78313_Auto_Edited-check.docx

WORD COUNT	8678	TIME SUBMITTED	19-OCT-2022 11:08PM
		PAPER ID	91743019

24
Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 78313

Manuscript Type: REVIEW

Mitochondrial carnitine palmitoyltransferase-II dysfunction: A possible novel mechanism for nonalcoholic fatty liver disease in hepatocarcinogenesis

Yao M *et al*. CPT-II in NAFLD

Min Yao, Ping Zhou, Yan-Yan Qin, Li Wang, Deng-Fu Yao

Abstract

Nonalcoholic fatty liver disease (NAFLD) or metabolic-associated fatty liver disease has been characterized by the lipid accumulation with injury of hepatocytes, and become one of the most common chronic liver diseases in the world. The complex mechanisms of NAFLD formation are still under identification. Carnitine palmitoyltransferase-II (CPT-II) on inner mitochondrial membrane (IMM) regulates long chain fatty acid β-oxidation, and its abnormality has been paid more and more attention by basic and clinical research in NAFLD. The sequences of its peptide chain and DNA nucleotides have been identified, and the catalytic activity of CPT-II is affected on its gene mutations, deficiency, enzymatic thermal instability, circulating carnitine level and so on. Recently, the CPT-II dysfunction has be discovered in models of liver lipid accumulation. Meantime, the malignant transformation of hepatocytes related $CD44^+$ stem T cell activation, high levels of tumor-related biomarkers (AFP, GPC3), and abnormal activation of Wnt3a expression as a key signal molecule of the Wnt/β-catenin pathway were parallel to the alterations of hepatocyte pathology. This review focuses on the some progresses of CPT-II inactivity on IMM with liver fatty accumulation as a possible novel pathogenesis for NAFLD in hepatocarcinogenesis.

Key Words: Carnitine palmitoyl transferase-II; Nonalcoholic fatty liver disease; Fatty acid β-oxidation; Carnitine; Hepatocyte malignant transformation; Mitochondrial membrane

Yao M, Zhou P, Qin YY, Wang L, Yao DF. Mitochondrial carnitine palmitoyltransferase-II dysfunction: A possible novel mechanism for nonalcoholic fatty liver disease in hepatocarcinogenesis. *World J Gastroenterol* 2022; In press

Core Tip: The complex mechanisms of nonalcoholic fatty liver disease formation are still under identification. Hepatic carnitine palmitoyl transferase-II (CPT-II) on inner mitochondrial membrane (IMM) regulates long chain fatty acid (LCFA) β-oxidation, and its abnormality has been paid more and more attention by basic and clinical research. The sequences of its peptide chain and DNA nucleotides have been identified, and the catalytic activity of CPT-II is affected on its gene mutations, deficiency, enzymatic thermal instability, circulating carnitine level and so on. CPT-II dysfuction has be discovered in models of lipid accumulation. Meantime, the malignant transformation of hepatocytes related CD44+ stem T cell activation, high levels of tumor-related biomarkers, and abnormal Wnt3a expression as a key signal molecule of the Wnt/β-catenin pathway were parallel to the alterations of hepatocyte pathology.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) or metabolic-associated fatty liver disease (MAFLD) is a general term of liver diseases characterized by inflammation, fatty accumulation and hepatocyte dysfunction, except of alcohol or other clear liver injury factors [1-3]. Up to now, NAFLD has become a potentially serious liver disease that affects approximately 25% of the adult population in the world [4], and divided into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) with or without liver fibrosis [4,5]. They showed balloon like hepatocyte injury based on NAFL. Most patients have no obvious symptoms, and may not be diagnosed until they

develop into liver cirrhosis or progression to hepatocellular carcinoma (HCC), and the effect of early clinical screening is poor [6]. Once NAFLD progresses to liver cirrhosis, it is difficult to reverse, and there is a risk of HCC that can't be ignored. Not only that, it also involves the occurrence of multiple systemic diseases in the body, which is closely related to cardiovascular disease, chronic kidney disease, colorectal tumor and so on, threatening human health [7,8]. Therefore, finding the monitoring target in the malignant transformation of NAFLD has practical clinical significance for the prevention of NAFLD related liver malignant diseases[9].

Lipid metabolism rearrangements in NAFLD contribute to disease progress that has emerged as one of the most risks for HCC, where metabolic reprogramming is a hallmark[10]. Hepatic carnitine palmitoyl transferases (CPTs) are critical for long-chain fatty acids (LCFAs) β -oxidation, as they are capable of transport through the mitochondrial membrane [11]. CPT made up of two separate proteins (CPT-I and CPT-II). CPT-I is located in the outer mitochondrial membrane (OMM) with three isoforms (liver CPT1a, muscle CPT1b and brain CPT1c) and CPT-II is in the inner mitochondrial membrane (IMM)[12,13]. The amino acid (a.a.) and cDNA nucleotide (nt) sequences of the ubiquitous CPT-II have been elucidated. The mutations or dysregulation of the CPTs, which are associated with many serious and even fatal diseases, are promising targets for the developing drugs to treat type 2 diabetes (T2D) and obesity [14,15]. Dysregulated lipid metabolism is involved in human diseases, including chronic inflammatory diseases and inflammatory-related tumors[16]. CPTs play an important role in lipid metabolism and fatty acid oxidation (FAO) in mitochondria. CPT-II has been confirmed as a rate-limiting enzyme and regulating host immune responses [17,18]. However, the pathological role of CPT-II alteration with NAFLD remains to be identified[19]. This review summarizes the latest research findings of CPT-II, which are important for accurate or early monitoring of NAFLD malignant transformation.

***CPT2* STRUCTURE**

The most important function of the *CPT* family is to ensure that fatty acids enter the mitochondria for β-oxidation. Transmembrane protein CPT-I is located in OMM and CPT-II is in IMM. Human CPT-II gene (*CPT2)* as autosomal recessive trait encoded gene localizes on chromosomes 1 (1p32) and the gene full length contains 3090 nucleotides (n.t.) and 5 exons, which can encode enzyme protein peptide chain composed of 658 amino acids (a.a.)[20]. Summaries of *CPT2*, CPT-II and total numbers of its reported mutated sites are shown in Table 1. Human CPT-II (NM_000098) is a mitochondrial protein in IMM. CPT-II together with CPT-I oxidizes LCFA in the mitochondria and play pivotal roles in the LCFA transport across the mitochondrial membrane for β-oxidation[21]. In molecular genetic aspects, *CPT2* is identified in about 70% of mutant alleles. There are variations in the *CPT2* genome, most of which are single-base substitutions, small insertions or discrete deletions[22,23]. Among the enzymatic system, CPT-II plays a rate-limiting role in the entry of fatty acids into mitochondrial FAO and is considered to be a key component of cellular metabolic homeostasis[24]. Anti-cancer drug oxaliplatin can activate CPT-II in gastrointestinal cancer cells and promote the catabolism of fatty acids [18]; Knocking down of *CPT2* by patient-derived xenograft models confirmed the regulating role of mitochondrial FAO in Src activation and metastasis of breast cancer[25]. However, a subset of substitutions, insertion or delete tend to cluster in all exons, especially in exon 4 and exon 5, suggested that CPT2 clustering is due to a combination of factors such as the rate of heterogeneous mutations in the genome, biophysical characteristics of exogenous carcinogens, endogenous dysregulation, and large mutation events related to genome instability [26].

