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Type 2 Diabetes and Hepatocellular Carcinoma: Risk Factors and Pathogenesis

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Abstract

Purpose of review: This review aims to assess the epidemiological evidence for a link between type 2 diabetes and hepatocellular carcinoma, and to investigate possible pathophysiological mechanisms.

Recent findings: The presence of type 2 diabetes significantly increases the risk of developing hepatocellular carcinoma, and treatment with metformin may be associated with a lower risk. Treatment with insulin and sulphonylureas may be associated with increased risk. The pathophysiology underlying development of hepatocellular carcinoma in this context is complex and is likely to involve increased proinflammatory mediators, oxidative stress, JNK-1 activation, increased IGF-1 activity, altered gut microbiota and immunomodulation.

Summary: Hepatocellular carcinoma incidence is increasing and this is likely to be linked to the increasing incidence of type 2 diabetes, obesity and the metabolic syndrome. These conditions increase the risk of developing hepatocellular carcinoma and a greater understanding of the underlying pathophysiology may help with the development of novel treatments.

Introduction

Type 2 diabetes and hepatocellular carcinoma (HCC) are closely linked due to their association with non-alcoholic fatty liver disease (NAFLD) and obesity. HCC has emerged as one of the commonest cancers worldwide and now accounts for 700,000 deaths per year (1). Global incidence of HCC has also increased significantly over the previous 2 decades (2, 3). There is now robust evidence showing a strong association between NAFLD and type 2 diabetes, with the development of insulin resistance being a key pathophysiological factor linking both diseases (4). It has been estimated that more than 70% of individuals with type 2 diabetes also have NAFLD (5, 6). NAFLD represents a wide spectrum of clinical liver disease, ranging from hepatic steatosis, to significant fibrosis, steatohepatitis (NASH) and end stage liver cirrhosis. It has recently been shown that up to 20% of individuals with both NAFLD and type 2 diabetes will have clinically significant hepatic fibrosis (7, 8), conferring

increased risk of progression to more severe forms of liver disease. This review will examine the evidence for a link between diabetes, NAFLD, obesity and HCC from an epidemiological and a pathophysiological perspective.

Type 2 Diabetes and Obesity as Risk Factors for Hepatocellular Carcinoma

A recent systematic review and meta analysis from 2012 looked specifically at diabetes as a risk factor for HCC (9). The authors included data from 27 case control and cohort studies including a total of over 2 million individuals with diabetes, follow-up over 80 million person years, detecting over 21,000 cases of incident HCC. The results of this study showed that people with diabetes had a 2.31 fold increased risk of developing HCC, compared to those subjects without diabetes. Additionally, the authors reported a 2.43 fold increased risk of death due to HCC in those subjects with diabetes compared to those without. A previous systematic review and meta-analysis had been performed 6 years previously and had concluded that diabetes was associated with a 2.5 fold greater risk of developing HCC (10).

A number of studies have been published since these meta-analyses. A study from 2013 investigated data from the Singapore Chinese Health Study which was initially set up to examine links between diet and cancer (11). This study included over 63,000 individuals aged 45-74 years, and over the period of study, 499 developed HCC. Having a diagnosis of type 2 diabetes at baseline was associated with a hazard ratio of 2.14 for the development of HCC as compared with those with no baseline diagnosis of diabetes. An Italian study published in 2015 studied 224 patients with HCC and compared these subjects with 389 control patients (12). 30.9% of cases in the HCC group had diabetes, as compared with 13.5% of the control group, and this corresponded to an adjusted odds ratio of 2.25. A study from Taiwan examined over 19,000 individuals newly diagnosed with diabetes and compared these subjects to a control group without diabetes (13). The authors observed a 2 fold higher incidence of HCC in the group with diabetes, and this was associated with an adjusted odds ratio of 1.73. Data from these studies are summarised in **Table 1**.

