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# Impact of diet and gut microbiota changes in the development of hepatocellular carcinoma

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## Abstract

Hepatocellular carcinoma (HCC) is a primary liver cancer that occurs with a frequency of 85% in patients with liver cirrhosis. It is the sixth most common type of cancer globally. Asia is the continent with the highest incidence (72%), followed by Europe (8%) and Africa (5%). Men are four times more likely than women to develop this cancer, especially in the 70-80 age group. Risk factors include alcoholic liver disease, tobacco use, genetic predisposition, dysmetabolic comorbidities such as type 2 diabetes mellitus and obesity, hepatitis B virus and hepatitis C virus infections, and non-alcoholic fatty liver disease. Unhealthy dietary regimens and gut dysbiosis are additional risk factors that have been recently investigated. These two factors are closely related because the gut microbiota performs several biological functions, including nutrient metabolism, a process that promotes gut homeostasis, known as eubiosis. With regard to the correlation between diet, gut microbiota, and HCC development, there are several mechanisms that have not yet been fully elucidated. This narrative review aims to evaluate the impact of diet and gut microbiota changes in the development of HCC. Our analysis, performed on several clinical and pre-clinical studies, showed that a high-fat diet promotes gut dysbiosis and hepatic fat accumulation, leading to the progression from simple steatosis to HCC, while the Mediterranean diet, rich in fiber and monounsaturated fatty acids, had a protective role. For this reason, international employment of this dietary regimen for therapeutic purposes should be encouraged.

**Keywords:** Liver diseases, dysbiosis, leaky gut, pathogenesis, cirrhosis

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary liver cancer that occurs with a frequency of 85% in patients with liver cirrhosis[1,2]. Men are four times more likely than women to develop HCC, especially in the 70-80 age group[3]. Risk factors are alcoholic liver disease (ALD), tobacco use, genetic predisposition, type 2 diabetes mellitus (T2DM), obesity, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and non-alcoholic fatty liver disease (NAFLD)[4,5]. In the case of HCV infection, mutations in the viral genome that prevent the achievement of a sustained virologic response after antiviral therapy are increasingly common[6-8]. This event is closely related to the circulation of specific viral genotypes[9]. With regard to HBV, its prevention through vaccination has been successful in globally reducing the incidence of HCC[10]. NAFLD represents a possible first stage of liver damage with a specific natural history ranging from accumulation of fat in the liver to necroinflammation, fibrosis, cirrhosis, and HCC[11]. Recently investigated additional risk factors are an unhealthy dietary regimen and gut dysbiosis: these two factors are closely related because the gut microbiota performs several biological functions, including nutrient metabolism, a process that promotes gut homeostasis, known as eubiosis[12]. For instance, the gut microbiota was recently involved in the treatment of HCC[13-15]. Changes in gut bacteria abundance in correlation with the progression from NAFLD to HCC have been investigated in recent studies. In particular, an increase in *Ruminococcus* and *Escherichia* and a decrease in *Lactobacillus* and *Bifidobacterium* have been seen in patients with advanced fibrosis[16]. In addition, alcohol-associated dysbiosis is linked to a reduced biosynthesis of long-chain fatty acids by the *Lactobacillus* genus[17]. At the same time, infective liver cirrhosis showed an increased abundance of *Prevotella*, *Streptococcus*, *Staphylococcaceae*, and *Enterococcus*, as well as decreased *Ruminococcus* and *Clostridium*[18]. Certain dietary regimens have been proven to have a positive effect on liver diseases such as NAFLD; in particular, the Mediterranean diet is rich in monounsaturated fatty acids, which reduce risk factors for metabolic syndrome related to NAFLD, such as waist circumference, high-density lipoproteins, and triglycerides, and has been shown to grant a protective effect against cardiovascular events[19-22]. Risk factors for HCC development are summarized in Figure 1.

As for the correlation between diet, gut microbiota, and HCC development, there are several mechanisms that have not yet been fully elucidated. This narrative review aims to evaluate the impact of diet and gut microbiota changes in the development of HCC.

## DIET AND HCC DEVELOPMENT

Diet refers to the total amount of food individuals consume[23]. In this context, the gut is involved in the fermentation of carbohydrates as it is related to short-chain fatty acids (SCFAs) production, cleavage of proteins into amino acids, synthesis of vitamins, and metabolism of polyphenols[24]. Diet plays a dual role in HCC development: while a diet rich in polyphenols, fiber, and omega-3 grants a protective effect, a diet high in saturated fat, red meat, and fried food may predispose to the development of the disease[25].