Enzymatic system that facilitates the transfer is known as CPT mainly in OMM or IMM and plays an important role in maintaining its structural and functional integrity. Liver cells must keep related-metabolic homeostasis in a wide range of conditions and meet their ATP needs depending on FAO[27,28]. CPT-II catalyzes transesterified acylcarnitines transferred from cytosol into intermembrane space (IMS) and the remaining acyl of acylcarnitine is changed back to CoA on IMM, which is next available for FAO. Meanwhile, the released carnitine is returned to IMS of mitochondrion via

CACT and is available for fatty acid re-transport[29]. However, the deficiency or gene mutation of CPT-II can significantly affect mitochondrial FAO. Bezafibrate, as a well-known hypolipidemic drug, was tested to stimulate CPT2 mutation, but it should be a challenge to restore normal LCFA oxidation from series of other fatty acid mitochondrial diseases [30,31], indicated that CPT-II not only provides ATP for liver cells via FAO, but also its down-regulated expression affects the growth and malignant transformation of hepatocytes via cell damage, related-signal molecules, stem cells, immunology and so on[32]. Therefore, the study of CPT-II will help to understand the pathogenesis and to develop a promising treatment of NAFLD.

Previous studies of *CPT2* mutations have identified the presence of single-base substitutions, and many other events such as double-base and multiple-base substitutions, insertions, or deletions. The reported mutations among all five exons of *CPT2* and 89 mutated sites are shown in Table 2[33,34]. Most of *CPT2* or CPT-II mutations are located in exon 4 or exon 5. Biochemical consequences of these mutations are still controversial. The c.338 C>T (P.S113L) variant can be detected in most cases of Caucasians; in Japanese, c.1148 T>A (P.F383Y) is the most frequent variant allele, and can obviously cause severe infant forms of symptoms. Among them, It might include deficiency of enzyme protein, enzyme inactivity or abnormality of enzymatic regulation. Protein encoded by this gene is a nuclear protein which is transported to the IMM. Due to the low activity, thermal instability, and short half-life of CPT-II, the CPT II variant exerts a dominant negative effect on homologous tetrameric proteins associated with mitochondrial LCFA oxidation impairment[35]. Recently, based on animal models or clinical studies, the crystal structures of CPT-II were determined in uninhibited forms and in complexes with inhibiting substrate analogs with anti-diabetic features. The crystal structures have a deep understanding of the enzymatic structure-function relationship, which is conducive to the discovery of new inhibitors through structure-based drug design[36].

MITOCHONDRIAL CARNITINE SHUTTLE SYSTEM

Using fatty acids as ATP requires more than 20 enzymes and transporters, which are involved in the activation and transport of fatty acids to mitochondria. Membrane transport system of fatty acid β-oxidation in mitochondria is shown in Figure 1. Mitochondrial FAO is one of the major pathway for fatty acid degradation and is critical for maintaining ATP balance in the human body[37,38]. When the glucose supply is limited, fatty acids are an important source of energy after absorption and during fasting. But even when glucose is sufficient, FAO still is the main source of energy for human tissues. A series of enzymes, transporters, and other facilitating proteins with biochemistry and physiological functions are involved in FAO (Figure 1 left). The role of CPT in the LCFA oxidation, and this system includes CPT-I, carnitine-acylcarnitine translocase (CACT) and CPT-II. The acyl-CoA synthetase located in OMM catalyzes fatty acids to form acyl-CoA with ATP and CoA participation, and then, transport long-chain acyl-CoA by the delivery system into mitochondria, that is carnitine shuttle system to enter the process for β-oxidation. Most genes encoding CPT-II are known to be recessive genetic defects, and the clinical manifestations of the related diseases may include hypoglycemia, cardiacopathy, arrhythmias and rhabdomyolysis; It also illustrates the importance of FAO during fasting and in liver and (heart) muscle function[39,40].

The carnitine shuttle system controls fatty acid translocation across the mitochondrial membrane. Key enzymes determine the competition of glycolysis *vs* mitochondrial FAO defined by the Randle cycle. This transport system with CACT is an important part for fatty acid esterification through OMM and IMM of mitochondria (Figure 1 right). First, CPT-I at OMM catalyzes long-chain acyl-CoA along with carnitine conversion to long-chain acylcarnitine and CoA transported to mitochondrial interior with help of translocase on mitochondrial intima[41,42]. After that, the transesterified acylcarnitines are transported from cytosol into IMM space through by CACT acylcarnitine releases carnitine by CPT-II catalysis, and converts to acyl group from carnitine to acyl-CoA, available for β-oxidation. Released carnitine returns to IMM space of mitochondria for fatty acid re-transport[43]. CPT-II plays an important regulatory role in FAO, its function

affects fatty acid metabolism. More importantly, CPTs in carnitine shuttle system can be used as a drug target to reduce gluconeogenesis or restore liposome balance. Therefore, it has the potential value of gene therapy or immunotherapy, and the further study of the mechanism of CPTs could provide useful ideas for clinical treatment of related diseases[44,45].

EFFECT FACTORS OF CPT-II ACTIVITY

Lipid metabolism involves a variety of biological processes, including the most important lipid metabolic pathway (FAO with carnitine shuttle system). The mutations or dysregulation of hepatic CPT-II have been linked to many serious or even fatal human diseases, and it should be a promising target for developing drugs to treat T2D or obesity[46]. However, the deficiency, over-expression, or inactivation of liver CPT-II might ultimately lead to disruption of immune homeostasis, thereby increasing the risk of various inflammatory diseases and even tumors. There are some evidences that CPT-II or the associated mitochondrial LCFA are involved in the development and progression of these related diseases. Thus, the agonists or inhibitors targeting the CPTs or carnitine shuttle system have emerged as novel therapies for these diseases [47]. Normal function of FAO in IMM is closely depend on the catalytic activity of CPT-II, that could be affected by *CPT2* variation, the amino acid substitution of enzyme, inhibition of enzyme activity, circulating carnitine level and so on.

CPT-II deficiency

Hepatic CPT-II deficiency is one of the most common forms of mitochondrial FAO disorders (FAODs) and have several clinical presentations those have been known for longer times. However, its phenotypic variability remains fascinating[48]. The clinical phenotypes of CPT-II deficiency are classified into muscular, severe infantile and fatal neonatal type. In addition, neonatal-onset CPT-II deficiency is often accompanied by brain and kidney organ dysfunction features, such as in the first month of life, and is almost always fatal. Three different phenotypes (neonatal, infant and adult onset) have

been identified, all with autosomal recessive inheritance patterns[49]. The clinical phenotype of adult CPT-II deficiency is mostly benign, and only with additional external stimuli, such as high-intensity exercise, can cause major myopathy symptoms. However, the perinatal and infantile CPT-II deficiency usually involves multiple organ systems, especially occurring in the perinatal period is the most serious form and is often fatal[50]. The application of mass spectrometry technology to analyze acylcarnitine profiles in blood has revolutionized for FAOD diagnosis, including CPT-II deficiency. In most cases, the number of *CPT2* mutations is increasing and there is a clear genotype-phenotype correlation. However, the clinical variants in some patients might contain other genetic or environmental factors[51].

