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Review

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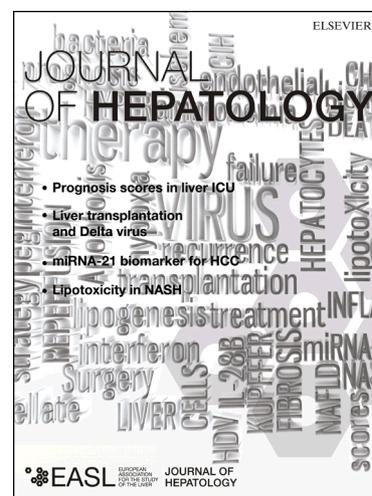
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Hepatocellular Carcinoma and Lifestyles

Uttara Saran^{1,2}, Bostjan Humar³, Philippe Kolly^{1,2} and Jean-François Dufour^{1,2}

¹Hepatology, Department of Clinical Research, University of Berne, Berne, Switzerland

²University Clinic of Visceral Surgery and Medicine, Inselspital Berne, Berne, Switzerland

³Department of Visceral & Transplantation Surgery, University Hospital Zürich, Zürich, Switzerland

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Corresponding Author

Prof. Jean-François Dufour, M.D.

University Clinic for Visceral Surgery and Medicine,
Inselspital, Bern, Switzerland

Phone: +41 31 632 26 95

Fax: +41 31 632 97 65

E-mail : jean-francois.dufour@insel.ch

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Abbreviations ACC: acetyl coenzyme A carboxylase, BMI: body mass index, DM: diabetes mellitus, FAS: fatty acid synthase, β -HAD: β -hydroxyacyl-CoA dehydrogenase, HBV: hepatitis B virus, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, IHF: intrahepatic fat, MDA: malondialdehyde, NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis, FFA: free fatty acids, OLETF: Otsuka Long-Evans Tokushima Fatty, NEFA: nonesterified fatty acids, PA: physical activity, SCD-1: stearoyl-CoA desaturase-1, SREBPF: sterol regulatory element-binding transcription factor 1, TNF- α : tumor necrosis factor- α .

Keywords: Liver cancer, exercise, obesity, diabetes, insulin, AMPK, mTOR

Abstract

The majority of hepatocellular carcinoma occurs over pre-existing chronic liver diseases that share cirrhosis as an endpoint. In the last decade, a strong association between lifestyle and hepatocellular carcinoma has become evident. Abundance of energy-rich food and sedentary lifestyles have caused metabolic conditions such as obesity and diabetes mellitus to become global epidemics. Obesity and diabetes mellitus are both tightly linked to nonalcoholic fatty liver disease and also increase hepatocellular carcinoma risk independent of cirrhosis. Emerging data suggest that physical activity not only counteracts obesity, diabetes mellitus and nonalcoholic fatty liver disease, but also reduces cancer risk. Physical activity exerts significant anticancer effects in the absence of metabolic disorders. Here, we present a systematic review on lifestyles and hepatocellular carcinoma.

Key points

- The growing epidemic of metabolic conditions such as obesity and DM and their close link to NAFLD in turn contribute to the increased risk of HCC development independent of cirrhosis.
- Both human and animal studies have demonstrated an inverse association between physical activity and liver cancer.
- Smoking increases the risk of developing HCC.
- Coffee intake is associated with a decreased risk of developing HCC.
- The molecular mechanisms underlying the effects of lifestyles and HCC involve changes in metabolism, in particular, the activation of AMPK, changes in the immune system and in inflammation.

Cancers result from the interactions of host features with environment factors. Lifestyles, which comprise the habits by which a person chooses to live, define these interactions. Therefore, lifestyles such as dietary choices, smoking, alcohol consumption and physical activity have a profound influence on cancer development, including hepatocellular carcinoma (HCC). The capacity to survive famine was one of the strongest selection traits during evolution. This changed drastically about 50 years ago with generalization of a lifestyle characterized by abundance of food and lack of exercise. Human physiology has not changed in such a short period of time. As a consequence, we are maladapted to our new environment and this maladaptation leads to the epidemics of obesity and diabetes mellitus (DM). Obesity has been consistently associated with a 1.5–4.5 times increase of HCC risk [1-7]. Even an increase in body mass index (BMI) during childhood was associated with an elevated risk of HCC during adulthood [8]. DM was also linked to a 2–3-fold increase of HCC risk [9-11], independently of the underlying liver disease [11] and even in lean individuals [12]. Moreover, treating diabetic patients with insulin and/or insulin sensitizers may further increase the risk to develop HCC. This highlights how strongly lifestyles influence the risk of developing HCC.

Smoking

Smoking is associated with the development of several types of cancers, particularly those arising in organs directly exposed to smoke. Smoking also increases the risk of developing HCC (Table 1). Tobacco smoke contains chemicals that become activated as carcinogens when metabolized in the liver [13]. A linear relation between 4-aminobiphenyl-DNA adduct levels in liver tissue and HCC risk was reported, which was also significant after adjustment for covariates, including hepatitis B surface antigen status [14]. In a large Chinese retrospective study, smokers had a higher risk ratio for HCC than nonsmokers; this concerned males as well as females and the risk correlated with the degree of cigarette consumption [15]. This was confirmed in two Asian prospective studies which adjusted for alcohol consumption [16], [17]. Data from the European Prospective Investigation into Cancer and Nutrition (EPIC) suggested that, in Europe, smoking contributes to nearly half the cases of HCC, which is actually more than hepatitis B and C viruses [18]. Moreover, smokers who underwent HCC resection had a higher rate of recurrence and liver-specific mortality [19].