### Junk food and HCC

Unhealthy foods, high in calories and low in nutritional value, are referred to as "junk food"[26,27]. Current literature lacks clinical data about the impact of junk food on the development of HCC. However, a recent study was performed by Hymel *et al.* on non-alcoholic steatohepatitis (NASH)-HCC mice models fed with junk food[28]. The Authors reported that a diet high in trans fat, cholesterol, and fructose contributes to HCC development in both lean and obese mice. The design of these mice models of NASH-HCC may be of critical importance for new studies in humans in the near future. Overall, a dietary regimen with junk food promotes the development of metabolic comorbidities, which supports tumorigenesis. At the same time, adipocytes become hypertrophic due to lipid accumulation and activate a series of biochemical pathways, including the overproduction of oxygen-free radicals (ROS) and the synthesis of pro-inflammatory

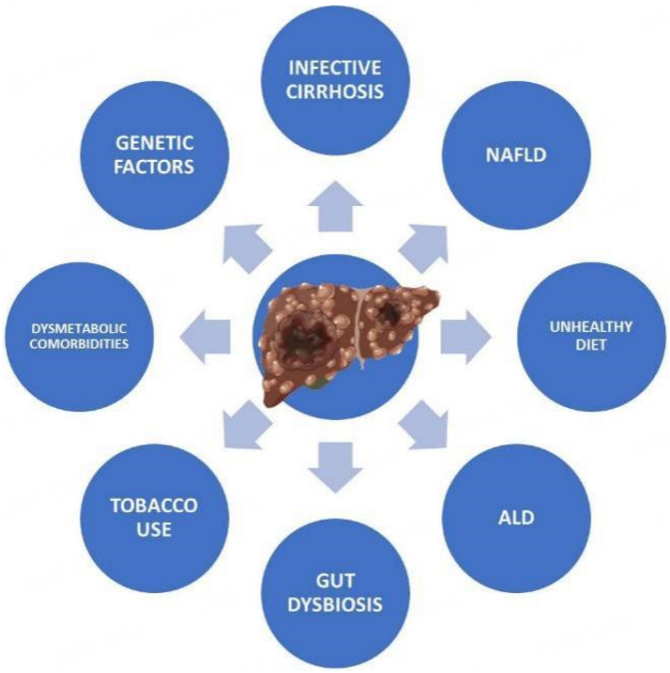


**Figure 1.** Risk factors for HCC development. ALD: alcoholic liver disease; NAFLD: non-alcoholic fatty liver disease; HCC: hepatocellular carcinoma.

cytokines. In addition, pro-inflammatory cytokines activate ROS production by monocytes and macrophages, promoting systemic inflammation and creating an ideal tumor microenvironment[29].

### Ketogenic diet and HCC

The ketogenic diet, characterized by a high fat and a low carb intake, prompts ketosis[30-34]. However, data on its impact on HCC are scarce. Healy *et al.* studied mice fed ketogenic, obesogenic, or control diets, revealing lower tumor burdens in the ketogenic group, regardless of obesity[35]. Elevated interleukin-6 levels correlated with tumor burden, while serum adiponectin inversely was related to sugar intake. Huang *et al.* further investigated ketone body exploitation by HCC, revealing OXCT1's correlation with HCC staging[36]. Ketone body catabolism in HCC cells promoted ATP increase and inhibited AMP-activated protein kinase activation. Byrne *et al.* showed no significant HCC staging alterations in mice fed with ketogenic diets[37]. Despite delayed initiation, limited glucose availability effectively hindered cancer cell reliance on glycolysis. The clinical study performed by Motta *et al.* supported findings in mice models, showing tumor disappearance and clinical improvements after ketogenic diet monotherapy. Furthermore, blood ketone bodies rose, while glucose levels decreased, emphasizing the diet's potential to limit tumor growth by reducing glucose availability[38]. These studies collectively shed light on the ketogenic diet's potential in HCC management, suggesting further research is needed for comprehensive understanding and application.