In clinical, the manifestations of patients with CPT-II deficiency include severe infant liver, myocardial infarction, fatal neonates, and myopathy (usually mild, from infancy to adulthood). Some patients have a serious multi-system disease that includes liver function failure, cardiomyopathy, epilepsyhy, poglycemia and premature death, while the other is characterized by muscle pain and weakness, sometimes accompanied by myoglobinuria[52]. The proband was diagnosed for CPT-II deficiency by finding a decrease in muscle CPT activity or by identifying a biallelic variant of *CPT2* in a molecular genetics test. A total of six mutations have been identified, including four new ones. Among those mutations, the S113 L mutation is common in about 50% of the mutant alleles. Three of the six mutations (3/6) have been found in a few unrelated patients, while others have been found in only one family with genetic heterogeneity. To date, about 100 *CPT2* mutations have been discovered. Prenatal diagnosis is provided when the risk of infant/severe CPT-II deficiency is 1/4. Infantile CPT-II presents as a severe hypoglycemic episode of ketoacidosis, occasionally associated with heart damage, usually resulting in sudden death before age 1[50]. Treatment for *CPT2* deficiency includes a low-fat diet in rich triglyceride or carnitine and avoiding fasting or hyperkinesis[53].

Thermal instability of CPT-II

CPT-II activation is associated with disorders of mitochondrial β-oxidation of LCFA in IMM. Based on the crystal structure of mouse CAT, the active site of CPT-II is located at the interface between two domains, extending in tunnels through the enzyme protein centres, alone or in complex with its substrate carnitine or CoA[54]. In this tunnel, carnitine combine with CoA and its opposite is catalytic His^{343} residue. The information of CPT-II structure provides a molecular basis to understand the catalytic activity of CAT or design their inhibitors. In addition, the carnitine might contribute to the catalytic stabilization of oxygen ions in the reaction intermediates. Hepatic CPT-II is sensitive to be inhibited by metabolites of fatty acids, Triton X-100, or malonyl-CoA[55].

Artificially recombinant His6-N-hCPT2 and His6-N-hCPT2/S113L showed the same enzymatic activity for wild-type or S113L variants of CPT-II [56]. However, the mutant CPT-II exhibited abnormal destabilization at 40° C or 45° C and was more sensitive to be inhibited by malonyl-CoA. The thermal solubility of mutant CPT-II, which may explain the symptoms of CPT-II deficiency may mainly occur during prolonged exercise, infection, and exposure to cold. In addition, CPT II abnormalities are likely to be largely suppressed when fatty acid metabolism are stressed[54]. The unstable CPT II variants with enzymatic inactivity might low mitochondrial fuel utilization under the phenotypic threshold during patients with hyperthermia, thus suggesting that hepatic CPT-II should play a pathological role in NAFLD progression.

High-risk patients have thermolabile genetic backgrounds of CPT-II in LCFA metabolism. However, until to now, no related mutation of CPT-II was reported in NAFLD patients[57,58]. Almost fatal or handicapped virus-associated encephalopathy cases exhibited transiently higher serum LCAC levels during fever more than 40 °C. The specific activity of patients' CPT-II (0.4 ± 0.06 nMol/min/mg) was 36% of normal control (1.1±0.3 nMol/min/mg protein) at 37 °C. The CPT-II specific activity in the patient group was down to 50% for 2 h at 41 °C, and CPT-II in the normal control group

still was 91.4%, and the sequencing analysis of patients' *CPT2* gene revealed compound (1055T>G/F352C) + (1102G>A/V368I) heterozygous variations[46,59]. F352C substitution was only reported in Japanese, and V368I polymorphic variation has relatively mildly effects related to CPT-II deficiency[47,60]. The CPT-II mutation or dysregulation has been linked to more serious, even fatal diseases, and these data should be promising molecule targets to develop therapeutic agents for NAFLD in future.

Carnitine level

Carnitine as a substance has a wide range of biological functions, including transport of LCAD from the cytoplasm to the mitochondrial matrix, regulation of acetyl-CoA/CoA, control of acyl transport between organelles, and prevention of oxidative stress[58]. Maintaining normal fat metabolism depends on carnitine concentration that is synthesized in most eucaryotic organisms[61]. The methylation of lysine initiates the biosynthesis of carnitine. The formed trimethyllysine is then converted to butylbetaine in all tissues and finally hydroxylated to carnitine in the liver and released from the tissues, which are then actively absorbed by all other tissues[62,63]. This transfer requires the enzyme and transporter that accumulates carnitine within the cell (OCTN2 carnitine transporter) , which is conjugated to LCFA (CPT-I) , to transfer acylcarnitines through IMM (CACT), and to transfer carnitines through the IMM (CACT) , fatty acids were conjugated back to CoA for subsequent β-oxidation (CPT-II). The regulation of carnitine synthesis is still incompletely understood because the turnover of carnitine in human body is slow[64].

Carnitine is essential for proper fat metabolism, producing ATP, and the transport of LFAC or medium fatty acid chains (MFAC). It attracts LFAC and MFAC, after breaks them down, and then takes them to the cell's mitochondria for FAO. And as it turns out, that the body burns more fat, providing the body with more natural energy in the process[65,66]. According to the previous study, using carnitine antagonist 3-(2,2,2-trimethylpropionate hydrazine dihydrate, THP, figure 2) resulted in lipid accumulation with increased liver weight in wild-type mice. The competition between THP and

carnitine inhibited CPT-II activity, resulting in carnitine deficiency, acyl CoA and fat accumulation [67]. Clinical data showed that the blood carnitine concentration in NAFLD patients was lower than those in healthy people, and the level in NAFLD cases with liver cirrhosis accounted for only 22% of normal people[68,69]. The concentration of carnitine in patients with liver disease is low, the fat accumulation in rat liver tissue, the content of total fatty acids, free fatty acids, short chain fatty acids (SCFC) and LCFA in liver, and the content of chain, long chain, short chain and total fatty acids in circulating blood also change. During patients with hepatitis B or hepatitis C virus infection, or with mitochondrial FAODs present with NAFLD or severe liver diseases, enough carnitine should play an important role in the mitochondrial carnitine shuttle system, suggesting that circulating carnitine level affects FAO, ameliorates mitochondrial dysfunction, reduces insulin resistance, and improves NAFLD progression[70,71].

CPT-II INACTIVITY IN NAFLD

NAFLD pathogenesis are much complicated with multi-factorial events. Recently, the low activity of CPT-II on IMM during NAFLD progression has attracted much attention both in basic and clinical aspects[72,73]. Although many theories of NAFLD with abnormal lipid metabolism[74,8,75] such as insulin resistance (IR), lipid peroxidation, cytokine expression, iron overload, genetics, environment, immunity, drugs, living habits and so on. However, there are still many problems in study of NAFLD pathogenesis. According to these theories, the IR stimulates liver fat accumulation and triglycerides, resulting in first trike to NAFLD formation; Then oxidative stress and lipid peroxidation aggravate hepatocyte injury to develop into second trike that starts with asymptomatic steatosis, and continues to cell inflammatory, steatohepatitis, fibrosis or hepatocyte malignant transformation[76,77]; Hence a "multiple hit" hypothesis seems a more accurate proposal[78,79]. Up to now, the new discovery of loss CPT-II activity has been confirmed in lipid accumulating models that should be as one of the NAFLD mechanisms.