Alcohol

Alcohol is linked to HCC via the development of cirrhosis. The published evidence does not support a role for alcohol as a direct carcinogen for HCC. Alcohol-induced liver disease is one of the most prevalent causes of cirrhosis and alcohol-induced cirrhosis is associated with a 5-year cumulative risk for HCC of 8% [20]. The odds ratios for HCC increase linearly with alcohol intake and are higher in cases of DM or infection with hepatitis B or C virus[21], [22].

Coffee

Since 2002, when a protective effect of coffee against HCC was first reported [23], epidemiological studies, covering different geographical areas and different HCC etiologies and with different designs, have substantiated this observation. Three meta-analyses comprising studies from Europe and Asia found a statistically significant association between coffee consumption and an approximately 40% reduced liver cancer risk[24], [25], [26]. Prospective studies confirmed the benefit of coffee consumption. A prospective cohort that enrolled Finnish male smokers reported that coffee intake (boiled or filtered) was inversely associated with incident liver cancer [27]. Comparing high coffee consumers with low coffee consumers in the EPIC study, Bamia et al. found a decreased risk for HCC with a hazard ratio of 0.28 [28]. Finally, a large, multiethnic, population-based prospective cohort found a dose-dependent protective effect of coffee intake [29].

Diet

More than specific nutrients, it is the promotion of obesity and DM by overnutrition and energy-rich diets which increases the risk of HCC. Two case-control studies from southern Europe found a positive association between high dietary glycemic load and HCC among patients with chronic HBV or HCV infections [30], [31]. Although the latter study found that this positive association was present in patients without chronic hepatitis infection, this link was weaker and not statistically significant [31]. There is growing evidence that adherence to a healthy diet plays a role in delaying HCC development in at-risk populations. Epidemiological studies have

suggested that increased consumption of fruits decreases the risk of HCC [32] and low vegetable intake was significantly associated with an increased risk of HCC [33]. An Italian case-control study reported an inverse relation between intakes of fruits, milk/yoghurt, white meats, eggs and HCC risk [34]. Higher intake of total dietary fiber and a lower intake of dietary sugar were associated with decreased risk of HCC [7]. Finally, the degree of adherence to a "Mediterranean" diet was significantly inversely related to HCC risk. Turati et al. scored adherence to a "Mediterranean" diet in 518 cases of HCC and 712 controls from Italy and Greece [35]. They found that good adherence is associated with a 50% reduction in HCC incidence and that this effect is particularly striking in patients with a chronic viral hepatitis B or C.

Physical activity

Regular exercise reduces the negative consequences associated with overconsumption of an energy-dense diet, including insulin resistance, weight gain, and obesity [12, 36, 37]. The recognition that physical activity can also prevent cancer has motivated growing interest in this area of research.

Preventive benefits of exercise (primary prevention)

Epidemiological studies have indicated that physical activity lowers the risk of various carcinomas (esophagus, colon, breast, bladder, lung, kidney, prostate, pancreas, endometrium and ovary). While risk reductions seem to be small for endometrial [38] and prostate cancer [39], a pronounced benefit was shown for breast [40], colon [41], and lung cancer [42]. Physical activity may even reduce lung cancer incidence in smokers [43], and breast cancer risk in *BRCA1/2* mutation carriers who are genetically predisposed to the disease [44], illustrating the powerful impact of exercise. In a recent prospective study of a large Taiwanese cohort, Wen et al. observed a gradual correlation between decline in HCC risk and degree of physical activity [45], an observation which has been duplicated in an NIH study by Behrens et al. [46]. In terms of primary prophylaxis, HCC associated mortality appears to be reduced (relative risk [RR] 0.71; 95% confidence interval [CI] 0.52–0.98) in patients on moderate-to vigorous-intensity physical activity regimes (>7 h/week) before the diagnosis of cancer relative to inactive subjects [47].

Benefits of exercise post cancer diagnosis

In addition to its preventive effects, physical activity also favorably impacts on outcomes following cancer diagnosis. Mounting evidence indicates an improved quality of life, a decreased risk of recurrence, and up to 50% reduced risk of cancer-related mortality in physically active breast, prostate or colorectal cancer survivors compared with their less active peers. In men diagnosed with early prostate cancer, regular vigorous-intensity exercise (≥ 3 h/week) was associated with a 61% and 57% decreased risk of cancer-specific mortality and progression, respectively [48], [49]. As for liver cancer, one can consider the beneficial effects of lifestyle changes in patients with cirrhosis as secondary prevention. At the level of tertiary prevention, there is presently no evidence that exercise decreases HCC recurrence.

Hepatic effects of exercise

The benefits of physical activity have been consistently observed in a number of studies that are summarized in Table 2. Regular physical activity reduces steatosis and improves insulin sensitivity even in the absence of weight loss [50-58]. Exercise improves adipocytic insulin sensitivity, reducing the flow of fatty acids (FAs) to the liver irrespective of BMI [59-61]. Correspondingly, elevated physical activity is inversely associated with the onset of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) [53], [56], [57], [62], [63], [64], [65], [66], [67], [68], [69], [70]. Although currently speculative, the increased energy expenditure should further mitigate the procarcinogenic features of lipotoxicity and excess lipids, while improved insulin sensitivity should counteract the glucose-addicted phenotype of cancer cells. Kaibori et al. observed greater loss of body fat through exercise compared with dietary modification in a cohort of HCC patients, with insulin sensitivity improving only in the group with the highest exercise intensity [71].