### Mediterranean diet and HCC

The Mediterranean diet was conceptualized by Ancel Keys in the 1960s as a diet that is low in saturated fat and rich in vegetable oils[39,40]. This diet grants several beneficial effects by reducing the risk of cardiometabolic comorbidities and liver diseases[41-44]. However, there are few clinical studies about its role in preventing the development of HCC. The first study was performed by Turati *et al.*, who evaluated the possible protective effect of the Mediterranean diet in 518 patients with HCC and 772 controls[45]. The study showed that HCC patients were less adherent to the Mediterranean diet. Furthermore, strong adherence to this dietary regimen disfavored the development of HCC among patients with liver cirrhosis of infectious

etiology. In the same year, Li *et al*. assessed the risk of developing HCC in a cohort of 494.942 enrolled patients[46]. Higher adherence scores were associated with a lower risk of HCC and chronic liver disease mortality. Overall, a strong commitment to the Mediterranean diet reduced the incidence of HCC. Likewise, Ma *et al*. evaluated the impact of different dietary scores in a large cohort of patients[47]. The results showed that adherence to the Mediterranean diet did not correlate with the development of HCC. Bogumil *et al.* performed an observational study with a more focused approach to ethnic groups[48]. The dietary regimen of 169.806 individuals belonging to different ethnic groups was analyzed with a clinical follow-up of 17 years. The results showed that a better adherence to the Mediterranean diet reduced the incidence of HCC. This can be explained by its composition, as it primarily comprises foods that are abundant in polyphenols, known for their antioxidant and anti-inflammatory properties. These substances decrease the concentration of ROS and suppress the proliferative pathways of phosphatidylinositol 3-kinase, mitogen-activated protein kinases (MAPK), and nuclear factor kappa B (NF-kB). Furthermore, vitamins C and E, along with flavonoids, prevent DNA damage. The high content of omega-3 reduces cell proliferation, the inflammatory process, and tumor angiogenesis. Moreover, a limited intake of fats hinders the development of hyperinsulinemia and consequently inhibits the growth of cancer cells[49].

### Alcohol intake and HCC

The mechanisms underlying alcohol-related hepatocarcinogenesis are multifactorial. As such, chronic alcohol exposure leads to oxidative stress, DNA damage, and impaired DNA repair mechanisms, which promote the accumulation of genetic mutations and genomic instability, the two hallmarks of cancer development[5,50]. Additionally, alcohol metabolism generates acetaldehyde, a highly reactive and toxic compound that can directly damage DNA and proteins, further contributing to carcinogenesis. Moreover, alcohol-induced liver injury triggers compensatory regenerative responses, leading to the proliferation of hepatocytes and the activation of hepatic stellate cells, which promotes fibrogenesis and creates a pro-carcinogenic microenvironment. Chronic inflammation further fuels carcinogenesis by promoting cell proliferation, angiogenesis, and evasion of immune surveillance. Importantly, the risk of HCC development in individuals with ALD is influenced by various factors, including the duration and intensity of alcohol consumption, concomitant viral hepatitis, and genetic predisposition[51,52]. Furthermore, several factors contribute to the heightened risk of HCC in individuals with combined ALD and NAFLD, especially in individuals with dysmetabolic comorbidities[53]. The combined insult of alcohol-induced liver injury and metabolic dysregulation can synergistically promote hepatic inflammation, fibrosis, and carcinogenesis due to oxidative stress through the previously mentioned shared common pathways[54,55]. Table 1 shows the different pre-clinical and clinical studies on the development of HCC and different dietary regimens.

## GUT DYSBIOSIS AND HCC

The gut microbiota plays an important role in keeping humans healthy through immunity and metabolic processes, but it is also related to several pathologies[56]. As such, it has been suggested that commensal bacteria may be intricately involved both in the pathogenesis and in the prevention of HCC. Gut homeostasis is linked to gut barrier status, with a possible disruption or dysfunction associated with local or systemic consequences[57]. Gut microbiota dysbiosis promotes the development of HCC by increasing the permeability of the gut barrier and triggering liver inflammation[58]. In particular, the leaky gut is defined by the intestinal barrier's increased permeability, which allows lipopolysaccharide (LPS) entry through the portal vein to the liver[59]. This results in the activation of hepatic natural killer T (NKT) cells and an increase in toll-like receptor (TLR) 2 expression by hepatic stellate cells and TLR4, triggering chronic inflammation that promotes liver fibrosis and tumorigenesis. Furthermore, the assessment of endotoxin levels and oxidant status is crucial in comprehending the pathophysiology of NAFLD and its complications. Serum soluble phagocytic NADPH oxidase 2 (sp-NOX2) and urinary 8-iso-prostaglandin F2 alpha (8-iso-PGF2α) evaluation offers an insight into the oxidative stress mechanisms underlying NAFLD