Ideal NAFLD models should correctly reflect both histopathology and pathophysiology, and imitate certain aspects of NAFLD are divided into genetic, dietary, and combination models referring to advantages and disadvantages[80,81]. Also, the models based on biological knowledge are reliable and reproducible, having low mortality, and being compatible with a simple and feasible method, not only in elucidating pathogenesis for understanding NAFLD but also in examining therapeutic effects of various agents to develop tools and giving crucial information. Inhibiting CPT-II activity is related to disorder of lipid metabolism, which may be related to NAFLD pathogenesis and down-regulating CPT-II in liver tissues. Gene defects are associated with mitochondrial LCFA oxidation disorders[82,83].

In order to determine the independent and interdependent roles of triglyceride (TG) hydrolysis and FAO, liver-specific defects in mice were generated in TG hydrolysis (*AtglL*$^{-/-}$) , FAO (*CPT2L*$^{-/-}$) , or both (double knockout)[73,84]. Loss of a single component of FAO [*CPT2*, adipose TG lipase (*Atgl*), and peroxisome proliferators-activated receptor-α (PPAR-α)] results in a major independent effect on the morphology of liver cells, gene expression, and intermediate metabolism in response to fasting [84]. However, the mice in high-fat diet (HFD) model revealed an interdependent role for *Atgl* and *CPT2*, as deletion of only one gene leads to NAFLD; But loss of both components leads to significant hepatocyte inflammation and liver fibrosis[85].

CPT-II IN HEPATOCARCINOGENESIS

During NAFLD progression, the transcription factors E2f1 and E2f2 contributed to NAFLD-associated mice HCC and their involvement in metabolic recombination [72, 85]. The expressions of E2f1 and E2f2 were significantly increased in the NAFLD-associated HCC in mice induced with HFD plus diethylnitrosamine (Den). However, the *E2f1*$^{-/-}$ and *E2f2*$^{-/-}$ mice were resistant to DEN-HFD-induced hepatocarcinogenesis and were associated with lipid accumulation. The administration of DEN-HFD in the *E2f1*$^{-/-}$ and *E2f2*$^{-/-}$ mice enhanced FAO and increased expression of *CPT2* because of CPT2 as an essential enzyme for FAO, whose down-regulation was linked to the NAFLD-related

hepatocarcinogenesis[86]. The mouse models of obesity-driven and NASH-driven HCC typically exhibit robust steatosis in HCC cells, as seem to be seen in the human NASH-HCC. The livers and HCC tissues from diethylnitrosamine-injected mice fed either control or HFD were subjected to comprehensive metabolome analysis[80,87]. Extensive acylcarnitines accumulation was seen in liver cancer tissue and in the sera of HFD-fed mice. A similar increasing level was seen in sera from patients with NASH-associated liver cancer. The increase of acylcarnitine might be related to the CPT-II down-regulation, suggesting that acylcarnitine is a surrogate marker of the down-regulation of CPT-II and directly participates in the development of hepatocarcinogenesis.

The down-regulation of CPT-II caused FAO inhibition, which might be the cause of steatosis in HCC. The knockdown of *CPT2* gene in HCC cells could inhibit the Src-mediated activation of JNK and produce anti-lipotoxicity. Furthermore, oleylcarnitine promotes spheroid formation in HCC cells through STAT3 activation[88,89]. HFD feeding and carnitine supplementation synergistically enhance hepatocarcinogenesis with acylcarnitine accumulation *in vivo*. The CPT-II level in HCC mice was significantly lower than those in control or NAFLD mice, and was negatively correlated with degree of hepatocyte malignant transformation[90,91]. A series of experiments confirmed that CPT-II was inactivated, suggesting that low CPT-II expression in IMM might lead to liver lipid accumulation, participate in promoting NAFLD malignant transformation[92].

CPT-II INVERSE-CORRELATED WITH HCC MARKERS

Based on clinical and basic evidences, abnormal lipid accumulation was associated with NAFLD malignant transformation. However, only few studies has been reported on the relationship between CPT-II activation and HCC progression. The alteration of CPT-II expression might be an important link in the obstruction of FAO and abnormal lipid accumulation[93]. Based on above findings, some scholars sequenced the whole gene of mitochondrial CPT-II in NAFLD patients, and found that *CPT2* variation was significantly associated with CPT-II activity that might be the key factor of NAFLD or related cirrhosis/HCC because its inactivation are closely related to energy production

disorder in models of NAFLD[94,95]. The dynamic alterations of CPT-II expression located on IMM during malignant transformation of hepatocytes in SD rats induced by chemical carcinogens (2-fluorenylacetamide, 2-FAA) were investigated under lipid accumulation[19]. The alterations of liver histopathology and HCC biomarkers in rat hepatocarcinogenesis are shown in Figure 3. At first time, the progressively decreasing expression of CPT-II at mRNA or protein level were reported, and the significantly increasing HCC related to molecular markers were confirmed during the rat hepatocarcinogenesis.

There has been a rise in the prevalence of NAFLD, paralleling a worldwide increase in MAFLD and HCC[96]. According to the dynamic pathological alterations of the model, a continuum of morphological abnormalities on liver sections (Figure 3) has a variable course, with from normal hepatocytes, lipid accumulation, cell denaturation, precancerous lesion and HCC formation (Figure 3A). Compared with the control liver sections, there was a large amount of fat in the hepatocytes of the model rats (Figure 3B) by the oil red O staining; In the meantime, transmembrane glycoprotein CD44 activation (Figure 3C) promotes inflammatory cell recruitment and plays a key player in linked to NAFLD progression to HCC[97-99]. CPT-II has been demonstrated to interact in forming supramolecular complexes that facilitate passage of acylcarnitine and its expression was gradually decreased in malignant trans-formation of hepatocytes in NAFLD (Figure 3D). However, the reported HCC biomarkers such as AFP (Figure 3E), GPC3 (Figure 3F)[100] and Wnt3a (Figure 3G)[101] were significantly increasing expressions in hepatocarcinogenesis except of CD44 as one of the most frequently reported cancer stem-like cell markers[102]. These data suggested that the alteration of CPT-II expression should be associated with the malignant progression of NAFLD.

Metabolically related NAFLD is emerging as a major cause of HCC in Western countries [103-105]. This presents an additional challenge, as NAFLD-related HCC tend to be advanced in elderly patients with comorbidities and their prognosis is very poor [106]. The pathogenesis of NAFLD-associated HCC is multifactorial and remains to be identified, although the risk of hepatocarcinogenesis is undoubtedly increased as

NAFLD progresses to NASH and cirrhosis[107]. The new findings of CPT-II are useful for understanding NAFLD and HCC and should hopefully lead to the development of clinically relevant biomarkers and strategies to help identify high-risk patients; Early use of preventive measures or better treatment[108]. Energy metabolism is a prerequisite for maintaining normal life activities. NAFLD is caused by excessive lipid accumulation in hepatocytes, and is regarded as one of the most common liver diseases[109,110]. The down-regulation of *CPT2* in IMM was one of the main causes of acyl carnitine accumulation, which was also seen in malignant transformation of hepatocytes, suggesting that CPT-II inactivity or dysfunction might become a new mechanism of blocked lipid oxidation for HCC.

