximate rate of 45%, while the participant group exhibited a 63% rate. The overall survival of CRC patients was 19 months. In terms of PET (see Figure 5) response, at the three-month mark, 13.5% of patients had a partial response. At the one-year interval, 18.9% had a partial response, and 8% had a complete response.

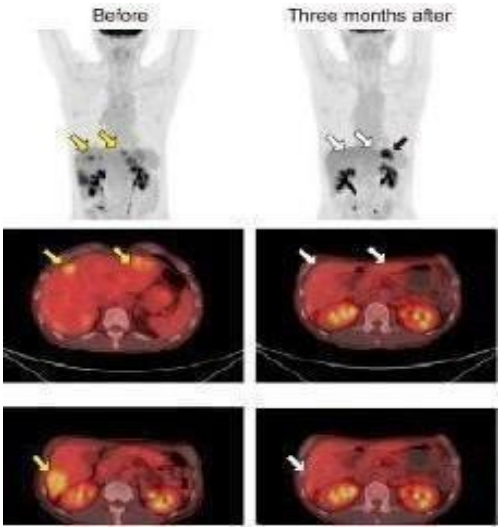

Figure 5. Pre and post ketogenic diet PET imaging in a patient with CRLM **(**Hagihara et al., 2020[20])

Adverse effects. Regarding quality of life and adverse effects, the Gastrointestinal Symptoms Rating Scale (GSRS), which ranges from 1 to 7, exhibited only a slight increase from 1.64 at baseline to 1.95 during the induction phase, and subsequently decreased to 1.8 during the maintenance phase, with most of the symptoms being attributed to constipation. Furthermore, 32% of the participants experienced hyperuricemia, while 29% developed hypercholesterolemia, which was characterized by an elevation in total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), but a decrease in triglycerides (TG).

In addition, there was a decrease in hemoglobin A1c (HbA1c) levels, while aspartate aminotransferase (AST) levels increased until the first month, indicating potential liver injury, before subsequently decreasing. Other liver enzymes, such as gamma-glutamyltransferase (γ-GTP), alanine aminotransferase (ALT), and glutamyl transpeptidase (GGT), exhibited reductions without an initial increase.

The findings of this trial underscore some of the well-documented adverse effects associated with the ketogenic diet, such as gastrointestinal symptoms and dyslipidemia, as well as hyperuricemia, which may be attributed to the high protein content of the diet. Nevertheless, hyperuricemia and elevated LDL levels were effectively managed using standard pharmaceutical interventions.

2.2 The Interplay of KD, Microbiota, and NAFLD in CRLM

KD resolves NAFLD, hence prevents CRLM. The KD has been demonstrated to effectively resolve non-alcoholic fatty liver disease (NAFLD), thereby preventing CRLM [1]. Employed primarily for weight management, the KD has been shown to alleviate and reverse NAFLD through multiple mechanisms, including weight loss, diminished food cravings [6, 9, 15], reduced insulin

resistance, and decreased glucose levels [6, 8-11, 13, 16, 20-22]. Additional benefits of the KD in addressing NAFLD include the reduction of inflammation and the promotion of mitochondrial development and health [5, 7, 11, 21] due to fatty acid oxidation, which in turn unloads the fatty liver [5, 21].

While the weight reduction effect of the KD remains a subject of debate, with some attributing it to the overall decrease in caloric intake compared to a Western diet and others arguing that the weight loss effect is temporary and fades upon discontinuation of the KD, the importance of weight loss in NAFLD and CRC development cannot be disregarded. A human trial conducted by Luukkonen et al. [5] involving 10 obese adults followed a KD regime consisting of 68% fat, 28% protein, and 8% carbohydrates, amounting to 1,444 kcal/d for six days. The findings revealed a significant positive impact of the KD on hepatic fat content, insulin and glycemic control, and mitochondrial health, as well as other biological measurements relevant to metabolic health (leptin, T3, liver enzymes, VLDL, and LDL) [1]. Additionally, lactate levels, which play a crucial role in the Warburg effect, were affected.

The trial also reported a 12% reduction in blood glucose levels, with ketone bodies (βHB and ACAC) elevated 10 and 6-fold from the control, respectively. Furthermore, intrahepatic triglyceride (IHTG) decreased by 31%, unloading the fatty liver, and body weight was reduced by 3% within just six days. Free fatty acid (FFA) concentrations increased by 35%, which could be attributed to the enhanced net hydrolysis of IHTG and the partitioning of the resulting fatty acids toward ketogenesis (a 232% increase) due to reductions in serum insulin concentrations (53%) and hepatic citrate synthase flux (38%).

Luukkonen et al. [5], presented a study that examined the impact of various factors on insulin sensitivity and liver function, in addition to the influence of a KD on obese and NAFLD conditions.

Notably, a 36% reduction in C-peptide and a 57% decrease in homeostasis model assessment of insulin resistance (HOMA-IR) were observed, which significantly influenced insulin sensitivity. Liver function was also affected, with glucose and lactate production witnessing a 22% and 18% reduction, respectively. Liver enzymes, including alanine aminotransferase (ALT) and GGT, declined by 10% and 20% from their baseline levels. However, a significant increase of 34% from the baseline in the AST to ALT ratio indicated a possible temporary liver injury.

Additionally, the study reported a 25% reduction in fasting TG indicative of a decrease in very-low-density lipoprotein (VLDL) in fasting conditions. Leptin and triiodothyronine (T3) levels decreased by 45% and 21%, respectively, which correlated with a decline in metabolic rate because of ketosis and weight loss. Protein oxidation was found to increase, as demonstrated by a 13% rise in urea nitrogen in urine, corresponding to an additional 9 grams of protein.