**Table 1. Summary of the different studies on the development of HCC and different dietary regimens**

| Authors | Study model | Dietary regimen | Outcome |
|---|---|---|---|
| Hymel *et al.*, 2022[28] | NASH-HCC mice | Junk food | Contribution of hepatocarcinogenesis in lean and obese mice |
| Healy *et al.*, 2014[35] | C57BL/6N mice | Ketogenic diet | Lower tumor burden compared with high-carbohydrate diets |
| Huang *et al.*, 2016[36] | HCC mice | Ketogenic diet | Early use of the ketogenic diet in mice models has an antitumor effect in the liver |
| Byrne *et al.*, 2018[37] | C57BL/6 mice | Ketogenic diet | A delayed dietary regimen did not lead to any benefit |
| Motta *et al.*, 2020[38] | Two patients with HCC | Ketogenic diet | Disappearance of the tumor, with significant clinical improvements after one year of therapy |
| Turati *et al.*, 2014[45] | 518 patients with HCC and 772 controls | Mediterranean diet | A strong adherence to the Mediterranean diet hindered the development of HCC among patients with liver cirrhosis of infectious etiology |
| Li *et al.* 2014[46] | 494.942 enrolled patients; 509 incident cases of HCC | Mediterranean diet | Strong adherence to the Mediterranean diet reduced the incidence of HCC |
| Ma *et al.*, 2019[47] | 160 patients with incident HCC | Mediterranean diet | The adherence to the Mediterranean diet did not correlate with the development of HCC |
| Bogumil *et al.*, 2019[48] | 169.806 enrolled patients; 605 incident cases of HCC | Mediterranean diet | Strong adherence to the Mediterranean diet reduced the incidence of HCC, independently of ethnicity |

NASH: non-alcoholic steatohepatitis; HCC: hepatocellular carcinoma.

progression. Endotoxins, derived from Gram-negative bacteria of the gut microbiota, induce inflammation in NAFLD by TLR4, which leads to ROS generation within hepatocytes. Elevated serum sp-NOX2 levels lead to NADPH oxidase 2-mediated oxidative stress, which exacerbates liver injury. Urinary 8-iso-PGF2α, a lipid peroxidation end-product, serves as a reliable oxidative stress biomarker in NAFLD, reflecting ROS-induced damage to cellular membranes and organelles. Assessing these biomarkers aids in diagnosing, prognosticating, and devising therapeutic strategies for NAFLD[60]. Overall, several studies analyze the changes in gut microbiota composition during HCC.

## Gut microbiota changes in patients with HCC

Gut dysbiosis is found in HCC patients. One of the first studies was performed by Ponziani *et al.* in patients with liver cirrhosis and NAFLD[61]. A high level of fecal calprotectin was observed in HCC patients, which showed a higher abundance of *Bifidobacterium* and *Akkermansia*. Bacteria belonging to *Ruminococcus* and *Bacteroides* genera were more abundant in HCC patients compared to cirrhotic patients. A strong correlation between gut microbiota profile and systemic inflammation, a process that leads to tumorigenesis, was found among groups. Indeed, cirrhotic patients with NAFLD and HCC lacked protective bacteria, thus engendering a subsequent inflammatory process in the gut. In order to characterize the gut microbiota that triggers the transition from chronic liver disease to HCC, Effenberger *et al.* compared the bacterial profile of patients with HCC and liver cirrhosis to that of patients with NAFLD[62]. Fecal samples