CONCLUSION

Owing to its high prevalence and potential risk of NAFLD has become a major health concern worldwide. Hepatocyte CPT-II variation or activity alteration undoubtedly has a significant impact on aggravating liver fatty accumulation, inducing activation of cancer-related stem cells, and malignant transformation of hepatocytes (Figure 4), especially in patients with HBV chronic infection. The phenomenon of CPT-II inactivation as warning signs of NAFLD malignant transformation needs attention. However, its specific regulation mechanism is unknown, so it has a good research prospect. At present, the exact relationship between CPT-II and NAFLD remains to be explored. It is believed that with the vigorous development of molecular biology theory and technology, the understanding function of CPT-II physiology will continue to deepen, which has guiding significance for CPT-II alteration during NAFLD progression. At the same time, it also brings hope and provides theoretical basis for the assumption of early intervention of NAFLD or human related diseases with CPT-II that as a molecule target for monitoring or therapy.

Hepatocyte CPT-II variation or activity alteration undoubtedly has a significant impact on aggravating liver fatty accumulation, inducing activation of cancer-related stem cells, and malignant transformation of hepatocytes.

REFERENCES

1 **Stefan N**, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. *Lancet Diabetes Endocrinol* 2022; **10**: 284-296 [PMID: 35183303 DOI: 10.1016/S2213-8587(22)00003-1]

2 **Segura-Azuara NLÁ**, Varela-Chinchilla CD, Trinidad-Calderón PA. MAFLD/NAFLD Biopsy-Free Scoring Systems for Hepatic Steatosis, NASH, and Fibrosis Diagnosis. *Front Med (Lausanne)* 2021; **8**: 774079 [PMID: 35096868 DOI: 10.3389/fmed.2021.774079]

3 **Rohm TV**, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity* 2022; **55**: 31-55 [PMID: 35021057 DOI: 10.1016/j.immuni.2021.12.013]

4 **Lazarus JV**, Mark HE, Anstee QM, Arab JP, Batterham RL, Castera L, Cortez-Pinto H, Crespo J, Cusi K, Dirac MA, Francque S, George J, Hagström H, Huang TT, Ismail MH, Kautz A, Sarin SK, Loomba R, Miller V, Newsome PN, Ninburg M, Ocama P, Ratziu V, Rinella M, Romero D, Romero-Gómez M, Schattenberg JM, Tsochatzis EA, Valenti L, Wong VW, Yilmaz Y, Younossi ZM, Zelber-Sagi S; NAFLD Consensus Consortium. Advancing the global public health agenda for NAFLD: a consensus statement. *Nat Rev Gastroenterol Hepatol* 2022; **19**: 60-78 [PMID: 34707258 DOI: 10.1038/s41575-021-00523-4]

5 **Tacke F**, Weiskirchen R. Non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH)-related liver fibrosis: mechanisms, treatment and prevention. *Ann Transl Med* 2021; **9**: 729 [PMID: 33987427 DOI: 10.21037/atm-20-4354]

6 **Tamaki N**, Ajmera V, Loomba R. Non-invasive methods for imaging hepatic steatosis and their clinical importance in NAFLD. *Nat Rev Endocrinol* 2022; **18**: 55-66 [PMID: 34815553 DOI: 10.1038/s41574-021-00584-0]

7 **Scorletti E**, Carr RM. A new perspective on NAFLD: Focusing on lipid droplets. *J Hepatol* 2022; **76**: 934-945 [PMID: 34793866 DOI: 10.1016/j.jhep.2021.11.009]

8 **Foerster F**, Gairing SJ, Müller L, Galle PR. NAFLD-driven HCC: Safety and efficacy of current and emerging treatment options. *J Hepatol* 2022; **76**: 446-457 [PMID: 34555422 DOI: 10.1016/j.jhep.2021.09.007]

9 **Du D**, Liu C, Qin M, Zhang X, Xi T, Yuan S, Hao H, Xiong J. Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. *Acta Pharm Sin B* 2022; **12**: 558-580 [PMID: 35256934 DOI: 10.1016/j.apsb.2021.09.019]

10 **Metwally M**, Berg T, Tsochatzis EA, Eslam M. Translation Reprogramming as a Novel Therapeutic Target in MAFLD. *Adv Biol (Weinh)* 2022; **6**: e2101298 [PMID: 35240009 DOI: 10.1002/adbi.202101298]

11 **Bonnefont JP**, Djouadi F, Prip-Buus C, Gobin S, Munnich A, Bastin J. Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Mol Aspects Med* 2004; **25**: 495-520 [PMID: 15363638 DOI: 10.1016/j.mam.2004.06.004]

12 **Weber M**, Mera P, Casas J, Salvador J, Rodríguez A, Alonso S, Sebastián D, Soler-Vázquez MC, Montironi C, Recalde S, Fucho R, Calderón-Domínguez M, Mir JF, Bartrons R, Escola-Gil JC, Sánchez-Infantes D, Zorzano A, Llorente-Cortes V, Casals N, Valentí V, Frühbeck G, Herrero L, Serra D. Liver CPT1A gene therapy reduces diet-induced hepatic steatosis in mice and highlights potential lipid biomarkers for human NAFLD. *FASEB J* 2020; **34**: 11816-11837 [PMID: 32666604 DOI: 10.1096/fj.202000678R]

13 **Sangineto M**, Bukke VN, Bellanti F, Tamborra R, Moola A, Duda L, Villani R, Romano AD, Serviddio G. A Novel Nutraceuticals Mixture Improves Liver Steatosis by Preventing Oxidative Stress and Mitochondrial Dysfunction in a NAFLD Model. *Nutrients* 2021; **13** [PMID: 33671262 DOI: 10.3390/nu13020652]

14 **Li L**, Yang X. The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. *Oxid Med Cell Longev* 2018; **2018**: 7580707 [PMID: 29849912 DOI: 10.1155/2018/7580707]

15 **Zhou R**, Lin C, Cheng Y, Zhuo X, Li Q, Xu W, Zhao L, Yang L. Liraglutide Alleviates Hepatic Steatosis and Liver Injury in T2MD Rats *via* a GLP-1R Dependent AMPK Pathway. *Front Pharmacol* 2020; **11**: 600175 [PMID: 33746742 DOI: 10.3389/fphar.2020.600175]

16 **Dall M**, Hassing AS, Treebak JT. NAD^+ and NAFLD - caution, causality and careful optimism. *J Physiol* 2022; **600**: 1135-1154 [PMID: 33932956 DOI: 10.1113/JP280908]

17 **Petrick HL**, Holloway GP. Cytosolic reverse CrAT activity in cardiac tissue: potential importance for fuel selection. *Biochem J* 2018; **475**: 1267-1269 [PMID: 29632149 DOI: 10.1042/BCJ20180121]

18 **Wang Y**, Lu JH, Wang F, Wang YN, He MM, Wu QN, Lu YX, Yu HE, Chen ZH, Zhao Q, Liu J, Chen YX, Wang DS, Sheng H, Liu ZX, Zeng ZL, Xu RH, Ju HQ. Inhibition of fatty acid catabolism augments the efficacy of oxaliplatin-based chemotherapy in gastrointestinal cancers. *Cancer Lett* 2020; **473**: 74-89 [PMID: 31904482 DOI: 10.1016/j.canlet.2019.12.036]