Experimental data regarding the impact of exercise on the livers of diet-induced animal models predisposed to NAFLD, NASH, and HCC are summarized in Table 3. Despite the heterogeneity of the experimental set-ups (particularly the composition of diets), the sum of evidence confirms the beneficial effects of exercising. Exercise programs improved adipose mass, steatosis, insulin

resistance, inflammation or other parameters associated with the metabolic syndromes, which may also be improved when exercise is introduced midway through a high-fat diet regimen [72-75], [76]. When comparing exercise with calorie restriction, Rector et al. noted elevated mitochondrial β -oxidation, oxidative enzyme function, improved glucose tolerance, and suppression of hepatic *de novo* lipogenesis in the exercise group, providing support to the claim that exercise has effects superior to those of dietary modification [77]. Interestingly, halting exercise for short periods (7 days) does not appear to hamper its benefits, although longer interruptions (4 weeks) caused deterioration of the overall metabolic phenotype in hyperphagic rats [78]. In a genetic mouse model of NASH-induced HCC, regular exercise had a positive effect in delaying the onset of HCC [79].

Molecular mechanisms

Lifestyles, in particular exercise, affect several aspects of hepatocarcinogenesis. They modify the metabolism, influence the immune system and affect inflammation (Figure 1).

Metabolic programming

Exercise reduces the cellular ATP:AMP ratio and hereby activates AMP-activated protein kinase (AMPK). AMPK inhibits mammalian target of rapamycin complex 1 (mTORC1) and activates peroxisome proliferator-activated receptor- α (PPAR α) [80, 81] (Figure 2). mTORC1 is a key metabolic growth promoter, which in situations of nutrient and insulin availability activates sterol regulatory element-binding protein (SREBP), a transcription factor which controls the expression of lipogenic genes such as fatty acid synthase (FAS) [82]. In contrast, PPAR α induces genes required for β -oxidation including carnitine palmitoyltransferase I (CPT1) [39, 83-85]. mTORC1 stimulates glutamate dehydrogenase (GDH), possibly via the downregulation of sirtuin 4 (SIRT4) [86, 87]. GDH converts glutamine to α -ketoglutarate, which enters the tricarboxylic acid (TCA) cycle for ATP generation [88]. In muscle, exercise downregulates SIRT4; this releases its inhibitory effects on malonyl-CoA decarboxylase (MCD) resulting in reduced levels of malonyl-CoA, an inhibitor of β -oxidation [89-91]. It remains to be investigated whether exercise has similar effects in the liver and to what extent they occur in HCC. Wang et al. reported reduced expression of SIRT4 in HCC samples [92]. In HCC, decreased AMPK activity

has been associated with poor outcome and AMPK activation-induced apoptosis [93]. Likewise, mTORC1 activity has been suggested to regulate lipogenesis in hepatocarcinogenesis, with the lipogenic phenotype of HCC cells correlating to clinical aggressiveness [94]. Hence exercise could counteract HCC risk/progression in part by upregulating AMPK and downregulating mTORC1.

Interestingly, the exercise-induced changes in AMPK/Akt-mTORC1 do not require the presence of obesity/DM, indicating an independent effect of exercise on HCC inhibition [80]. Both calorie restriction and exercise have been shown to independently lower circulating insulin and insulin growth factor 1 (IGF-1) levels [95] which, apart from generally dampening PI3K-Akt-mTOR activities [81, 96] may also play a role in preventing the initiation and propagation of malignant tumors in the liver [97].

Immune system

Exercise is known to have immunostimulatory effects in cancer patients; however, no study has yet addressed this in HCC patients. In breast cancer survivors, regular exercise increased the percentage of CD4(+)CD69(+) cells and increased DNA synthesis after stimulation of these cells [98]. Circulating natural killer (NK) cells have key functions in the immunological defense against cancer. Brief bouts of exercise seem to be sufficient to increase the number of circulating NK cells by 4–5-fold, at least in young healthy adults [99]. Experimentally, exercise may induce relatively long-lasting changes in NK cells, with elevations sustained for up to 3 weeks following cessation of exercise in mice. Furthermore, these exercised animals developed resistance to lung tumor formation compared with sedentary controls [100]. Significant differences in T-cell proliferation between sedentary and exercised tumor-bearing rats have been reported, with the latter demonstrating higher macrophage cytotoxic antitumor action [101].

Inflammation

Experimental models of diet-induced and genetic-induced obesity promote low-grade hepatic inflammation, which leads to the development of HCCs [102]. HCC progression was reversed when the hepatic inflammation was reduced by deletion of interleukin-6 (IL-6) and tumor

necrosis factor- α (TNF- α). Clinically, modification of diet has been shown to reduce inflammation. A study with obese individuals reported an association between caloric-restricted weight reduction and decreased plasma C-reactive protein (CRP) levels [103]. Different diets were able to decrease IL-6 levels as long as weight loss was achieved [104]. Physical activity also reduces systemic inflammation, either directly or in combination with weight loss [105]. Even in low-intensity exercise groups of cancer patients, decreased levels of oxidative DNA damage have been observed [106]. The nuclear factor erythroid 2-related factor (NRF2) system is likely to provide an important contribution to the antioxidative properties of exercising; the increased production of reactive oxygen species (ROS) during exercise leads to NRF2 activation, which in turn activates a number of antioxidant enzymes [107]. Exercise primed against exercise-unrelated oxidative stress and significantly blunted carcinogenic stimuli [80, 106-114]. Physical intervention programs can reduce serum IL-6 levels independently of BMI and DM in men [115, 116]. In healthy adults, high-intensity training reduces responses of blood cells to TNF- α [111], while moderate exercise in cancer patients alters inflammatory cytokine responses [113].