In the context of obesity and NAFLD, the study noted that insulin's failure to suppress lipolysis leads to a continuous delivery of FFA to the liver. This accumulation results in IHTG through lipogenesis, culminating in a fatty liver. Surprisingly, the KD led to a decrease in IHTG despite elevated FFA levels. It is hypothesized that the liver's hepatic mitochondria metabolize FFA into ketone bodies, rather than esterifying them into complex lipids. Acetyl CoA can enter the ketogenic cycle or form citrate by condensing with oxaloacetate through citrate synthase. During the experiment, the ratio of pyruvate carboxylase to citrate synthase increased by 52%, attributed to a 38% reduction in citrate synthase. Pyruvate carboxylase is responsible for the formation of oxaloacetate from pyruvate. those findings are supported by another trial that is described in this paper, “BHB is known for its favorable use in the TCA cycle by replacing succinyl-CoA and increasing the free pool of succinate, improving mitochondrial efficiency and TCA cycle turnover” [16].

In conclusion, although a reduction in specific liver enzymes, such as GGT and ALT, was observed, there was a significant increase in AST levels and the AST/ALT ratio, suggesting the possibility of transient liver injury resulting from rapid weight loss. The role of leptin and T3 as indicators of mitochondrial redox state is noteworthy, with their decline associated with a decreased metabolic rate during weight loss, as evidenced in previous studies [5, 20]. These hormones appear to promote citrate synthase mitochondrial flux over pyruvate flux. However, this study is limited by its small sample size and short duration. Consequently, the regimen's potential benefits and drawbacks beyond the six-day intervention period remain unknown. Furthermore, the study does not provide insights into the progression of non-alcoholic fatty liver disease (NAFLD) following the cessation of the regimen, leaving questions regarding recurrence and long-term applicability unanswered.

KD and gut microbiota in CRLM. It has been hypothesized that some of the advantages associated with the KD may be mediated by the gut microbiota and their metabolites [8, 9, 15]. The effects of both gut microbiome and ketosis are not yet fully understood, and the literature presents conflicting findings, partly due to the absence of a standardized, safe, and easily adhered-to KD regimen. Stool sample analyses have shown that KD conditions can lead to a reduction in alpha diversity, which is an unfavorable prognostic indicator for bowel health [8, 9]. However, an increase in SCFA and SCFA-producing bacteria, such as Akkermansia muciniphila, has also been observed. This finding is not universally reported, with some authors noting a decrease in the bacteria [9]. One interpretation of these results is that despite the reduction in phyla richness, a higher quality of mycobacterial growth is promoted, which may increase beta diversity while reducing alpha diversity. Another theory posits that in the absence of dietary fibers, bacteria can transition to protein fermentation [9].

Furthermore, a decrease in the growth of Bifidobacterium has been observed, which was proportional to both the increase in βHB and the reduction in IL-17 [9]. However, it remains unclear whether the beneficial effect is attributable to the βHB or the Bifidobacterium. Bifidobacterium is a beneficial strain often used as a probiotic [8, 9] suggesting that IL-17 suppression occurs despite its decrease, rather than as a direct result. In a separate study involving 48 obese individuals following a KD, the authors reported a decrease in Firmicutes and an increase in Bacteroidetes, indicative of a healthier microbiome balance. This effect was more pronounced in participants consuming non-animal source proteins [9, 15].

In a comprehensive meta-analysis conducted by Paoli A et al. [8], the effects of the KD, practiced with varying ketosis ratios and durations (see Figure 6), were examined in patients suffering from a wide range of potential diseases. Upon excluding pediatric and non-human populations, the following effects of KD on gut health were observed: a decrease in ROS production, ghrelin, mammalian target of rapamycin (mTOR), gamma-glutamyl amino acid, lactate, glycemia, and lipogenesis; and an increase in adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, gamma-aminobutyric acid (GABA), adiponectin, cholecystokinin (CCK), BBB integrity, SCFA, vagus activation, H2S, lipid oxidation, and insulin sensitivity.

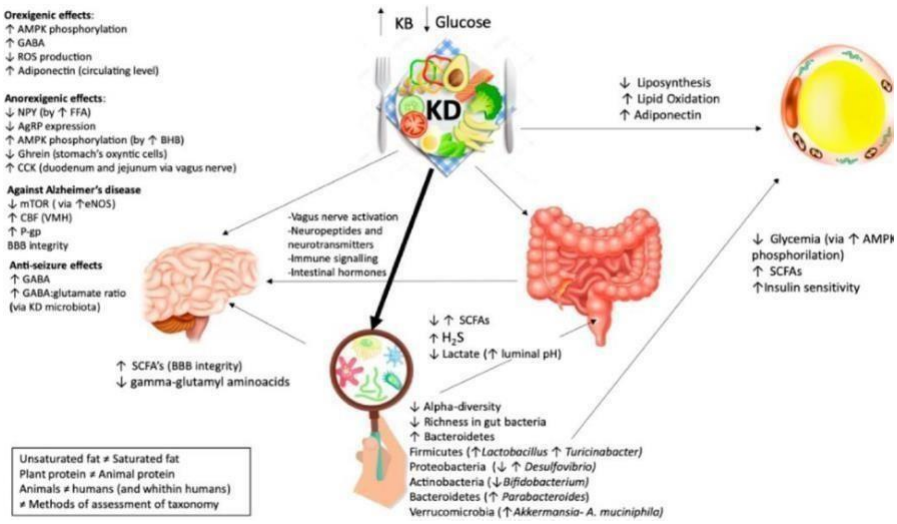

Figure 6. Effect of KD on gut microbiome (Paoli et al., 2019[8])

In terms of bacterial population changes, both alpha and beta diversity decreased (alpha diversity may improve within 12 weeks); Proteobacteria [Desulfovibrio] (pro-inflammatory) either increased or decreased; the Firmicutes to Bacteroidetes ratio improved (both increased); Actinobacteria [Bifidobacterium] decreased (correlating with the prevention of colorectal cancer); and Akkermansia, Muciniphila, and Lactobacillus increased (fermenting SCFA).