were collected from patients with liver cirrhosis and HCC; both showed a reduced abundance of different taxa, including SCFAs-producing genera such as *Blautia* and *Agathobacter*. Using 16S coupled ribosomal RNA gene and transcriptome sequencing, a direct association was identified between the abundance of the gut bacterial genus and the host transcriptome response within liver tissue. This evidence indicates that perturbations of the gut-liver resident microbiota are a critical determinant of patients with HCC. In these patients, the gut microbiota is characterized by potentially pathogenic bacteria and the development of liver disease is mediated by bacterial metabolites, such as the inhibition of 7α-dehydroxylation, a process that promotes deoxycholic acid synthesis, which is a rate-limiting step of tumorigenesis. In addition, significantly different levels of α-diversity were identified in liver tissue between patients with NAFLD and cirrhosis/HCC, indicating that the tissue-specific microbiota changes at different tumor stages. To understand this microbial abundance, they evaluated the expression of different bacterial genes. Among these, the MT1B gene, which encodes the metallothionein that activates ROS to create oxidative stress, was upregulated during carcinogenesis. However, further studies are needed to better evaluate the molecular mechanisms driving the process. Ma *et al.* discussed a study using summary statistics from whole-genome association studies of gut microbiota and liver cancer[63]. The aim was to explore the causal role of the gut microbiota in the development of primary liver cancer, including HCC and intrahepatic cholangiocarcinoma. The results showed that healthy controls had a higher relative abundance of *Ruminococcaceae* ($P$ = 0.00033), *Porphyromonadaceae* ($P$ = 0.0055), and *Bacteroidetes* ($P$ = 0.021) than patients with liver cancer. In particular, the decrease of *Firmicutes* in favor of *Bacteroidetes* leads to a decrease in the production of trimethylamine-N-oxide, which has been linked to cancer as it induces inflammation through the activation of MAPK-NF-kB signaling to induce angiogenesis[64]. Furthermore, these taxa were correlated with a reduced risk of liver cancer, suggesting potential significance for its prevention and control.

### Gut microbiota changes in HCC patients after immunotherapy

Commensal bacteria may improve the efficacy of cancer therapy by inducing antitumor immune responses. Several studies showed that *Bacteroides fragilis* has a positive influence on immune checkpoint inhibitors (ICI) treatment through activation of CD4+ T cells and interferon (IFN)-γ producing dendritic cells[65,66]. *Bifidobacterium*, *Bacteroides*, *Eubacterium*, and *Fusobacterium* genera have also been shown to increase the efficacy of immunotherapy by raising IFNγ+ and CD8+ T cells' responses[67]. Fecal samples from patients who responded to immunotherapy showed higher taxa richness than those from non-responders. Indeed, *Akkermansia muciniphila* and *Ruminococcaceae* were the most abundant in patients who responded to ICI[68]. According to the Authors, *Akkermansia muciniphila* degrades mucin to produce SCFAs with subsequent activation of G protein-coupled receptors and inhibition of histone deacetylases. The bacterial-induced signal transduction mechanism involves TLR-2, glucagon-like peptide 2, and natural killer cell group 2 member D ligand. This step leads to a decrease in pro-inflammatory cytokines by disfavoring the establishment of a suitable tumor microenvironment. It also promotes the integrity of the intestinal barrier, and for this reason, it is used as a next-generation probiotic. As for *Ruminococcaceae*, its synergistic action in the production of butyrate promotes intestinal balance. Furthermore, this evidence suggests the gut microbiota's ability to promote an antitumor response by activating CD8+T cells, which are essential in controlling HCC growth. In this regard, early changes in gut microbiota could be analyzed to predict immunotherapy outcomes. In the study performed by Lee *et al.*, supportive therapy with *Bifidobacteria* was used in patients with HCC undergoing perioperative hepatectomy[69]. According to linear discriminant analysis, HCC patients with progressive disease after ICI therapy showed a higher abundance of *Prevotella*, while *Veillonella*, *Lachnoclostridium*, and *Streptococcaceae* were the most abundant taxa in the responder's group. Bile acids showed significantly higher concentrations in patients who responded to ICI and were positively related to *Lachnoclostridium* relative abundance. The latter, acting in synergy with other intestinal microorganisms, promotes bile acid homeostasis. However, under imbalanced conditions of gut microflora

and bile acid secretion, there is pathway blockage involving CYP7A1 and SHP, promoting fat synthesis. At the same time, an excess of secondary bile acids derived from the gut microbiota promotes the establishment of an inflammation process, with the activation of mTOR signaling in hepatocytes and subsequent tumorigenesis. Such evidence confirms the role of the gut microflora as an enhancer in immunotherapy[15].