Physical activity may dampen inflammatory states by decreasing the circulating levels of proinflammatory cytokines such as leptin and IGF-1 levels [95, 117]. In rats bearing mammary tumors, both calorie restriction and/or voluntary exercise decreased serum insulin, IGF-1, and tumor burden, along with Akt pathway downregulation and increased AMPK activity in tumors as well as in other tissues such as liver [80, 81]. Exercise reduces circulating leptin levels independent of metabolic conditions [109, 118]. Leptin opposes the beneficial effects of adiponectin and AMPK in cancer patients, extending its role beyond proinflammatory signaling [118, 119]. Experimental studies observed that impairment of leptin signal transduction mediated by Janus-activated kinase-2 (JAK-2) and the mitogen-activated protein kinase (MAPK) pathway occurs specifically in fructose-fed rats but not in glucose-fed rats [120, 121].

Diet and/or genetic obesity also induces alterations of gut microbiota, resulting in increased levels of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage. Enterohepatic circulation of DCA provokes senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSC), which in turn secrete various inflammatory and tumor-promoting

factors. Yoshimoto et al. reported that SASP promoted obesity-associated HCC development in mice [122]. Subsequent blocking of DCA production or decreasing gut bacteria efficiently prevented HCC development in obese mice. Mice lacking SASP inducers or depleted of senescent HSCs also showed similar results, indicating that the DCA-SASP axis in HSCs plays a key role in obesity-associated HCC development [122].

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Conclusion

The preventive and therapeutic impact of lifestyle on cancer is remarkable and its exploitation should be further promoted. HCC is a cancer tightly linked to lifestyle. We need multicenter, prospective studies on large patient cohorts with different levels of intervention. We further need more detailed experimental studies on signaling pathways involved in liver carcinogenesis that may be negatively or positively modified by lifestyles. The implementation of policies favoring the adoption of healthier lifestyles should be an integral part of our efforts against HCC.

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ACCEPTED MANUSCRIPT

Figure Legends

Fig. 1. Schematic representation of the beneficial cirrhosis-independent effects of exercise in liver cancer. Excess calories lead to lipid accumulation in hepatocytes, mainly due to increased lipogenesis, reduced β -oxidation, and import of fatty acids from adipose tissue. Excess lipids induce lipotoxicity, with ER stress being tightly linked to the development of insulin resistance and inflammation. Deregulated adipokine signaling from adipose tissue additionally promotes inflammatory conditions and insulin resistance in liver. The resulting hyperinsulinemia and hyperglycemia are important drivers of malignancy and increase the HCC risk, together with other processes induced by caloric overload. These include reduced blood perfusion or inflammatory/oncogenic signals from altered gut microbiota composition. By increasing calorie expenditure, physical activity counteracts the procancerous consequences of excess calories. In addition, exercise inhibits HCC development and progression through a number of processes (*blue*) that are independent of caloric overload and comprise improved perfusion and immune function, metabolic alterations (e.g. increased β -oxidation at the expense of glycolysis), activation of tumor suppressors, and a general amelioration in systemic inflammation and insulin resistance. These processes reduce cancer risk in nonobese individuals also. Although many organs are affected by exercise, the main contribution to insulin sensitivity and antiinflammatory conditions is believed to originate from muscle – the prime target of exercise. Akt: protein kinase B, AMPK: AMP-activated protein kinase, HCC: hepatocellular carcinoma, ER: endoplasmic reticulum, IGF: insulin growth factor, IGFR: IGR receptor, IL-6: interleukin-6, INSR: insulin substrate receptor, MAPK: mitogen-activated protein kinase, mTOR: mammalian target of rapamycin, mTORC1: mTOR complex 1, NK: natural killer, NRF2: nuclear factor erythroid 2-related factor, PTEN: phosphatase and tensin homolog deleted on chromosome 10, ROS: reactive oxygen species. STAT3: signal transducer and activator of transcription-3, TNF: tumor necrosis factor.

Fig. 2. Hepatocellular signaling pathways affected by exercise. Increased nutrient intake and metabolism elevates levels of circulating insulin and growth factors, which activate the PI3K/Akt pathway. Exercise induces AMPK activation, which in turn targets mTORC1 via phosphorylation, subsequently decreasing PI3K-Akt activities. At high circulating insulin, mTORC1 is activated to promote glutamine metabolism. Glutamine is catabolized by GDH into