While GABA and serotonin have primarily been studied in relation to epilepsy, it could be intriguing to explore the connections between these two neuromediators and adipokines (adiponectin and leptin) and gut microbiota under ketosis conditions.

There remains much to be discovered regarding the potential benefits of KD and the role of microbiota in these benefits. Randomized control trials on the subject are needed. A deeper understanding of the role of microbiota in metabolism and cancer pathology could lead to improved health recommendations or even the development of new probiotics. Finally, it is important to note that even if all the knowledge of "beneficial" bacteria in terms of CRLM were combined into a single probiotic formula, individual and uncharted factors could still lead to varying results and impede progress.

2.3 Direct Effects of KD on CRLM and Potential Supplements

The Western diet, with its glucose and insulin alterations, contributes to CRC through oncogenic promotion and immune suppression. Observations indicate that advanced CRC patients on a high-fat diet experience lower survival rates, with obesity being a factor in up to 40% of all cancers [19]. A metabolic shift observed in cancer cells, known as the Warburg effect, involves an increased conversion of glucose to lactate, even under aerobic conditions [10, 14, 16-20]. This process is less efficient in ATP production compared to normal cells' oxidative phosphorylation [18]. Inflammation-induced mTOR protein amplification is suggested to facilitate this shift to aerobic glycolysis [18, 19].

The KD has been linked to suppression of various cancers, particularly CRC through several mechanisms [6, 7, 10-12, 18-20]. The mechanisms involving ketone bodies, mainly βHB, are still being studied [7, 8, 11, 12]. Among the established pathways (see Figure 7) are:

1. Hyperinsulinemia from a Western diet, triggering insulin-like growth factor 1 (IGF1) overexpression, activating PI3K/AKT pathway, and promoting inflammation and angiogenesis [1, 9, 10, 19, 20];
2. Histone deacetylase 3 (HDAC3) regulating IL-6 and mTOR secretion [19], and successful CRC suppression trials targeting HDAC3 [7, 11, 12, 19];
3. HMGCS2 enzyme reduces TNFα-induced intestinal cell apoptosis and enhancing PD-1 modality effectiveness in CRLM;[19]
4. Homeodomain-only protein (HOPX) gene and hydroxycarboxylic acid receptor 2 (HCAR2) inhibiting colon growth through epigenetic regulation, correlating with βHB levels, and leading to tumor size and number reduction [7, 11, 12, 19];

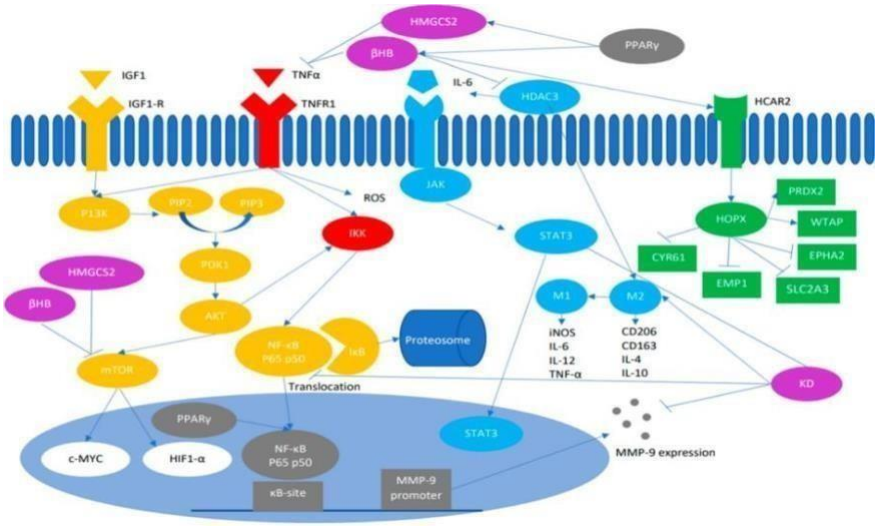

Figure 7. Mechanisms of KD in CRLM (Tamraz et al., 2023[19])

In a study by Dmitrieva-Posocco et al. [11], different fat-to-carbohydrate ratios showed significant tumor suppression as the ratios increased. In the KD group, the survival rate at 120 days was 56.2%, significantly higher than the 30% in the non-KD group. The mean tumor score in the KD group was also lower at 0.5, compared to 2.1 in the non-KD group. Furthermore, the KD group exhibited lower levels of IL-17 and NLRP3 inflammasome. Also, the KD group had more anti-apoptotic protein Mcl1, reducing epithelial cell turnover regulated by Lgr5+ at crypt bottoms.

The KD has been shown to significantly inhibit colorectal tumor growth in both prevention and treatment contexts. KD led to a substantial reduction in IL-17 levels (4.8-fold) and NLRP3 inflammasome levels (2.5-fold). Increased ki67 staining in the non-KD group and a 150% rise in anti-apoptotic protein Mcl1 in the KD group were also observed, implicating a decrease in epithelial cell turnover regulated by Lgr5+.

These findings suggest βHB, acting via the Hcar2 receptor, induces Hopx expression, reducing epithelial growth. This antiproliferative effect, seen in organoid cells and the HT-29 cell line, is enhanced under glucose restriction and exogenous βHB.