Table 1 – Characteristics of studies linking type 2 diabetes with incident hepatocellular carcinoma

Study	Study design	Sample size	Number of HCC cases	Country / population	Relative risk of developing HCC (95% CI)	Notes
El-Serag 2006	Meta-analysis	615,569	3229	Various	2.5 (1.9 - 3.2)	Based on cohort studies, no RCTs
Wang 2012	Meta-analysis	2,000,000+	21,000	Various	2.31 (1.87 - 2.84)	All observational studies, no RCTs
Lai 2012	Retrospective population cohort study	96,745	1939	Taiwanese	1.73 (1.47 – 2.03)	Retrospective study at higher risk of bias
Koh 2013	Prospective cohort	63,257	499	Singapore Chinese	2.14 (1.69 – 2.71)	No differentiation between T1DM and T2DM, only considered DM at baseline
Miele 2015	Case control study	613	224	Rome, Italy	2.25 (1.42 – 3.56)	Small sample size, did not adjust for overweight / obesity

A further systematic review was published in 2016 investigating risk of HCC amongst those subjects with a pre-existing diagnosis of chronic hepatitis C infection and the effect of diabetes (14). Of the 7 studies included, 5 demonstrated significantly increased risk of HCC in patients with chronic hepatitis C infection who also had diabetes as compared with those without diabetes. These data would suggest a synergistic, additive interaction between diabetes and the other major risk factor for HCC, and indeed there are similar data to suggest this synergism also exists with alcoholic liver disease (15).

There is also evidence to suggest that certain anti-diabetic medications may have a modifying effect on the risk of developing HCC. This issue was recently addressed in a comprehensive meta-analysis (16). The authors included 10 studies in their review; 5 case control studies, 3 cohort studies and 2 randomised controlled trials. These studies included

over 330,000 individuals with diabetes and over 22,000 cases of HCC. The authors demonstrated an overall 50% reduction in incidence of HCC associated with the use of metformin from 8 studies which reported use of this drug. This significant positive effect persisted after adjustment for the effects of other anti-diabetic medications. Eight of the included studies addressed the effect of sulfonylureas, and the authors reported an overall increased risk of 62% associated with the use of this class of drug as compared with non-use with a significant adjusted odds ratio of 1.62. Use of thiazolidinediones was also examined on the basis of data from 4 studies, but no statistically significant risk modifying effect was seen. Finally, use of insulin was assessed. Insulin use was compared with non-use of insulin using data from 7 studies, and the authors observed a 161% increased risk of developing HCC associated with insulin use. This effect persisted when only using results from studies that accounted for risk-modifying effects of other anti-diabetic medications. It is unclear whether this effect is related to toxicity associated with the medication, or if it is simply reflective of increased risk in individuals with more severe disease.

There is also an association more generally between other features of the metabolic syndrome and HCC. A meta-analysis of 11 studies was published in 2007 which demonstrated a relative risk of developing HCC of 1.17 for overweight individuals as compared with normal weight controls, and a relative risk of 1.89 for obese individuals as compared with normal weight controls (17). A further meta analysis published 5 years later assessed 26 prospective studies including over 25,000 cases of primary liver cancer and showed that those with excess body weight (as defined by a body mass index greater than 25 kg/m^2) had a summary relative risk of HCC of 1.48, while those with obesity had a summary relative risk of HCC of 1.83 (18). A systematic review based on 9 studies purely assessing Japanese populations revealed a relative risk of 1.74 for overweight or obese people as compared with those who were either normal weight or low weight (19). Insulin resistance and the metabolic syndrome are specifically associated with abdominal obesity which is more closely correlated with visceral adiposity (20). A recent prospective cohort study reported an association between abdominal obesity and HCC with waist-to-height ratio having the strongest association, an association which was found to be independent of general body weight (21).

The presence of an association between NAFLD and HCC is well established, although quantifying the exact level of risk is problematic due to high variability of outcomes in reported data. Data is variable due in part to the vagaries of diagnosing NAFLD, however it is reported that the risk of progressing to cirrhosis in NAFLD is between 4 and 20% (22, 23). The risk of then going on to develop HCC from cirrhosis secondary to NAFLD has been reported as being between 2.4 and 12.8% (24-26). The risk of developing HCC would appear to be related to the severity of hepatic fibrosis, which itself is related to adverse features of the metabolic syndrome. It is also known that HCC can develop in individuals with NAFLD in the absence of cirrhosis, and it is important for clinicians to be aware of this phenomenon (27-29). Interestingly, some investigators have suggested that non-cirrhotic HCC may account for as many as 50% of all NAFLD related HCC cases (30).

Pathophysiology of Hepatocellular Carcinoma and Links with Diabetes and Insulin Resistance

The epidemiological associations between diabetes, NAFLD and HCC are proven. Although the pathophysiology underpinning this association is not clear, understanding of the pathophysiology of HCC in this context has improved in recent years.