α KG, an intermediate of the TCA cycle. mTORC1 may mediate glutamine metabolism via suppression of SIRT4, an inhibitor of GDH. SIRT4 also plays a vital role in lipid metabolism; both exercise and calorie restriction inhibit SIRT4 expression in liver cells. Inhibition of SIRT4 in turn seems to promote the activities of AMPK, SIRT1, and SIRT3. AMPK and SIRT4 also regulate cellular malonyl CoA levels by targeting ACC and MCD levels, respectively. Loss of SIRT4 results in decreased malonyl-CoA levels and improved exercise capacity, suggesting that SIRT4 plays a role in metabolic reprogramming during exercise training. Altogether, SIRT4 may function upstream of AMPK and SIRT1, and its inhibition may improve hepatic insulin sensitivity by enhancing fat oxidation. Activation of AMPK is strongly associated with tumor suppressor effects, while potential anticancer functions of the other energy sensors (SIRT1/3/4) need further investigation. ACC: acetyl coenzyme A carboxylase, Akt: protein kinase B, AMPK: AMP-activated protein kinase, GDH: glutamate dehydrogenase, α KG: α -ketoglutarate, MCD: malonyl-CoA decarboxylase, mTORC1: mammalian target of rapamycin complex 1, PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase, SIRT: sirtuin, TCA: tricarboxylic acid.