A positive correlation was observed between βHB levels and HOPX expression in epithelial cells, suggesting that βHB, via Hcar2, elevates HOPX levels and reduces proliferation in these cells. Exogenous βHB supplementation showed therapeutic effects, which can be enhanced by glucose restriction. βHB also functions as a signaling molecule during nutrient scarcity, delaying peripheral tissue growth. Combining this with glucose restriction, histone deacetylase inhibitors, and DNA methylation inhibitors showed additive benefits.

A study by Plotti et al. [18] highlighted the benefits of fasting or a KD in metastatic cancer patients, including improved quality of life, enhanced chemotherapy efficacy, and increased survival. Despite higher dropout rates, the KD reduced chemotherapy-induced toxicity, and cravings, appetite, and fatigue, while enhancing the efficacy of bevacizumab. Interestingly, the American Cancer Society

(ACS) diet resulted in inferior outcomes compared to the KD in terms of hunger control and synergistic benefits with systemic modalities.

KD downregulates insulin/IGF1 [9, 10, 19, 20] and HAC3 [7, 10, 11, 19] and suppresses TNFα [19], possibly inhibiting CRC progression and development. SCFAs can produce butyrate through bacterial fermentation of dietary fibers [19]. Advanced cancer patients on a KD have shown disease stability or partial remission, with minimal adverse effects. KD also suppresses pro-inflammatory and pro-metastatic IL-6 levels, preserving muscle mass and potentially preventing cachexia. Studies show that combining chemotherapy and KD results in a three-fold increase in chemotherapy response.

Exogenous supplementation, including plant-based oils like omega-3 fatty acids, ketone esters, ketone salts, and medium-chain triglycerides (MCTs) [7, 11, 12, 15, 19, 21], offers an alternative to endogenous ketosis, reducing insulin and inflammation similar to animal-based proteins [9, 15, 19, 23]. This approach, however, is less profound and can be disrupted by an unknown intake of carbohydrates [11, 19, 21]. Given the negative effects of simple carbohydrates on gut microbiota [8, 9, 19] and the role of fructose in CRLM [1], along with the increased cardiovascular and CRC risk from animal-source proteins and fats [6, 10, 15, 19], plant-based fats and proteins are favored. Although exogenous supplementation allows more dietary fibers without disrupting ketosis, carbohydrate limitation remains advisable to maximize its effectiveness [6-9, 10-12, 15, 18, 19, 21, 23].

2.4 Adverse Effects, Age, and Gender Differences in KD Application

The metabolic transition to ketosis, potentially occurring at about 2,000 Kcl of glucose reservoir depletion, is under debate [6]. Adhering to the KD is challenging due to the presence of refined sugars in most foods, which can disrupt ketosis [6, 13, 15, 19]. Success in KD often requires education and professional assistance, with weight loss results appearing over time and fading upon diet cessation [6, 13, 15, 22]. KD is recommended for short-term use, especially with a 4:1 ratio [6, 13, 15].

Shalabi et al. [13] found in a study with 226 obese individuals that most attempted KD short-term multiple times, with around 70% unable to commit for more than six months. Guidance was mostly sought from social media (50%), with only 2.2% consulting dietitians.

KD is unsuitable for certain populations, including athletes, vegans, pregnant and breastfeeding women, those who have had a cholecystectomy, and people with advanced kidney disease [6, 9, 10, 15, 16]. Unplanned pregnancy while on KD can lead to neural tube defects and gestational diabetes, and individuals with type 1 diabetes mellitus risk hypoglycemia events [15].

Keto Flu Symptoms and its impact. The KD may cause short-term adverse effects, including a prevalent phenomenon known as the “keto flu”, characterized by symptoms such as fatigue, poor concentration, gastrointestinal issues, and others, typically lasting a week after the diet's initiation [6, 13, 15, 19]. These symptoms can reoccur with each attempt to re-enter ketosis after breaking the diet, and acidosis is a rare complication [6, 13, 19]. Discontinuation of KD often leads to a significant increase in body mass [6, 9, 10, 15].

Shalabi’s et al. cross-sectional observational study [13] on 226 obese individuals confirmed the severity and prevalence of KD's adverse effects. Further research is needed to understand these short-term impacts of the KD (for the results see Table 2).

Long-term KD adherence can lead to health concerns like dehydration, kidney injury, nutrient deficiencies, and potential dyslipidemia [6, 8, 9, 15, 23]. Decreased dairy product consumption can elevate osteoporosis risk due to calcium insufficiency [6, 10, 13, 15]. The diet's impact on kidney health is disputed, with some studies showing protective effects, while others suggest harm [6, 9, 15, 16]. It's not recommended for those with advanced kidney failure or susceptibility to kidney stones [13, 15]. However, a study by Torres et al. [16] highlights the protective effects of βHB against the proliferation and inflammation associated with polycystic kidney disease (PKD). These effects also have relevance in the context of cancer. Dyslipidemia and elevated HDL levels have been observed among followers of the KD. While some researchers argue that an increase in LDL is non-pathogenic given the context of HDL and total cholesterol levels [15], others have successfully treated patients with statins to lower LDL levels [20].

Cancer patients may develop cachexia due to treatment effects. Extended unsupervised KD could potentially induce cachexia through malnutrition and protein catabolism. However, a well-managed KD can maintain muscle mass while promoting fat loss [5, 11, 12, 19-22]. Challenges in KD adherence stem from social, cultural, religious, and psychological factors associated with food, contributing to high dropout rates [6, 15, 18-20, 22]. The KD, a restrictive regimen, poses challenges to advanced cancer patients, with the dropout rate being a major obstacle. Supervision and education are critical for those attempting this diet, similar to guidance for other medical interventions. Symptoms of glucose withdrawal may resemble those from systemic therapies. No consensus exists on optimal KD parameters. Lane et al [10] suggested further research to address this (see Figure 8).