## INTERPLAY BETWEEN DIET, GUT MICROBIOTA AND HCC DEVELOPMENT

To date, most of the evidence on the role of different dietary regimens and gut microbiota in HCC development is based on functional studies in mice models[70]. More studies are needed to define the microbial and metabolite signatures of HCC in order to provide a potential therapy[71-73]. The metabolic role of the gut microbiota can influence several disease conditions, including liver cancer[74]. Yamada *et al.* assessed the promotion of HCC development using high-fat diets that induce steatohepatitis (STHD-01) in mice models[75]. Mice fed the STHD-01 diet developed HCC and, subsequently, HCC after 9 and 41 weeks. The impact of the gut microbiota was highlighted by antibiotic treatment, which not only dramatically reduced the accumulation of secondary bile acids but also significantly reduced liver disease and suppressed the tumor. The bacterial genera *Bacteroides* and *Clostridium* cluster XVIII were increased, while the *Bifidobacterium*, *Prevotella,* and *Streptococcus* genera decreased. The cause triggering the development of HCC is related to the products of the gut microbiota after following the STHD-1 high-fat diet, such as secondary bile acids: specifically, an increase in the transcription of cytochrome P450 cholesterol 7 alpha-hydroxylase, which is involved in the synthesis of bile acids in the liver. 7α-hydroxycholesterol is converted to 7α-hydroxy-4-cholesten-3-one, which is the precursor for the synthesis of cholic acid and chenodeoxycholic acid. In line with the accumulation of cholesterol in the liver, the concentration of total bile acids was significantly higher in the plasma and feces of mice fed with the STHD-01 diet [Figure 2A][76-78]. Zhang *et al.* showed that elevated dietary cholesterol could promote liver steatosis, steatohepatitis, fibrosis, and HCC in mice models[79]. The development of cholesterol-induced NAFLD-HCC was related to dysbiosis of gut flora. In mice fed with a high fat/cholesterol (HFHC) diet, increases in *Mucispirillum* and *Desulfovibrionaceae* taxa were observed. On the other hand, *Bacteroides* and *Bifidobacterium* decreased. Germ-free mice subjected to gastric probing of feces derived from HFHC-fed mice showed lipid accumulation and liver inflammation. As reported by the Authors, the development and progression of NAFLD-HCC in these mice models are triggered by a prolonged dietary regimen rich in cholesterol, supported by the gut microflora [Figure 2B]. In order to highlight the metabolic phenotypes involved in NAFLD-HCC, the Authors assessed the serum metabolites of mice fed HFHC and high fat low carb (HFLC) diets, respectively. They showed that bile acid metabolism was altered in mice after a HFHC diet. In addition, LPS levels in the portal vein were higher than in mice fed with the HFLC diet, indicating that the HFHC diet was able to impair intestinal barrier function. In addition, mice fed a cholesterol-rich diet developed insulin resistance and had elevated oxidative stress characterized by the activation of mediators of inflammatory responses. This was justified by an increased accumulation of hepatic ROS, CD8+ T cells, NKT cells, and their inflammatory cytokines. Moreover, atorvastatin, an anti-cholesterol treatment, suppressed cholesterol-induced gut microbial dysbiosis to prevent the development of NAFLD-HCC. Therefore, cholesterol inhibitors and gut microbiota manipulation may be an efficient preventive approach for NAFLD-HCC development.

Recent evidence shows that an excessive presence of cholesterol in the alimentary regimen is the main factor responsible for HCC development. In fact, it reduces the abundance of *Bifidobacterium* and *Bacteroides* genera and promotes gut dysbiosis; at the same time, triglycerides and very low-density lipoproteins are produced. The latter transport triglycerides from the liver to tissues, but if they are not correctly formed, triglycerides accumulate in hepatocytes, inducing NAFLD, which in turn can advance into HCC