Figure 1

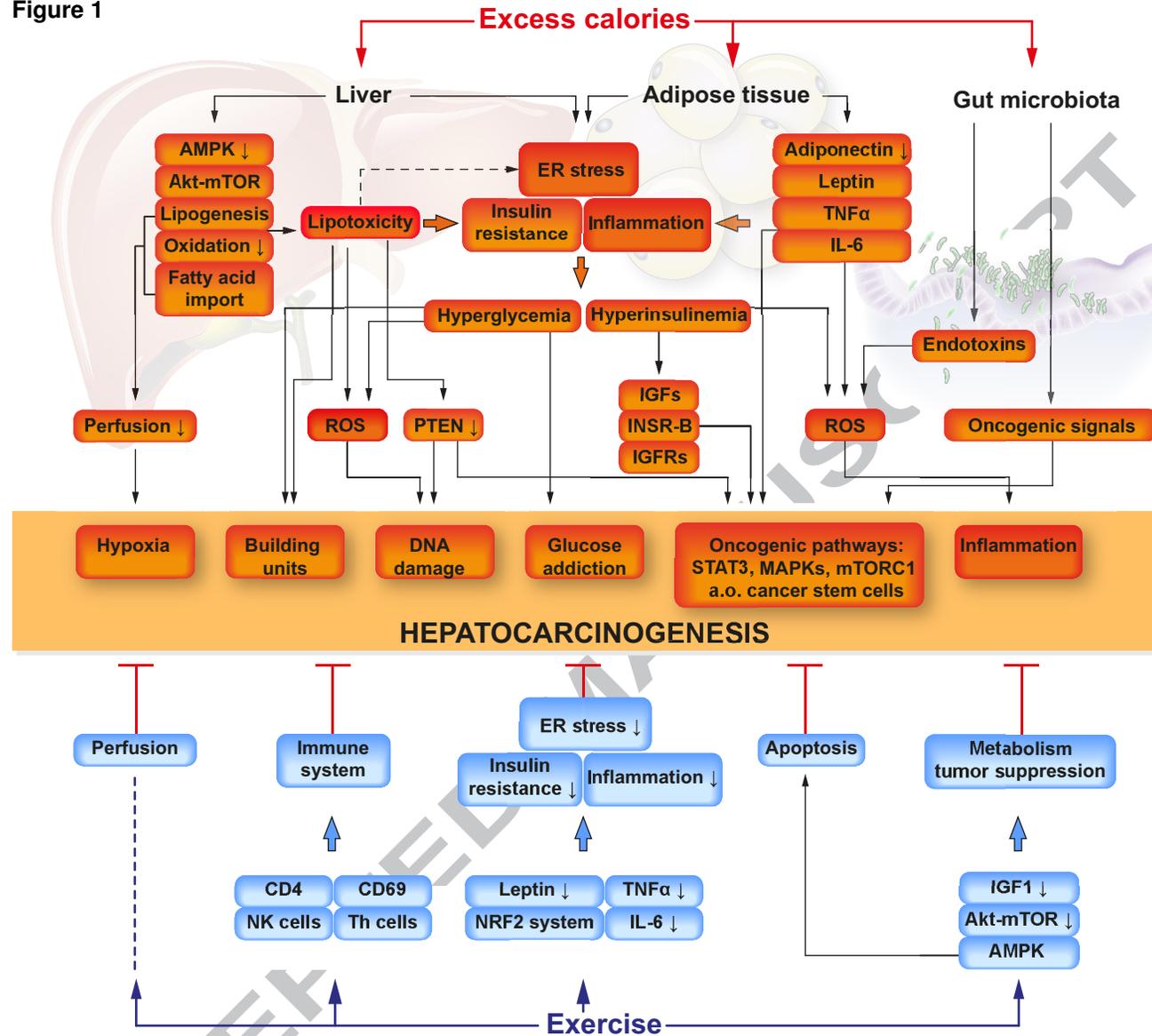


Figure 2

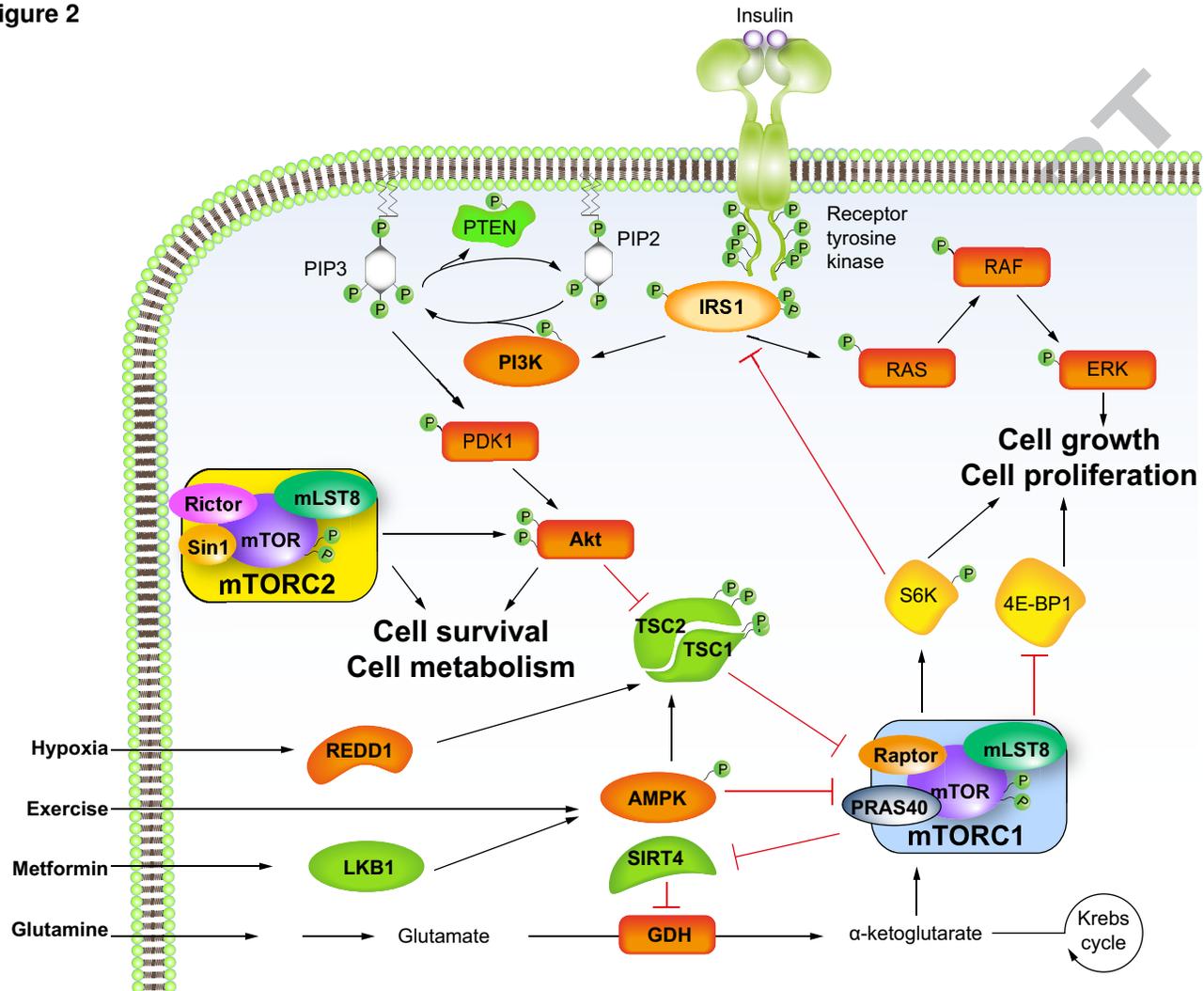


Table 1

[Ref.]	Design	Population	Total	Conclusions Drawn	Limitation of study
[14]	Case study	110 HCC patients and 42 patients with metastatic liver tumors / intrahepatic stones who underwent surgery between 1984-1995	152/110	4-aminobiphenyl exposure (result of cigarette smoking) plays a role in the development of HCC in humans. OR = 4.14 (1.15-15.50) and OR = 9.71 (2.82-34.86) for medium and high 4-aminobiphenyl-DNA adducts levels respectively.	Retrospective case control study, no clear definition of smoking, information about smoking duration/quantity was not available for all subjects.
[15]	Case control	36,000 adults who died from liver cancer (cases) and 17,000 who died from cirrhosis (controls)	53,000/ 36,000	For men smokers, RR = 1.36 (1.29-1.43) to die from liver cancer. Looking at consumption (cigarettes/day): RR = 1.5 (1.39-1.62) for 20/day and RR = 1.32 (1.23-1.41) for 10/days. For women smoker RR = 1.17 (1.06-1.29), RR = 1.45 (1.18-1.79) for 22/day and RR = 1.09 (0.94-1.25) for 8/day.	Retrospective study
[16]	Prospective cohort	63,257 adults aged 45-74 years in Singapore	61,321/394	Current vs never smokers have an increased risk of HCC HR = 1.63 (1.27-2.10) after adjusting for alcohol consumption and other cofounders. Result was dose-dependent ($p < 0.001$) and duration of smoking dependent ($p = 0.002$).	Smoke habit evaluated only at enrollment
[17]	Prospective nested case- control study	115 HCC matched with 229 controls from the European Prospective Investigation into Cancer and nutrition EPIC cohort.	115/229	Smokers have a higher risk to develop HCC. OR = 4.55 (1.90- 10.91). Former smokers have a higher risk to develop HCC. OR = 1.98 (0.90-4.39).	Information about comorbidities such as diabetes was not available for all subjects, HCC treatment was not taken into account
[18]	Prospective cohort	2273 HCC patients aged 20-75.	2273/ 2273	Looking at survival after HCC diagnosis, HR = 1.20 (1.05-1.37) for current smoker and 1.16 (0.98-1.38) for ex-smokers compared to never smokers.	Lack of evaluation of interactions with other possible factors (cirrhosis, diabetes, diet)
[19]	Prospective cohort	302 patients with HBV infection who underwent surgical resection for HCC	302/302	Heaving smoking (PY ≥ 20) was the most significant factor associated with HBV-related HCC recurrence after surgical resection ($p = 0.001$). Median recurrence-free survival was worse for ex- and current-smoker than for non-smoker (24, 26, 34 months respectively, $p = 0.033$).	Small number of ex-smoker (n=25), tumour burden in that specific group was worse than the other groups, Short-term follow-up.