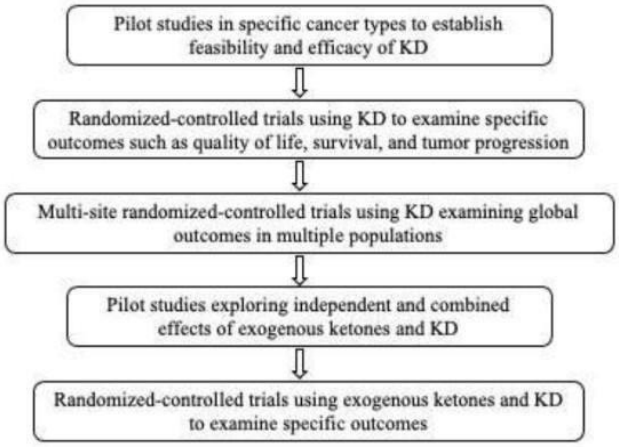

Figure 8. Recommendations investigating KD's role in cancer management validation (Lane et al., 2021[10])

Sex and gender differences under KD and ketosis. Studies indicate that ketosis, resulting from the KD, influences body metabolism differently across ages and genders. One trial [13] found that middle-aged healthy males (36-55 years) benefited most in terms of fewer and less severe adverse effects. However, the diet's specifics were not disclosed.

A different study by Kovac et al. [21] (see Table 2), used a murine model to measure glucose levels, βHB, and body weight over 17 months. These findings indicate that both glucose and βHB levels are influenced by age and gender. From the 10th month, weight loss was observed in both genders, suggesting normal growth. Males lost weight more rapidly, with a 21.9% decrease from their peak body weight, while females lost 17.6% of their peak body weight.

This study suggests KD effects may vary by age, sex, and gender, but accurate ketosis duration couldn't be determined due to infrequent measurements and short follow-up after feeding. Shalabi et al. [13] supports these findings, emphasizing that middle-aged males gain most from ketosis.

Human trials comparing gender differences in KD are limited. Majid et al. [22] conducted a study on 46 healthy individuals, measuring biochemical and anthropometric parameters. The most significant parameters (P value <0.001) included triceps circumference, body fat, water mass, basal metabolic rate, muscle mass, bone mass, and protein.

The 28-day KD trial was short for assessing the diet's anti-cachexic effect. However, it suggested males had a more favorable metabolic response, with a significant reduction in triceps circumference and body fat, and an increase in protein and water mass.

KD has shown potential to treat cachexia and preserve lean body mass in pre-clinical and clinical trials, but this typically requires a longer duration. Despite this study's short length, males showed a smaller reduction in muscle mass than females.

The study highlighted improved muscle and fat composition in males, with fewer metabolic adverse effects. However, its limitations include the short duration and exclusion of patients with comorbidities such as diabetes, hypertension, and obesity.

RESULTS

To address the concerns regarding the ketogenic diet, we conducted a literature review with strict inclusion and exclusion criteria (Table 1). Experimental trial results are presented in Table 2, while the roles of specific bacteria in CRLM are summarized in Table 3 based on various overviews. Data from the past five years suggest that ketosis, whether achieved through supplementation or endogenous carbohydrate restriction, may play a role in "starving" cancer cells, inhibiting the initial progenitor for CRC and CRLM (Lgr5), and reducing the toxicity and adverse effects of systemic therapies if implemented under controlled and supervised conditions. Ketone body supplementation could be a viable strategy for maintaining ketosis, alleviating strict dietary restrictions, and facilitating fiber consumption.

The aggressiveness of synchronous colorectal liver metastasis (CRLM) is a subject of debate in the literature. Engstrand et al. [2] reported that synchronous CRLM patients experienced a modest improvement in overall survival, even though their tumors were less likely to be resectable. Conversely, other researchers [4, 14, 17, 23] argue that synchronous CRLM is more aggressive and may be characterized by the presence of markers such as p27 and cyclooxygenase-2 (COX-2), both of which have been linked to poorer outcomes. [14]

In terms of prevalence, approximately 26.5% of colorectal cancer (CRC) patients are diagnosed with CRLM, while 50% will develop CRLM at some stage during the progression of their disease. Male patients are more likely to develop CRLM at a younger age. Additionally, left-sided tumors are more prevalent, representing 65% of all CRC cases [1, 2, 14, 17, 18, 20].

DISCUSSION

The findings from the reviewed literature indicate that ketosis, achieved either through exogenous supplementation or endogenous maintenance, is a viable and effective therapeutic approach with antitumorigenic, anti-inflammatory, antioxidant, and metabolic properties, all of which contribute to mitigating the burden of CRC and subsequent CRLM. In the state of ketosis, a decline in various pathophysiological drivers of CRLM is observed, including reduced insulin secretion and resistance, decreased body weight, clearance of excess fat from the liver, enhancement in beta diversity of gut microbiota, an increase in beneficial phylum, epigenetic modulation of stem cells Lgr5, and mitochondrial stability.

Moreover, the literature reveals the advantageous synergistic effects of combining ketosis with chemotherapy and biological treatments, resulting in diminished toxicity and improved treatment adherence efficacy. There is a scarcity of experimental data regarding the adaptation of the

ketogenic diet for adults, particularly for advanced cancer patients. The recent popularity of the ketogenic diet for weight management has led to a proliferation of unreliable and biased literature.