The availability of next generation sequencing (NGS) and other "omics" technologies have helped facilitate increased understanding of hepatocarcinogenesis in general. A recent whole exome sequencing study of 243 liver tumours could not identify any particular mutation signature unique to HCC in the context of NAFLD, in contrast to HCC secondary to alcoholic liver disease or hepatitis B virus where such signatures were identified (31). For HCC in general, mutations were most commonly found in *TP53* (a cell cycle regulator), *CTNNB1* and *AXIN1* (both components of the β-catenin / WNT signalling cascade), and the gene encoding for albumin.

Prior to the advent of NGS techniques, comparative genomic hybridisation was used in multiple studies to look for chromosomal insertions or deletions across the whole genome. These studies demonstrated a high level of genomic instability in individuals who had progressed to NASH; up to 20 fold higher than those with NAFLD alone (32). Such studies identified two genes specifically associated with HCC occurring due to NASH; phosphodiesterase 1B (*PDE1B*) and exportin 4 (*XPO4*) (33). Epigenetic changes have also been suggested to play a role in HCC pathogenesis, and it has been shown that methylation changes associated with *PDE1B* are associated with differences in clinical outcome and survival (34). Additionally, hypermethylation of the cadherin-1 (*CDH1)* gene has been associated with HCC secondary to NAFLD (35).

Genetic variation in the patatin like phospholipase domain-containing protein 3 (*PNPLA3*) gene has been shown to be of importance when considering the risk of metabolic liver disease in general. This gene encodes for a protein called adiponutrin which appears to be located in intrahepatic lipid droplets and both effects to promote lipogenesis (36) and lipolysis (37) have been described. It has been reported that genetic variation within the *PNPLA3* gene is strongly associated with increased hepatic inflammation and hepatic steatosis, and those subjects that have both alleles affected (i.e. homozygous for *PNPLA3* 148MM) have twice the hepatic fat content of non-carriers (38). It has subsequently been shown that carriers of the genetic polymorphism that encodes for the I148M variant allele are at greatly increased risk of developing HCC, and it has been suggested that testing for genotype status could contribute to risk stratification in certain populations (39).

Type 2 diabetes, obesity and NAFLD are all strongly associated with insulin resistance (40), and also with a chronic low grade inflammatory state (41). There is evidence to suggest that these factors can contribute significantly to the development of HCC by promoting cellular growth and proliferation. As insulin resistance develops, free fatty acids are increasingly released from adipocytes and there is increased release of proinflammatory cytokines such

as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), resistin and leptin, alongside decreased release of the adipokine adiponectin (42). These factors create an environment that leads to increased hepatic inflammation and steatosis (43). There is also increased release of the NF-κB family of transcription factors (44) which are known to play a major, if complex and incompletely understood, role in the initiation and progression of many forms of cancer (45). This relationship was assessed in a study which examined murine liver parenchymal cells which were deficient in a crucial activation factor for NF-κB (46). Removal of this factor led to the spontaneous development of HCC in the liver tissue, preceded by steatohepatitis. The authors speculated that the hepatocyte injury / cell death / regeneration cycle and its subsequent proliferative cellular response may potentially predispose to hepatic carcinogenesis.

As insulin resistance progresses, insulin concentrations in the blood increase leading increased production of insulin-like growth factor 1 (IGF-1), and this hormone is known to stimulate cellular proliferation and also inhibit apoptosis within the liver (47, 48). As well as IGF-1, insulin also stimulates insulin receptor substrate-1 (IRS-1) which is known to play a role in intracellular cytokine signalling pathways implicated in the development of HCC (49). Adiponectin is known to have anti-inflammatory properties, and has been shown in animal models to decrease angiogenesis and modify the apoptotic response (50). Collectively, these factors will lead to increased cellular growth and proliferation within the liver which could contribute towards carcinogenesis.

NASH, and the cellular environment in which it develops, is associated with oxidative stress and the production of reactive oxygen species and it is possible that this may also be contributing to tumourigenesis. Animal models have shown that hepatocytes with fatty infiltration demonstrate increased production of reactive oxygen species (51). 4-hydroxy-2-nonenal is a product of phospholipid peroxidation and is associated with considerable cytotoxicity and reactivity (52). It has been reported to have a causative role in the development of pathogenic mutations in the tumour suppressor gene p53 which is itself implicated in a wide variety of cancers including HCC (53).