Table1: Human studies focusing on the effect of smoking on HCC.

Footnotes: Total column: number of subjects in study/number of subjects with HCC.

Ref	Liver parameter	Type of exercise	Inclusion of diet	Low/High intensity	Time period	Conclusions Drawn	Limitations of study
[50]	Lipid content (n=23)	Aerobic cycling	–	Progressively increasing intensity	4 wk	Aerobic exercise reduced hepatic lipids thereby mitigating metabolic and cardiovascular consequences of fatty liver	No effects of long-term aerobic exercise assessed
[51]	Fat accumulation (n=15)	Controlled aerobic exercise program	–	High	12 wk	Decreased hepatic fat accumulation and thereby potential of fatty liver to progress to liver inflammation, fibrosis and cirrhosis.	–
[56]	Lipid Content (n = 15)	Habitual PA	–	Both	–	Higher level of PA correlated with lower IHF content	Cross-sectional study – no assessment of longitudinal effect; influence of diet on lipid content
[65]	Free fatty acids (FFA) (n = 16)	Conditioning exercise and general PA	–	Both	–	Lower hepatic FFA in more active twins	Low cohort number; long-term study required
[66]	Fat content (n = 18)	Conditioning exercise and general PA	–	Both	–	Twins with higher PA had 23% less hepatic fat	Low cohort number; long-term study required
[62]	NAFLD (n = 813)	Assorted (aerobic, leisure PA)	–	Moderate, High	–	Exercise intensity (vigorous) was inversely associated with decreased risk of developing NAFLD, NASH severity and fibrosis	Cross-sectional study; no establishment of an association between moderate exercise and disease severity
[61]	NAFLD (n = 19,921)	Patient-reported aerobic exercise	–	Moderate	–	Exercise intensity, duration and frequency was associated with less insulin resistance and decreased risk of NAFLD development	Self-reported information; cross-sectional study; use of ultrasound to diagnose fatty liver
[70]	NAFLD (n = 13)	Aerobic exercise	Normal diet	High intensity	7 d	Short-term exercise decreased circulating marker of hepatocyte apoptosis in obese NAFLD patients and increased insulin sensitivity	Influence of diet; long-term effect;
[57]	NAFLD (n = 218)	Cardiorespiratory fitness	–	Maximal treadmill test	–	Inverse association between fitness and NAFLD prevalence	Lack of serum hepatitis C status; cross-sectional study
[67]	NAFLD (n = 141)	Leisure PA	–	Low, moderate intensity	–	Increasing PA significantly improved metabolic parameters in people with NAFLD	Lack of objective measurement of change in PA
[63]	NAFLD (n = 37)	Compared health-related fitness (cardiorespiratory fitness, body composition, muscle strength) with general PA participation	–	–	–	Suboptimal health-related fitness and PA beneficial in reducing associated risk factors and preventing progression of NAFLD	Low cohort number; results not generalizable to all NAFLD patients
[53]	NAFLD (n = 375)	Assorted PA – aerobic; resistance	Yes	Both	–	Higher rate of PA associated with lower NAFLD prevalence	Cross-sectional study ; unclear whether resistance or aerobic PA more beneficial
[52]	NAFLD (n = 28)	Resistance exercise	–	–	8 wk	Resistance exercise increased insulin sensitivity and improved metabolic flexibility in NAFLD.	No assessment of long-term resistance exercise; exclusion of patients with fibrosis
[55]	NAFLD including steatosis (n = 31)	Aerobic exercise	Yes	High	15 mo	Exercise resulted in sustained improvement in liver enzymes and serum insulin levels	Extent of dietary effect
[68]	Steatosis and chronic hepatitis C (n = 19)	Individualized exercise regime	Yes- individualized diets	–	3 mo	Inverse correlation between weight reduction and steatosis/abnormal liver enzymes; improvement in fibrosis	Extent of dietary effect; long-term studies
[69]	NASH (n = 65)	Individualized aerobic exercise regime	Yes- individualized diets	Moderate – high intensity	3 mo	Moderate exercise normalised aminotransferase levels in NASH patients	Extent of dietary effect
[71] *	HCC patients under- going hepatectomy (n = 51)	Patient-specific exercise program	Patient-specific daily energy in- take: 25– 30 kcal/ kg body weight	Both	6 mo	Exercise group presented no clinical problems as well as significant improvement in both serum insulin and insulin resistance index.	No patients with advanced disease
[123] *	Advanced HCC (relapsed) (n = 1)	Aerobic cycling	–	Progressively increasing intensity	6 wk	Aerobic program increased peak work capacity of patient by 20.3% and improved quality of life	Results based on one individual; larger patient cohort required
[45] *	Risk prediction study for HCC (n = 428,584) HCC cases (n = 1668)	Patient-reported exercise	–	Both	8.5 yr	Correlation between decline in HCC risk and degree of physical activity	Additional validation of results from other populations needed. Participants belonged to above-average socioeconomic status
[46] *	Total participants n = 507,897 (Liver cancer n = 628; HCC n = 415)	Patient-reported vigorous physical activity	–	Both	10 yr	Decreased risk of total liver cancer and HCC by 36% and 44%, respectively	No data on chronic liver disease and hepatitis B/C virus infection status. Frequency of physical activity assessed using self-report questionnaire. No continuous assessment of physical activity status during 10-year follow-up

Table 2. Human studies focusing on the effects of exercise in the liver.