The existing literature exhibits considerable controversy surrounding the ketogenic ratio, metabolic effects of ketosis (primarily on body weight and NAFLD), and gut microbiota. However, the point at which ketosis' antitumor benefits are compromised by antinutrients or the acceptable carbohydrate intake threshold remains unclear, especially when supplements are taken. Despite advancements in diagnosis, screening, and management, the burden of CRC remains significant. The pathophysiology is not yet fully understood, and discoveries continue to emerge. The potential for the ketogenic diet to facilitate the conversion of unresectable lesions to operable ones and improve 5-year survival rates when used as a sole adjuvant or combined with other adjuvant therapies requires further investigation (see the recommendations summarized in Figure 8, Subchapter 2.4).

CONCLUSIONS

In conclusion, this literature review has highlighted the complex interplay of various factors in the management of metachronous colorectal liver metastasis, including the tumor microenvironment, liver's pre-metastatic niche, gut and intrahepatic microbiota, and non-alcoholic fatty liver disease. The ketogenic diet shows promise as a potential intervention for managing non-alcoholic fatty liver disease, preventing, and managing colorectal liver metastasis. However, further research is necessary to understand its impact on gut microbiota, suitability for patients of different ages, sexes, and genders, and optimal carbohydrate intake threshold.

The aggressiveness of synchronous CRLM and the role of specific biomarkers remain contentious issues, warranting further investigation. Additionally, more research is needed to assess the potential of the ketogenic diet in converting unresectable lesions to operable ones and improving 5-year survival rates when used alone or combined with other adjuvant therapies.

Overall, it is crucial to investigate novel management strategies for colorectal liver metastasis and adopt a comprehensive, personalized approach to enhance patient outcomes. As our understanding of the complex relationships between various factors and the ketogenic diet deepens, more effective personalized treatment strategies, improved health recommendations, and novel interventions for managing and treating metachronous colorectal liver metastasis can be developed. However, further research, especially randomized control trials, is necessary to expand our knowledge of the role of microbiota, the ketogenic diet, and their interplay with non-alcoholic fatty liver disease in the context of colorectal liver metastasis.

No.	Subjects, Type of Research	Country, University, Hospital	Times cited	Impact Factor of the Journal	Researchers	Year of Publication
1	Review	Ohio State University (USA)	10	6.78	Chakraborty D, Wang J.	2020
2	Cohort	Karolinska Institutet, Danderyd Hospital (Sweden)	38	8.77	Engstrand J, Strömberg C, Nilsson H, et al.	2019
3	Research on mice (in vivo and in vitro) Experimental Study	Hebei General Hospital (China)	7	4.3	Yuan N, Li X, Wang M, et al.	2022
4	Review	University of Manchester (UK)	3	23.12	Patel M, McAllister M, Nagaraju R, Foad Al Badran S, et al.	2022
5	Human Trial Experimental Study	Yale School of Medicine (USA)	118	12.79	Luukkonen PK, Dufour S, Lyu K, et al.	2020
6	Qualitative Methodology	College of Science, Qassim University, Buraydah (KSA)	20	3.55	Alharbi A, Al-Sowayan NS.	2020
7	Review	Army Medical University (China)	4	38.12	Xiang Y, Wang M, Miao H.	2022
8	Systematic Review	University of Murcia (Spain)	170	3.9	Paoli A, Mancin L, Bianco A, Thomas E, Mota JF, Piccini F.	2019
9	Review	Amsterdam University Medical Center (The Netherlands)	15	5.7	Attaye I, van Oppenraaij S, Warmbrunn MV, Nieuwdorp M.	2021
10	Literature Review	University of Alabama (USA)	13	5.7	Lane J, Brown NI, Williams S, Plaisance EP, Fontaine KR.	2021
11	Mice Pre-clinic experimental study	University of Pennsylvania (USA)	53	69.5	Dmitrieva-Posocco O, Wong AC, Lundgren P, et al.	2022
12	Informational article	National Cancer Institute	-	11.8	Reynolds S.	2022
13	Retrospective Cross-Sectional Study	University of Jeddah (KSA)	1	1.15	Shalabi H, Alotaibi A, Alqahtani A, Alattas H, Alghamdi Z.	2021
14	Review	Second Xiangya Hospital, Hunan Province, (China)	42	38.12	Zhou H, Liu Z, Wang Y, et al.	2022
15	Review	Committee for Responsible Medicine Washington D.C. (USA)	70		Crosby L, Davis B, Joshi S, et al.	
16	Pre-clinic Trial, xperimental study	University of California (USA)	115	27.28	Torres JA, Kruger SL, Broderick C, et al.	2019
17	Review	National University Health System (Singapore)	97	2.58	Kow AWC.	2019
18	Literature Review	University of Rome (Italy)	35	1.3	Plotti F, Terranova C, Luvero D, Bartolone M, Messina G, et al.	2020
19	Review	American University of Beirut Medical Center (Lebanon)	0	6.2	Tamraz M, Al Ghossaini N, Temraz S.	2023
20	Human Case Series Study, Experimental Study	Osaka University Graduate School of Medicine (Japan)	30	5.7	Hagihara K, Kajimoto K, Osaga S, et al.	2020
21	Rats Trial, Experimental Study	Savaria Department of Biology, Savaria University Centre, ELTE Eötvös Loránd University (Hungary)	6	5.5	Kovács Z, Brunner B, D'Agostino DP, Ari C.	2021
22	Experimental Study of Human	Peer Review Articles	1	2.96	Majid B, Maqsood AM, Majid M, Ahmad Z, Mehmood A, Raza SS.	2022
23	NCCN Clinical Practice Guidelines in Oncology	-	523	11.9	National Comprehensive Cancer Network (NCCN)	2022