Another factor which is likely to play an important role is the c-Jun amino terminal kinase 1 (JNK1), which has been linked with HCC in the context of insulin resistance, NAFLD and obesity. JNK1 is part of the mitogen associated protein kinase (MAPK) family and is known to have an important regulatory role in many cellular processes including apoptosis (54). There is evidence to link the obese state with pathologically increased JNK1 activity, and factors associated with insulin resistance such as increased free fatty acids, TNF-α and oxidative stress are all known to activate JNK1 (55). It has been shown that individuals with NASH exhibit higher levels of the phosphorylated, active form of JNK1 than those with just NAFLD (56). An interesting experimental study from 2009 showed that JNK1 double knockout mice are resistant to weight gain and the development of insulin resistance and steatohepatitis which normally occurs in response to an experimental high fat diet (57). There is also clear evidence of a link between JNK activity and development of HCC. JNK1 has been shown to be highly active in 17 out of 31 human HCC biopsy samples (58), and there is hope that targeting JNK1 may in the future prove to be a key step in providing better treatments for people with HCC (59).

More recently evidence has emerged that the adaptive immune response may contribute towards the development of HCC secondary to NAFLD. A recent study from Chi Ma et al demonstrated that impaired regulation of lipid metabolism in NAFLD is associated with a selective loss of CD4+ T lymphocytes within the liver but not CD8+ T lymphocytes resulting in accelerated carcinogenesis (60). The authors also observed that the CD4+ T cells had a higher mitochondrial mass than the CD8+ T cells and generated higher levels of reactive oxygen species leading to increased oxidative stress. Crucially, they also observed that blockade of the formation of reactive oxygen species in this setting reversed the depletion of CD4+ cells and attenuated the development of HCC. In an additional study, an experimental model was developed by feeding mice a choline-deficient high fat diet to generate NASH and it was noted that this activated CD8+ T lymphocytes, natural killer cells and inflammatory cytokines (61). In this instance, CD8+ T cells and natural killer cells were found to promote both NASH and the subsequent development of HCC.