Footnotes: d = days, wk = weeks, mo = months, yr = years, PA = physical activity, FFA = Free fatty acids

Bold font * = studies specifically focused on effects of exercise on HCC development

[Ref]	Liver condition	Animal model	Inclusion of diet	Type of exercise	Forced or voluntary exercise	Low/high intensity	Time period	Conclusions drawn	Limitations
[39]	NAFLD	KK/Ta and BALB/cmice	High sucrose diet	Treadmill running	Forced	Progressively increased	12 wk	Exercise prevents fatty liver and subsequent NAFLD development by improving hepatic lipid metabolism	Mechanism underlying the inhibitory effect of exercise remains unclear; not considered if exercise prevented necroinflammation and fibrosis
[73]	NAFLD	C57BL/6 mice	High fat/standard chow	Swimming	Forced	Progressively increased	10 wk	Swimming improved fat oxidation and significantly reduced liver steatosis Reduction in all severe features of NAFLD	Mice of study demonstrate high levels of HDL-C which does not portray model of human metabolic syndrome; Role of exercise in increasing plasma adiponectin is unclear
[77]	NAFLD	OLETF rats	Normal and restricted diet	Running wheel	Voluntary	–	4–40 wk of age	Attenuated NAFLD development on daily exercise; more effective than restricted diet	Reason unknown for exercised animals to remain hyperphagic while calorie restricted diet animals demonstrated upregulated lipogenesis
[72]	Onset of steatosis	Rats	High fat/standard chow	Treadmill running	Forced	Progressively increased	Midpoint of 16-wk experiment	Exercise training significantly decreased fat accumulation, triacylglycerol, plasma nonesterified fatty acids, and leptin concentrations	Liver lipid infiltration did not progress linearly over 16 weeks of HFD
[76]	Onset of steatosis	Sprague-Dawley® rats	High fat/standard chow	Treadmill running	Forced	Progressively increased	8 wk	Complete prevention of steatosis	Hepatic insulin sensitivity was not determined; plasma β -hydroxybutyrate levels remained unaltered by exercise training
[75]	Onset of steatosis	OLETF rats	–	Running wheel	Voluntary	–	16 wk	Exercise training attenuates the progression of hepatic steatosis in OLETF rats	Wheel running did not seem to increase alter AMPK α or AMPK phosphorylation status nor specific enzymatic activity or increased mitochondrial content in the liver
[84]	Onset of steatosis	Mice	High fat/standard chow	Treadmill running	Forced	Progressively increased	8 wk	Exercise effectively decreased sFREBP-1c, FAS and SCD1 expressions while promoting increased ACC phosphorylation and CPT1 expression. Exercise reduced the total hepatic lipids and reversed the hepatic steatosis in obese mice.	The mechanism underlying exercise mediated decrease of SREBP-1c and FAS remains unclear
[78]	Onset of steatosis	OLETF rats	–	Running wheel	Voluntary	–	Wheels were locked for last 4 wk of experiment	While ceasing of PAdid not result in complete loss of PA-induced benefits like decreased serum lipids, markers for lipogenesis (SREBP-1, ACC, FAS and SCD-1), it caused development of steatosis and loss of markers of hepatic mitochondrial function (palmitate oxidation, citrate synthase and β -HAD)	Exercised animals remain hyperphagic; tissue specific changes in liver and adipose tissue were not noted
[80]	NASH	Rats	High fat/standard chow	Swimming	Forced	Progressively increased	12 wk	Exercised HFD rats demonstrated decreased hepatopathologic manifestations of steatosis and inflammation. The serum and liver parameters measured were decreased	Serum AST in high-fat trained rats remained unaltered
[79] *	Liver carcinogenesis	AlbCrePten ^{flox/flox}	Standard 10% fat chow	Treadmill running	Forced	Progressively increased	32 wk	Exercised mice showed decreased incidences of tumor development (71% Vs 100%), decreased mean number and total volume of tumor nodules.	Beneficial effect of exercise on tumor development was independent of improvement of steatosis and NASH lesions
[74] *	Liver carcinogenesis	Wistar rats	High fat/low fat chow	Swimming	Forced	Progressively increased	8 wk	Exercise postconditioning attenuated liver carcinogenesis under adequate low fat dietary regimen	Effect of exercise seems to be dependent on low fat diet, effect with control diet was not tested.

Table 3. The effect of exercise in animal models predisposed to liver pathologies.

Footnotes: OLETF=OtsukaLong-Evans Tokushima Fatty; d=days; wk=weeks; mo=months; yr=years; HFD = high fat diet; AMPK α = AMP-activated protein kinase- α

Bold font * = studies specifically focused on effects of exercise on HCC development