No.	Participant (number+type)	Duration	Ketogenic ratio/ Carb Restriction	Results
2	1,026 humans with CRC	5 years	-	Metachronous patients had similar tumor resectability at 3, 6, and 12 months (37%, 37%, 40%), while synchronous patients had lower rates (17%, 17%, 19%). However, synchronous patients showed slightly better OS during the 60-month trial.
3	60 mice	6 weeks	-	Antibiotics like colistin improved KC function, reduced CRLM nodules, and promoted a healthier microbiome. Increased B. vulgatus levels correlated with better KC function and fewer, smaller CRLM nodules (P=0.041).
5	10 obese humans	6 days	Carb restriction: 23g/day	Reductions in glucose (12%), IHTG (31%), body weight (3%), serum insulin (53%), citrate synthase flux (38%), C-peptide (36%), HOMA-IR (57%), fasting triglycerides (25%), leptin (45%), and T3 (21%). Elevations in ketone bodies (BHB, ACAC) by 10 and 6-fold, free fatty acids (35%), and ketogenesis (232%). Improved liver function with decreased glucose and lactate production (22%, 18%), ALT, GGT, and increased AST and AST/ALT ratio. A 13% rise in urinary urea nitrogen indicated protein oxidation.
8	80 humans with varied pathologies, split into groups of 6, 25, 29, and 20	Varied durations: 3 months, 6 months, 3 weeks, and 6 months	KD ratio: 1:1, increased to 2:1, 3:1, or 4:1. Carb restriction: ≥50g/day. Composition: 78% fat, 4:1 ratio.	KD impacted gut microbiota in non-pediatric populations by decreasing ROS production, ghrelin, mTOR, lactate, and lipogenesis, and increasing AMPK phosphorylation, GABA, adiponectin, CCK, BBB integrity, SCFA, vagus activation, H2S, and insulin sensitivity. Bacterial population changes included decreased alpha and beta diversity, variable Desulfovibrio levels, improved Firmicutes to Bacteroides ratio, decreased Bifidobacterium, and increased Akkermansia and Muciniphila.
11	10, 545 mice, human organoids, 41 CRC humans	4 weeks of each trial	90% fat (plant/animal-based)	Tumor numbers and sizes were suppressed substantially with increasing fat-to-carbohydrate ratios. 120 days survival was 30% and 56.2% in the control and KD group. results indicate that a KD potently suppressed colorectal tumor growth in both prevention and treatment models. βHB levels correlated positively with HOPX expression and negatively with cell cycle progression.
13	226 humans (symptoms severity)	30.1%<1 month, 34.5% 1-<3 months, 15% 3-<6 months, 12.8%6-<12 months 7.5% ≥12 months	unknown	Mild, moderate, and severe side effects as follows: nausea (29.2%, 16.4%, 5.8%), dizziness (39.8%, 27.4%, 11.5%), fatigue (29.6%, 27.4%, 14.6%), muscle pain (22%, 20%, 7%), constipation (19.9%, 38.9%, 10.2%), polyuria (26.5%, 29.2%, 16.4%), palpitation (29.6%, 12.8%, 1.8%), hypoglycemia (17.7%, 7.1%, 4.9%), and acidosis (1.8%, 0.9%, 0.4%).
16	8 M\ 12F KD group and 13M\ 8F control (41 total) mice	8 weeks	KD: 91% fat, 5% protein; normal diet: 62% carb, 25% protein, 13% fat. TRF vs free access	BHB levels rose and glucose fell over time, more pronounced in KD than TRF. Control was inferior to both. mTOR inhibition provided kidney protection, reversed PKD, and reduced inflammation. Despite studies suggesting kidney exhaustion, this research supports KD's protective role.
20	55 total, 5 dropped before KD onset, 50 started the regime, 11 dropped due to disease progression/personal reasons. All had stage IV cancer, 9 with CRC	≥3 months-almost 6 years first week=induction, slowly lowering restrictions=maintenance phase	Decelerated restriction: 2:1 first week (10 g/day carbs) 2nd week-3 months, 1.5:1 (20g/day), ≥ 3 months=1:1(30g/day)	Metabolic changes: ↑ Ketone bodies (AcAc, BHB), ↓ Glucose, insulin, ABC score, GKI; PET findings: Altered CRC activity Survival and safety: Improved 1-year and overall survival, Enhanced safety (↓ GSRS score).
21	8 females (F); 8 males (M); Control (C): First 4 days every month before force feeding	17-month study; Ketone supplements given once a month; Measurements taken before and after feeding	Intervention: Ketone ester + MCT oil (60% caprylic, 40% capric triglyceride supplements) KEMCT supplementation: 1:1 mix of ketone ester (KE) and medium-chain triglyceride (MCT) oil	Initial findings: F group showed advantages in βHB, glucose levels, and body weight; Long-term results: M group exhibited superior outcomes over time, F group's effect diminished with time;Limitation: βHB measurements taken before and after feeding, whole-month levels could better reflect ketosis duration.
22	46 healthy humans	28 days	3:1	(p<0.001): i) Triceps circumference (cm), ii) Body fat (%), ii) Water mass (kg), iv) Basal metabolic rate (J/(h·kg)), v) Muscle mass (kg); vi) Bone mass (kg), vii) Protein (kg); 1. Triceps Circumference: F -10%, M -13%; 2. Body Fat: F 95%, M 85% (baseline comparison); 3. Water Mass: F +2%, M +5%; 4. Basal Metabolic Rate: F & M decreased to 97%, 98% respectively; 5. Muscle Mass: F -3%, M -1%; 6. Bone Mass: Both -4%; 7. Protein: F +1%, M +8% (28 days); Overall, Males improved in muscle/fat composition with fewer metabolic effects.