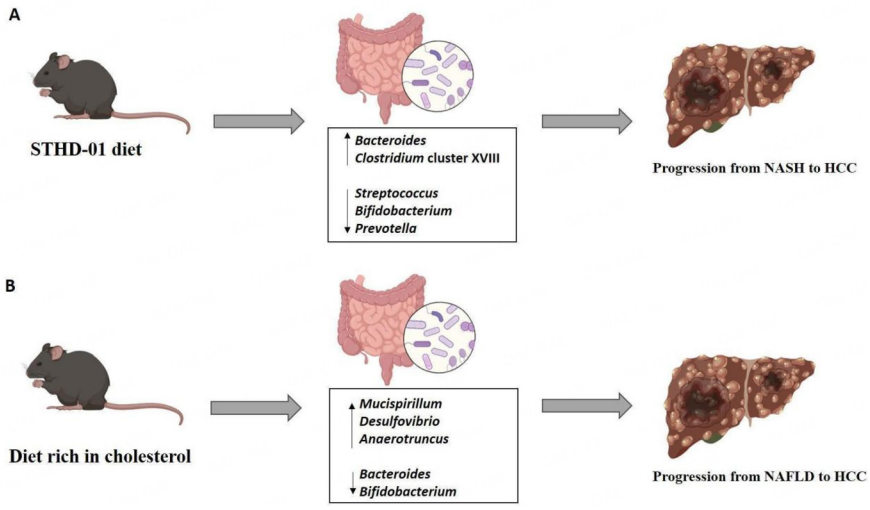


**Figure 2.** Gut microbiota changes and progression to HCC in mice models fed with STHD-01(A) and rich in cholesterol diets (B). STHD: high-fat diets that induce steatohepatitis; NASH: non-alcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease.

[Figure 3A][80]. Excessive sugar intake negatively affects the composition of the gut microbiota. The main culprit is fructose, widely contained in sweets and drinks. The predominance of acetate-producing gut bacteria, such as *Bacteroides* genus, allows its entry into the Acetyl-CoA production cycle with induction of hepatic lipogenesis. This step contributes to fatty liver formation and subsequent HCC [Figure 3B][81]. Proper nutritional intake of fiber, also supported by the Mediterranean diet, promotes the increased abundance of a healthy microbiota mainly comprised of *Bifidobacterium* and *Faecalibacterium* genera. Fibers such as inulin and pectin are fermented by gut-resident microorganisms into SCFAs, thus ensuring gut impermeability and consequently reducing the risk of liver damage [Figure 3C][82]. The association between dietary fats, gut microbiota composition, and HCC development is quite controversial. High saturated fat intake induces changes in the gut microbial flora composition by reducing the abundance of genera such as *Bacteroides*, *Bifidobacterium*, and *Eubacterium*, promoting insulin resistance, which is a risk factor for the development of HCC. On the contrary, omega-3 polyunsaturated fatty acids, contained in some foods in the Mediterranean diet, limit hepatic triglyceride accumulation, insulin resistance, and pro-inflammatory pathways, thus promoting gut eubiosis [Figure 3D][83]. Overall, further functional studies are needed to better understand this complex interplay.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Diet and gut microbiota are the main actors in the development of HCC, from as early as the onset of liver steatosis. However, functional studies are necessary to highlight the metabolic pathways that lead to this evidence. A healthy diet such as the Mediterranean diet can prevent the onset of liver cancer. It is therefore necessary to use this dietary regimen for therapeutic purposes and to promote its adherence internationally. Concurrently, probiotics, prebiotics, and symbiotics should be used for preventive purposes to restore gut eubiosis, while another approach could be the use of cholesterol inhibitors to prevent HCC[84]. Finally, all microbiology laboratories should implement the use of gut microbiota sequencing to evaluate the changes in bacterial composition, mainly in critical area patients[85-87]. Shotgun metagenome sequencing, especially when combined with deep learning tools, quantifies the abundance of gut bacteria with high resolution[88,89]. The application of these data, together with clinical and laboratory parameters, is crucial in diagnosis and targeted therapies. Consequently, there is a significant shift toward the use of machine learning approaches in the genomics discipline[90-92]. At the same time, machine learning finds wide application as a predictive