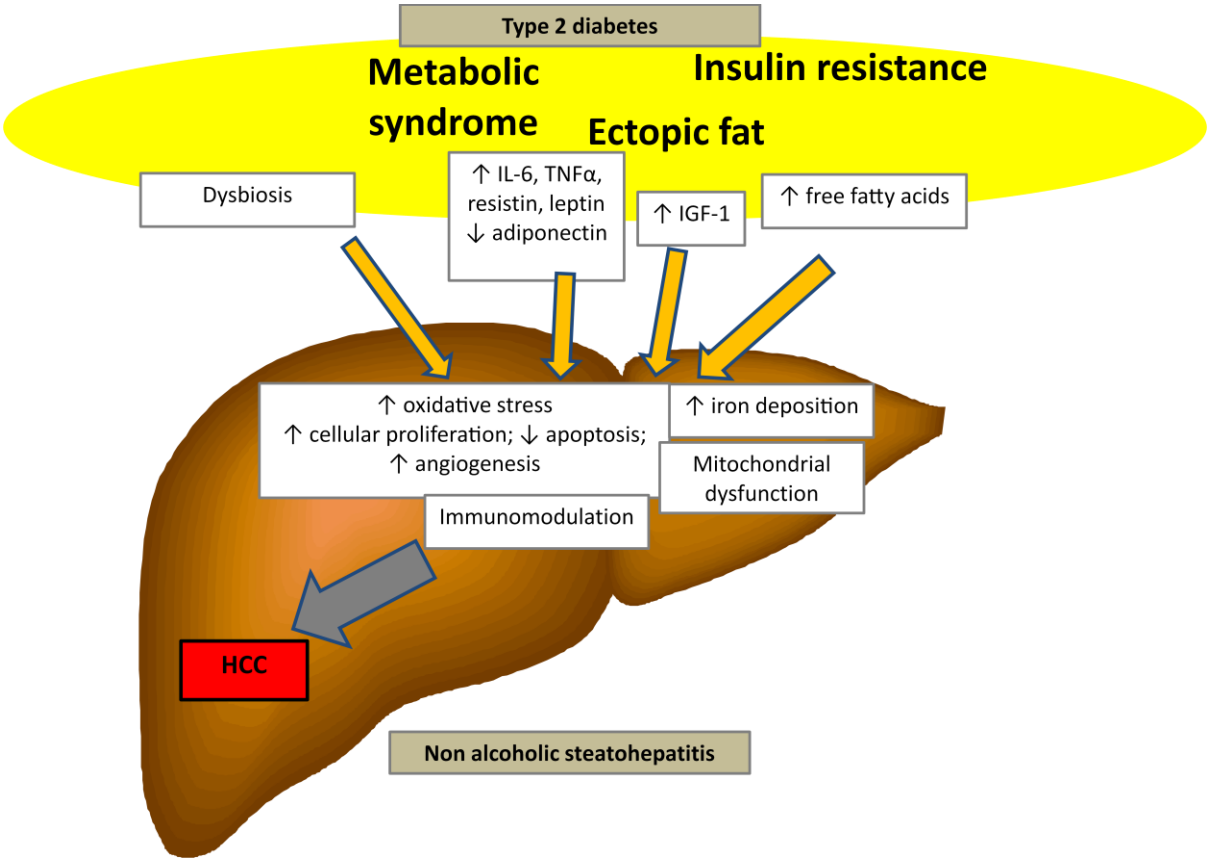
Alterations in gut microbiota have long been considered to play in role in the pathogenesis of metabolic disease associated with type 2 diabetes, obesity and NAFLD (62). There is also evidence to suggest that this may be linked to the development of HCC. A recent study investigated faecal samples from 105 individuals with HCC and compared faecal microbial composition with samples from 131 healthy control subjects (63). They observed evidence of dysbiosis associated with more advanced HCC; specifically decreases in Bacteroidetes and increases in Proteobacteria and Fusobacteria which are consistent with changes seen in animal studies. Evidence from animal studies suggests that the relationship between alterations in the intestinal microbiome and development of HCC is likely to be complex. One group used transgenic mice with non-functional toll-like receptor 4 (TLR4) genes to investigate this (64). They subjected these mice, along with wild type controls, to diethylnitrosamine and carbon tetrachloride to induce a chronic liver injury characterised by inflammation, fibrogenesis and increased levels of endotoxin. This injury typically leads to the development of HCC in experimental models. The TLR4 deficient mice showed no differences to control mice in terms of tumour initiation, but there was a marked difference in tumour promotion as wild type mice displayed increased cellular proliferation and increased expression of epiregulin which is part of the epidermal growth factor family of peptides and thought to contribute to the development of HCC. They also tested gut sterilization with a regime of oral antibiotics and found that this decreased hepatic proliferation and fibrogenesis, suggesting that intestinal microbiota are required for hepatic tumourigenesis. Finally, an additional study investigated the link between obesity-induced alterations in gut microbiota and development of HCC (65). The authors showed in an experimental mouse model that both dietary-induced and genetic obesity alters the gut microbiota and increases levels of the gut bacterial metabolite and secondary bile acid deoxycholic acid. This bile acid may then alter the secretory profile of hepatic stellate cells leading to increased production of fibrotic, inflammatory and tumourigenic factors facilitating the development of HCC. They also observed that blocking the production of deoxycholic acid, or reducing gut bacteria using oral antibiotics, effectively blocked the development of a chemically-induced experimental form of HCC.

Recently evidence has emerged indicating an association between iron excess, insulin resistance and HCC. It has long been recognised that iron overload is a significant risk factor for type 2 diabetes, with the underlying pathophysiology thought to relate to a defect in the early insulin response to glucose (66). Hereditary haemochromatosis is a common genetic condition leading to iron overload and untreated it can result in cirrhosis, it is therefore not surprising that HCC is responsible for up to 45% of deaths in this patient group (67). One group looked at hepatic iron content in biopsy specimens from 153 patients with NASH cirrhosis, 51 of which also had HCC (68). They observed that significant hepatic iron deposition was more common in those with HCC than those without. An earlier study had shown that the presence of hepatic iron in patients with NASH was associated with increased hepatic fibrosis (69). Clearly these observations are not evidence of causation but it is possible that iron metabolism is altered in patients with more severe metabolic liver disease and that this may predispose to poorer clinical outcomes and HCC.

It has long been thought that mitochondrial dysfunction plays a significant role in the development of NAFLD and this is thought to include altered mitochondrial DNA, structural mitochondrial lesions, decreased activity of the respiratory chain enzymes and abnormal beta-oxidation (70). Evidence also exists of mitochondrial dysfunction contributing to the development of HCC. Various somatic mitochondrial DNA mutations have been detected in human HCC biopsy samples including insertions, deletions and a significant decrease in copy number (71). Mitochondrial dysfunction can result in increased generation of reactive oxygen species which may also contribute to hepatic carcinogenesis as described above.