19 **Gu JJ**, Yao M, Yang J, Cai Y, Zheng WJ, Wang L, Yao DB, Yao DF. Mitochondrial carnitine palmitoyl transferase-II inactivity aggravates lipid accumulation in rat hepatocarcinogenesis. *World J Gastroenterol* 2017; **23**: 256-264 [PMID: 28127199 DOI: 10.3748/wjg.v23.i2.256]

20 **Rufer AC**, Thoma R, Hennig M. Structural insight into function and regulation of carnitine palmitoyltransferase. *Cell Mol Life Sci* 2009; **66**: 2489-2501 [PMID: 19430727 DOI: 10.1007/s00018-009-0035-1]

21 **Rufer AC**, Thoma R, Benz J, Stihle M, Gsell B, De Roo E, Banner DW, Mueller F, Chomienne O, Hennig M. The crystal structure of carnitine palmitoyltransferase 2 and implications for diabetes treatment. *Structure* 2006; **14**: 713-723 [PMID: 16615913 DOI: 10.1016/j.str.2006.01.008]

22 **Trauner M**, Fuchs CD. Novel therapeutic targets for cholestatic and fatty liver disease. *Gut* 2022; **71**: 194-209 [PMID: 34615727 DOI: 10.1136/gutjnl-2021-324305]

23 **Filali-Mouncef Y**, Hunter C, Roccio F, Zagkou S, Dupont N, Primard C, Proikas-Cezanne T, Reggiori F. The ménage à trois of autophagy, lipid droplets and liver disease. *Autophagy* 2022; **18**: 50-72 [PMID: 33794741 DOI: 10.1080/15548627.2021.1895658]

24 **Han S**, Wei R, Zhang X, Jiang N, Fan M, Huang JH, Xie B, Zhang L, Miao W, Butler AC, Coleman MA, Vaughan AT, Wang Y, Chen HW, Liu J, Li JJ. CPT1A/2-Mediated FAO Enhancement-A Metabolic Target in Radioresistant Breast Cancer. *Front Oncol* 2019; **9**: 1201 [PMID: 31803610 DOI: 10.3389/fonc.2019.01201]

25 **Park JH**, Vithayathil S, Kumar S, Sung PL, Dobrolecki LE, Putluri V, Bhat VB, Bhowmik SK, Gupta V, Arora K, Wu D, Tsouko E, Zhang Y, Maity S, Donti TR, Graham BH, Frigo DE, Coarfa C, Yotnda P, Putluri N, Sreekumar A, Lewis MT, Creighton CJ, Wong LC, Kaipparettu BA. Fatty Acid Oxidation-Driven Src Links Mitochondrial Energy Reprogramming and Oncogenic Properties in Triple-Negative Breast Cancer. *Cell Rep* 2016; **14**: 2154-2165 [PMID: 26923594 DOI: 10.1016/j.celrep.2016.02.004]

26 **de Carvalho Ribeiro M**, Szabo G. Role of the Inflammasome in Liver Disease. *Annu Rev Pathol* 2022; **17**: 345-365 [PMID: 34752711 DOI: 10.1146/annurev-pathmechdis-032521-102529]

27 **Rufer AC**, Lomize A, Benz J, Chomienne O, Thoma R, Hennig M. Carnitine palmitoyltransferase 2: analysis of membrane association and complex structure with a substrate analog. *FEBS Lett* 2007; **581**: 3247-3252 [PMID: 17585909 DOI: 10.1016/j.febslet.2007.05.080]

28 **Song A**, Park Y, Kim B, Lee SG. Modulation of Lipid Metabolism by Trans-Anethole in Hepatocytes. *Molecules* 2020; **25**: 4946 [PMID: 33114589 DOI: 10.3390/molecules25214946]

29 **Wang J**, Xiang H, Lu Y, Wu T, Ji G. The role and therapeutic implication of CPTs in fatty acid oxidation and cancers progression. *Am J Cancer Res* 2021; **11**: 2477-2494 [PMID: 34249411]

30 **Yao M**, Cai M, Yao D, Xu X, Yang R, Li Y, Zhang Y, Kido H, Yao D. Abbreviated half-lives and impaired fuel utilization in carnitine palmitoyltransferase II variant fibroblasts. *PLoS One* 2015; **10**: e0119936 [PMID: 25781464 DOI: 10.1371/journal.pone.0119936]

31 **Yao M**, Yao D, Yamaguchi M, Chida J, Yao D, Kido H. Bezafibrate upregulates carnitine palmitoyltransferase II expression and promotes mitochondrial energy crisis dissipation in fibroblasts of patients with influenza-associated encephalopathy. *Mol Genet Metab* 2011; **104**: 265-272 [PMID: 21816645 DOI: 10.1016/j.ymgme.2011.07.009]

32 **Gu JJ**, Yao M, Cai Y, Fang M, Wang L, Zheng WJ, Yao DB, Dong ZZ, Yao DF. [Dynamic expression of carnitine palmitoyltransferase II in the mitochondrial inner

membrane during hepatocyte malignant transformation induced by lipid accumulation]. *Zhonghua Gan Zang Bing Za Zhi* 2017; **25**: 279-284 [PMID: 28494547 DOI: 10.3760/cma.j.issn.1007-3418.2017.04.009]

33 **Stenson PD**, Ball EV, Mort M, Phillips AD, Shaw K, Cooper DN. The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. *Curr Protoc Bioinformatics* 2012; **Chapter 1**: Unit1.13 [PMID: 22948725 DOI: 10.1002/0471250953.bi0113s39]

34 **Xue Y**, Chen Y, Ayub Q, Huang N, Ball EV, Mort M, Phillips AD, Shaw K, Stenson PD, Cooper DN, Tyler-Smith C; 1000 Genomes Project Consortium. Deleterious- and disease-allele prevalence in healthy individuals: insights from current predictions, mutation databases, and population-scale resequencing. *Am J Hum Genet* 2012; **91**: 1022-1032 [PMID: 23217326 DOI: 10.1016/j.ajhg.2012.10.015]

35 **Lehmann D**, Zierz S. Normal protein content but abnormally inhibited enzyme activity in muscle carnitine palmitoyltransferase II deficiency. *J Neurol Sci* 2014; **339**: 183-188 [PMID: 24602495 DOI: 10.1016/j.jns.2014.02.011]

36 **Yao D**, Mizuguchi H, Yamaguchi M, Yamada H, Chida J, Shikata K, Kido H. Thermal instability of compound variants of carnitine palmitoyltransferase II and impaired mitochondrial fuel utilization in influenza-associated encephalopathy. *Hum Mutat* 2008; **29**: 718-727 [PMID: 18306170 DOI: 10.1002/humu.20717]

37 **Houten SM**, Violante S, Ventura FV, Wanders RJ. The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders. *Annu Rev Physiol* 2016; **78**: 23-44 [PMID: 26474213 DOI: 10.1146/annurev-physiol-021115-105045]

38 **Fucho R**, Casals N, Serra D, Herrero L. Ceramides and mitochondrial fatty acid oxidation in obesity. *FASEB J* 2017; **31**: 1263-1272 [PMID: 28003342 DOI: 10.1096/fj.201601156R]