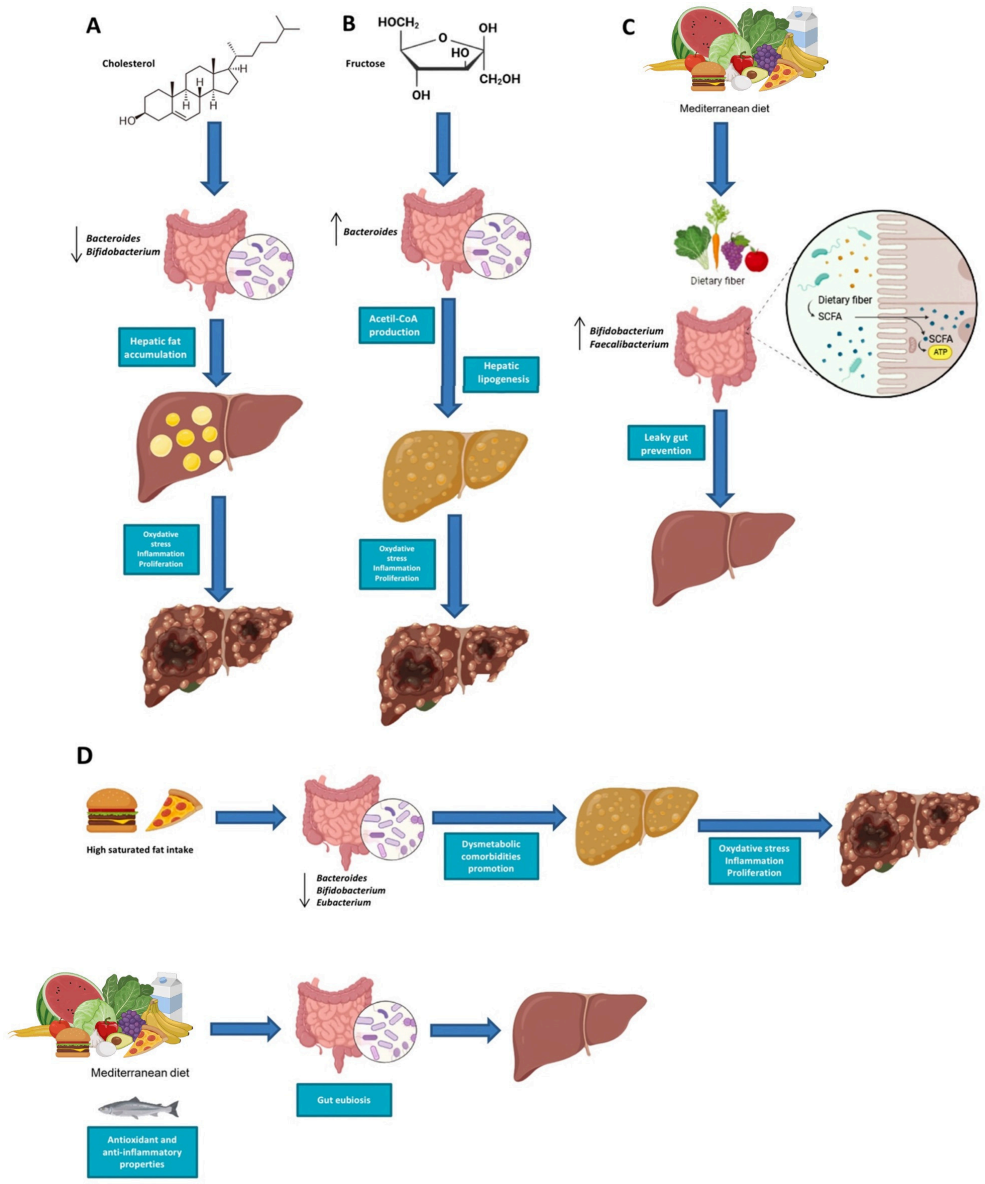


**Figure 3.** Pathogenetic ways involving gut microbiota composition, HCC development and cholesterol (A) fructose (B) fibers (C) and fats (D) dietary intake. HCC: hepatocellular carcinoma.

model of inflammatory bowel diseases in the gastroenterology field[93]. Diagnostic and therapeutic strategies focused on the gut microbiota are possible because of the continuous collaboration between gastroenterologists and microbiologists. In conclusion, we propose the following future perspectives in Table 2.

**Table 2. Take-home messages and future perspectives**

| Take-home messages | Future perspectives |
|---|---|
| Diet and gut microbiota are involved in HCC development from as early as the onset of liver steatosis | Functional studies are needed to highlight the metabolic pathways between diet, gut microbiota, and HCC development |
| Probiotics, prebiotics and symbiotics can be used for preventive purposes to restore gut eubiosis | Wide therapeutic use of the Mediterranean diet against HCC |
| Application of gut microbiota analysis in each microbiology laboratory | Increased application of machine learning to genome sequencing techniques |

HCC: hepatocellular carcinoma.

## DECLARATIONS

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### Authors' contributions

Wrote, reviewed, and edited the manuscript: Scarlata GGM, Cicino C
Provided figures and tables: Scarlata GGM, Cicino C
Reviewed and approved the final manuscript as submitted: Spagnuolo R, Marascio N
Read and approved the final manuscript: Quirino A, Matera G, Dumitrașcu DL, Luzza F, Abenavoli L
Conceptualized and designed the review: Abenavoli L

### Availability of data and materials

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.



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