Bacteria	Structure and Properties	Correlation to CRLM	Pathways Activated	Immune Suppression	Other Correlations	Comments
Fusobacterium	Gram-negative, obligate anaerobic, rod [1]	CRLM driver [1], poor prognostic factor [4, 14], increased number and size of liver nodules [3, 4]	NF-κB and Wnt/ß-catenin signaling [4]	↓CD8	↑ER stress [4]	Travels to the liver with CRC "seed" [4]
E. coli	Gram-negative [3, 4]	LPS as main virulence [1, 3, 4, 14]	TLR4/NF-κB, PI3K/AKT, β1 integrin-mediated cell adhesion [4, 14]	Polarizing macrophages into M2 phenotype → activates TLR4-mTOR [4]	↑EMT and EVGF [4, 14]	Reaches liver via "gut-liver axis" [1, 3, 4, 14]
Lactobacillus	Member of the Firmicutes phylum [1, 4, 9]	↓EVGF, MMP, reversing 5-FU resistance [4]	-	-	Exacerbates NAFLD [1]	Supplemented as probiotic [1, 4, 9], ↑GABA [4]

REFERENCES

1. Chakraborty D, Wang J. Nonalcoholic Fatty Liver Disease and Colorectal Cancer: Correlation and Missing Links. Life Sci. 2020;262.
2. Engstrand J, Strömberg C, Nilsson H, et al. Synchronous and metachronous liver metastases in patients with colorectal cancer—towards a clinically relevant definition. World J Surg Onc. 2019; 17:228.
3. Yuan N, Li X, Wang M, et al. Gut Microbiota Alteration Influences Colorectal Cancer Metastasis to the Liver by Remodeling the Liver Immune Microenvironment. Gut Liver. 2022; 16:575-588.
4. Patel M, McAllister M, Nagaraju R, Foad Al Badran SS, et al. The Intestinal Microbiota in Colorectal Cancer Metastasis – Passive Observer or Key Player? Crit Rev Oncol Hematol. 2022; 180:103856.
5. Luukkonen PK, Dufour S, Lyu K, et al. Effect of a ketogenic diet on hepatic steatosis and hepatic mitochondrial metabolism in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A. 2020;117(13):7347-7354.
6. Alharbi A, Al-Sowayan NS. The Effect of Ketogenic-Diet on Health. Food Nutr Sci. 2020; 11:301-313.
7. Xiang Y, Wang M, Miao H. Ketogenic diet: new avenues to overcome colorectal cancer. Sig Transduct Target Ther. 2022; 7:262.
8. Paoli A, Mancin L, Bianco A, et al. Ketogenic Diet and Microbiota: Friends or Enemies? Genes (Basel). 2019;10(7):534.
9. Attaye I, van Oppenraaij S, Warmbrunn MV, Nieuwdorp M. The Role of the Gut Microbiota on the Beneficial Effects of Ketogenic Diets. Nutrients. 2021;14(1):191.
10. Lane J, Brown NI, Williams S, et al. Ketogenic Diet for Cancer: Critical Assessment and Research Recommendations. Nutrients. 2021;13(10):3562.
11. Dmitrieva-Posocco O, Wong AC, Lundgren P, et al. β-Hydroxybutyrate suppresses colorectal cancer. Nature. 2022;605(7908):160-165.
12. Reynolds S. Keto Molecule Offers Clue for Preventing Colorectal Cancer. National Health Institute. 2022 June.
13. Shalabi H, Alotaibi A, Alqahtani A, Alattas H, Alghamdi Z. Ketogenic Diets: Side Effects, Attitude, and Quality of Life. Cureus. 2021;13(12).
14. Zhou H, Liu Z, Wang Y, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Sig Transduct Target Ther. 2022; 7:70.
15. Crosby L, Davis B, Joshi S, et al. Ketogenic diets, and chronic disease: Weighing the benefits against the risks. Front Nutr. 2021.

16. Torres JA, Kruger SL, Broderick C, et al. Ketosis ameliorates renal cyst growth in polycystic kidney disease. Cell Metab. 2019;30(6):1007-1023.e5.
17. Kow AWC. Hepatic metastasis from colorectal cancer. J Gastrointest Oncol. 2019 Dec;10(6):1274-1298.
18. Plotti F, Terranova C, Luvero D, et al. Diet and Chemotherapy: The Effects of Fasting and Ketogenic Diet on Cancer Treatment. Chemotherapy. 2020; 65:77-84.
19. Tamraz M, Al Ghossaini N, Temraz S. The Ketogenic Diet in Colorectal Cancer: A Means to an End. Int J Mol Sci. 2023;24(4):3683.
20. Hagihara K, Kajimoto K, Osaga S, et al. Promising Effect of a New Ketogenic Diet Regimen in Patients with Advanced Cancer. Nutrients. 2020 May 19;12(5):1473.
21. Kovács Z, Brunner B, D'Agostino DP, Ari C. Age- and Sex-Dependent Modulation of Exogenous Ketone Supplement-Evoked Effects on Blood Glucose and Ketone Body Levels in Wistar Albino Glaxo Rijswijk Rats. Front Neurosci. 2021 Jan 11; 14:618422.
22. Majid B, Maqsood AM, Majid M, Ahmad Z, Mehmood A, Raza SS. Effect of ketogenic diet on biochemical and anthropometric parameters. Int J Health Sci. 2022;6(S7):3224-3230.
23. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. NCCN Guidelines Version 2.2022.