39 **Angajala A**, Lim S, Phillips JB, Kim JH, Yates C, You Z, Tan M. Diverse Roles of Mitochondria in Immune Responses: Novel Insights Into Immuno-Metabolism. *Front Immunol* 2018; **9**: 1605 [PMID: 30050539 DOI: 10.3389/fimmu.2018.01605]

40 **Serra D**, Mera P, Malandrino MI, Mir JF, Herrero L. Mitochondrial fatty acid oxidation in obesity. *Antioxid Redox Signal* 2013; **19**: 269-284 [PMID: 22900819 DOI: 10.1089/ars.2012.4875]

41 **Schlaepfer IR**, Joshi M. CPT1A-mediated Fat Oxidation, Mechanisms, and Therapeutic Potential. *Endocrinology* 2020; **161**: bqz046 [PMID: 31900483 DOI: 10.1210/endocr/bqz046]

42 **Casals N**, Zammit V, Herrero L, Fadó R, Rodríguez-Rodríguez R, Serra D. Carnitine palmitoyltransferase 1C: From cognition to cancer. *Prog Lipid Res* 2016; **61**: 134-148 [PMID: 26708865 DOI: 10.1016/j.plipres.2015.11.004]

43 **Isackson PJ**, Bennett MJ, Vladutiu GD. Identification of 16 new disease-causing mutations in the CPT2 gene resulting in carnitine palmitoyltransferase II deficiency. *Mol Genet Metab* 2006; **89**: 323-331 [PMID: 16996287 DOI: 10.1016/j.ymgme.2006.08.004]

44 **Qu Q**, Zeng F, Liu X, Wang QJ, Deng F. Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. *Cell Death Dis* 2016; **7**: e2226 [PMID: 27195673 DOI: 10.1038/cddis.2016.132]

45 **Eng JM**, Estall JL. Diet-Induced Models of Non-Alcoholic Fatty Liver Disease: Food for Thought on Sugar, Fat, and Cholesterol. *Cells* 2021; **10** [PMID: 34359974 DOI: 10.3390/cells10071805]

46 **Cacciola NA**, Sgadari M, Sepe F, Petillo O, Margarucci S, Martano M, Maiolino P, Restucci B. Metabolic Flexibility in Canine Mammary Tumors: Implications of the Carnitine System. *Animals (Basel)* 2021; **11** [PMID: 34679988 DOI: 10.3390/ani11102969]

47 **Wang M**, Wang K, Liao X, Hu H, Chen L, Meng L, Gao W, Li Q. Carnitine Palmitoyltransferase System: A New Target for Anti-Inflammatory and Anticancer Therapy? *Front Pharmacol* 2021; **12**: 760581 [PMID: 34764874 DOI: 10.3389/fphar.2021.760581]

48 **Mccormick BJ**, Chirila RM. Carnitine palmitoyltransferase-II deficiency: case presentation and review of the literature. *Rom J Intern Med* 2021; **59**: 420-424 [PMID: 34118800 DOI: 10.2478/rjim-2021-0021]

49 **Sigauke E**, Rakheja D, Kitson K, Bennett MJ. Carnitine palmitoyltransferase II deficiency: a clinical, biochemical, and molecular review. *Lab Invest* 2003; **83**: 1543-1554 [PMID: 14615409 DOI: 10.1097/01.lab.0000098428.51765.83]

50 **Tajima G**, Hara K, Yuasa M. Carnitine palmitoyltransferase II deficiency with a focus on newborn screening. *J Hum Genet* 2019; **64**: 87-98 [PMID: 30514913 DOI: 10.1038/s10038-018-0530-z]

51 **Lehmann D**, Motlagh L, Robaa D, Zierz S. Muscle Carnitine Palmitoyltransferase II Deficiency: A Review of Enzymatic Controversy and Clinical Features. *Int J Mol Sci* 2017; **18** [PMID: 28054946 DOI: 10.3390/ijms]

52 **Kilfoyle D**, Hutchinson D, Potter H, George P. Recurrent myoglobinuria due to carnitine palmitoyltransferase II deficiency: clinical, biochemical, and genetic features of adult-onset cases. *N Z Med J* 2005; **118**: U1320 [PMID: 15776096]

53 **Taggart RT**, Smail D, Apolito C, Vladutiu GD. Novel mutations associated with carnitine palmitoyltransferase II deficiency. *Hum Mutat* 1999; **13**: 210-220 [PMID: 10090476 DOI: 10.1002/(SICI)1098-1004(1999)13:3<210::AID-HUMU5>3.0.CO;2-0]

54 **Joshi PR**, Deschauer M, Zierz S. Carnitine palmitoyltransferase II (CPT II) deficiency: genotype-phenotype analysis of 50 patients. *J Neurol Sci* 2014; **338**: 107-111 [PMID: 24398345 DOI: 10.1016/j.jns.2013.12.026]

55 **Jogl G**, Hsiao YS, Tong L. Structure and function of carnitine acyltransferases. *Ann N Y Acad Sci* 2004; **1033**: 17-29 [PMID: 15591000 DOI: 10.1196/annals.1320.002]

56 **Motlagh L**, Golbik R, Sippl W, Zierz S. Stabilization of the thermolabile variant S113L of carnitine palmitoyltransferase II. *Neurol Genet* 2016; **2**: e53 [PMID: 27123472 DOI: 10.1212/NXG.0000000000000053]

57 **Dang Y**, Xu J, Zhu M, Zhou W, Zhang L, Ji G. Gan-Jiang-Ling-Zhu decoction alleviates hepatic steatosis in rats by the miR-138-5p/CPT1B axis. *Biomed Pharmacother* 2020; **127**: 110127 [PMID: 32325349 DOI: 10.1016/j.biopha.2020.110127]

58 **Xu A**, Wang B, Fu J, Qin W, Yu T, Yang Z, Lu Q, Chen J, Chen Y, Wang H. Diet-induced hepatic steatosis activates Ras to promote hepatocarcinogenesis *via* CPT1α. *Cancer Lett* 2019; **442**: 40-52 [PMID: 30401637 DOI: 10.1016/j.canlet.2018.10.024]

59 **Yao D**, Yao M, Yamaguchi M, Chida J, Kido H. Characterization of compound missense mutation and deletion of carnitine palmitoyltransferase II in a patient with adenovirus-associated encephalopathy. *J Med Invest* 2011; **58**: 210-218 [PMID: 21921422 DOI: 10.2152/jmi.58.210]

60 **Jogl G**, Tong L. Crystal structure of carnitine acetyltransferase and implications for the catalytic mechanism and fatty acid transport. *Cell* 2003; **112**: 113-122 [PMID: 12526798 DOI: 10.1016/S0092-8674(02)01228-X]

61 **Abolfathi M**, Mohd-Yusof BN, Hanipah ZN, Mohd Redzwan S, Yusof LM, Khosroshahi MZ. The effects of carnitine supplementation on clinical characteristics of patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med* 2020; **48**: 102273 [PMID: 31987257 DOI: 10.1016/j.ctim.2019.102273]

62 **Li N**, Zhao H. Role of Carnitine in Non-alcoholic Fatty Liver Disease and Other Related Diseases: An Update. *Front Med (Lausanne)* 2021; **8**: 689042 [PMID: 34434943 DOI: 10.3389/fmed.2021.689042]