Figure 1 summarises these pathophysiological processes and illustrates the links between them.

Figure 1 – A summary of key risk factors and pathophysiological processes underlying the development of hepatocellular carcinoma



Metabolic syndrome, insulin resistance and increased ectopic fat are all strongly associated with type 2 diabetes. These factors lead to activation of JNK-1 which has a direct tumour promoting effect. Insulin resistance leads to increased IGF-1 which promotes hepatic cellular growth and proliferation. Insulin resistance also alters gut microbiota and increases circulating free fatty acids which promote hepatic steatosis. Increased free fatty acids also lead to increased proinflammatory cytokines which affect cellular proliferation and survival, and also have a tumour-promoting immunomodulatory effect. Hepatic steatosis is directly associated with development of HCC, and also indirectly through increased oxidative stress and a possible effect on iron metabolism. Increased oxidative stress is associated with NASH and may also result from mitochondrial dysfunction, all of which may contribute to hepatic carcinogenesis.

Hepatitis C is a major risk factor for HCC, and there is also evidence linking it with type 2 diabetes. A meta-analysis of 34 studies showed that people with hepatitis C are at increased risk of developing type 2 diabetes as compared with non-infected people (72). There is also evidence suggesting that type 2 diabetes may predispose to hepatitis C infection, although the mechanisms for this are not known (73). There is also now a significant body of evidence to suggest that the presence of type 2 diabetes and insulin

resistance is associated with significantly poorer outcomes in individuals with hepatitis C infection, increasing the risk of liver fibrosis and cirrhosis, worsening the response to antiviral therapy, increasing the risk of HCC, increasing the chance of death due to liver-related causes, and shortening the time from diagnosis to liver transplantation (74). As such it is clear that there is a bidirectional relationship between type 2 diabetes and hepatitis C infection, and both of these conditions act synergistically to increase the risk of developing HCC.

Treatment

There are 3 principle treatment options for HCC, and these are generally the same regardless of aetiology; orthotopic liver transplantation, resection and radiofrequency ablation. Liver transplantation rates for NAFLD-related HCC have increased dramatically in recent years, and numbers increased 4 fold in the US between 2002 and 2012 (75). There is evidence to suggest that individuals with NAFLD-related HCC are less likely to receive curative treatment than those with hepatitis C related HCC, and are more likely to receive no treatment at all (76). Good quality outcome data is severely lacking for NAFLD-related HCC patients who receive curative treatment, and all the studies that have investigated this involve very small numbers. From this limited data, 1 year overall survival has been reported as being 83-95%, and 5 year survival has been reported as being 54-65% (77). These figures are broadly concordant with survival for HCC of other aetiologies.

Conclusion

There is now robust evidence of an association between the development of HCC and the collection of metabolic disorders connected via insulin resistance; type 2 diabetes, obesity and NAFLD. These data are supported by genetic data from genome-wide studies as well as single gene studies such as those implicating variation in *PNPLA3* as a predisposing risk

factor for the more severe forms of NAFLD and for HCC. In the insulin resistant state production of pro-inflammatory mediators is dramatically increased and this can lead to the generation of a hepatic microenvironment in which cellular proliferation and growth is promoted which can facilitate the development of HCC. It can also lead to oxidative stress and the production of reactive oxygen species. In addition to this, insulin resistance and the associated conditions lead to alterations in gut microbiota and evidence from animal studies is beginning to emerge suggesting that this may also be a significant contributory factor to hepatic carcinogenesis. A better understanding of these pathophysiological processes and how they relate to insulin resistance, type 2 diabetes and obesity may help in the future development of rational, targeted treatments for patients with HCC.

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Compliance with Ethics Guidelines

Conflict of Interest

P. Wainwright and E. Scorletti declare that they have no conflict of interest.

C. D. Byrne is Principal Investigator for the INSYTE trial (**IN**vestigation of **SY**nbiotic **T**reatm**E**nt in NAFLD). The INSYTE randomized placebo-controlled double blind trial is testing the effects of a synbiotic on liver fat, disease biomarkers and intestinal microbiota in non-alcoholic fatty liver disease and is currently in recruitment phase (www.clinicaltrials.gov NCT01680640). The INSYTE trial is completely independent of Industry, but Christian Hansen (Denmark) are providing the synbiotic intervention and placebo at no cost to the Investigators.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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