63 **Savic D**, Hodson L, Neubauer S, Pavlides M. The Importance of the Fatty Acid Transporter L-Carnitine in Non-Alcoholic Fatty Liver Disease (NAFLD). *Nutrients* 2020; **12** [PMID: 32708036 DOI: 10.3390/nu12082178]

64 **Baker PR 2nd**, Friedman JE. Mitochondrial role in the neonatal predisposition to developing nonalcoholic fatty liver disease. *J Clin Invest* 2018; **128**: 3692-3703 [PMID: 30168806 DOI: 10.1172/JCI120846]

65 **Poulos JE**, Kalogerinis PT, Milanov V, Kalogerinis CT, Poulos EJ. The Effects of Vitamin E, Silymarin and Carnitine on the Metabolic Abnormalities Associated with Nonalcoholic Liver Disease. *J Diet Suppl* 2022; **19**: 287-302 [PMID: 33491528 DOI: 10.1080/19390211.2021.1874587]

66 **Sogabe M**, Okahisa T, Kurihara T, Takehara M, Kagemoto K, Okazaki J, Kida Y, Hirao A, Tanaka H, Tomonari T, Taniguchi T, Okamoto K, Nakasono M, Takayama T. Differences among patients with and without nonalcoholic fatty liver disease having

elevated alanine aminotransferase levels at various stages of metabolic syndrome. *PLoS One* 2020; **15**: e0238388 [PMID: 32866186 DOI: 10.1371/journal.pone.0238388]

67 **Gu J**, Yao M, Yao D, Wang L, Yang X, Yao D. Nonalcoholic Lipid Accumulation and Hepatocyte Malignant Transformation. *J Clin Transl Hepatol* 2016; **4**: 123-130 [PMID: 27350942 DOI: 10.14218/JCTH.2016.00010]

68 **Enooku K**, Nakagawa H, Fujiwara N, Kondo M, Minami T, Hoshida Y, Shibahara J, Tateishi R, Koike K. Altered serum acylcarnitine profile is associated with the status of nonalcoholic fatty liver disease (NAFLD) and NAFLD-related hepatocellular carcinoma. *Sci Rep* 2019; **9**: 10663 [PMID: 31337855 DOI: 10.1038/s41598-019-47216-2]

69 **da Silva RP**, Kelly KB, Al Rajabi A, Jacobs RL. Novel insights on interactions between folate and lipid metabolism. *Biofactors* 2014; **40**: 277-283 [PMID: 24353111 DOI: 10.1002/biof.1154]

70 **Giannakoulis VG**, Dubovan P, Papoutsi E, Kataki A, Koskinas J. Senescence in HBV-, HCV- and NAFLD- Mediated Hepatocellular Carcinoma and Senotherapeutics: Current Evidence and Future Perspective. *Cancers (Basel)* 2021; **13** [PMID: 34572959 DOI: 10.3390/cancers13184732]

71 **Das UN**. Beneficial role of bioactive lipids in the pathobiology, prevention, and management of HBV, HCV and alcoholic hepatitis, NAFLD, and liver cirrhosis: A review. *J Adv Res* 2019; **17**: 17-29 [PMID: 31193303 DOI: 10.1016/j.jare.2018.12.006]

72 **González-Romero F**, Mestre D, Aurrekoetxea I, O'Rourke CJ, Andersen JB, Woodhoo A, Tamayo-Caro M, Varela-Rey M, Palomo-Irigoyen M, Gómez-Santos B, de Urturi DS, Núñez-García M, García-Rodríguez JL, Fernández-Ares L, Buqué X, Iglesias-Ara A, Bernales I, De Juan VG, Delgado TC, Goikoetxea-Usandizaga N, Lee R, Bhanot S, Delgado I, Perugorria MJ, Errazti G, Mosteiro L, Gaztambide S, Martinez de la Piscina I, Iruzubieta P, Crespo J, Banales JM, Martínez-Chantar ML, Castaño L, Zubiaga AM, Aspichueta P. E2F1 and E2F2-Mediated Repression of CPT2 Establishes a Lipid-Rich Tumor-Promoting Environment. *Cancer Res* 2021; **81**: 2874-2887 [PMID: 33771899 DOI: 10.1158/0008-5472.CAN-20-2052]

73 **Fujiwara N**, Nakagawa H, Enooku K, Kudo Y, Hayata Y, Nakatsuka T, Tanaka Y, Tateishi R, Hikiba Y, Misumi K, Tanaka M, Hayashi A, Shibahara J, Fukayama M, Arita J, Hasegawa K, Hirschfield H, Hoshida Y, Hirata Y, Otsuka M, Tateishi K, Koike K. CPT2 downregulation adapts HCC to lipid-rich environment and promotes carcinogenesis *via* acylcarnitine accumulation in obesity. *Gut* 2018; **67**: 1493-1504 [PMID: 29437870 DOI: 10.1136/gutjnl-2017-315193]

74 **Cheung KP**, Taylor KR, Jameson JM. Immunomodulation at epithelial sites by obesity and metabolic disease. *Immunol Res* 2012; **52**: 182-199 [PMID: 22160809 DOI: 10.1007/s12026-011-8261-7]

75 **Dhamija E**, Paul SB, Kedia S. Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: An increasing concern. *Indian J Med Res* 2019; **149**: 9-17 [PMID: 31115369 DOI: 10.4103/ijmr.IJMR_1456_17]

76 **Oseini AM**, Sanyal AJ. Therapies in non-alcoholic steatohepatitis (NASH). *Liver Int* 2017; **37 Suppl 1**: 97-103 [PMID: 28052626 DOI: 10.1111/liv.13302]

77 **Stefan N**, Häring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol* 2019; **7**: 313-324 [PMID: 30174213 DOI: 10.1016/S2213-8587(18)30154-2]

78 **Pinweha P**, Rattanapornsompong K, Charoensawan V, Jitrapakdee S. MicroRNAs and oncogenic transcriptional regulatory networks controlling metabolic reprogramming in cancers. *Comput Struct Biotechnol J* 2016; **14**: 223-233 [PMID: 27358718 DOI: 10.1016/j.csbj.2016.05.005]

79 **Yi YS**. Regulatory Roles of Caspase-11 Non-Canonical Inflammasome in Inflammatory Liver Diseases. *Int J Mol Sci* 2022; **23** [PMID: 35563377 DOI: 10.3390/ijms23094986]

80 **Lee J**, Choi J, Selen Alpergin ES, Zhao L, Hartung T, Scafidi S, Riddle RC, Wolfgang MJ. Loss of Hepatic Mitochondrial Long-Chain Fatty Acid Oxidation Confers Resistance to Diet-Induced Obesity and Glucose Intolerance. *Cell Rep* 2017; **20**: 655-667 [PMID: 28723568 DOI: 10.1016/j.celrep.2017.06.080]

81 **Selen ES**, Choi J, Wolfgang MJ. Discordant hepatic fatty acid oxidation and triglyceride hydrolysis leads to liver disease. *JCI Insight* 2021; **6** [PMID: 33491665 DOI: 10.1172/jci.insight.135626]

82 **Liu X**, Zhang J, Ming Y, Chen X, Zeng M, Mao Y. The aggravation of mitochondrial dysfunction in nonalcoholic fatty liver disease accompanied with type 2 diabetes mellitus. *Scand J Gastroenterol* 2015; **50**: 1152-1159 [PMID: 25877002 DOI: 10.3109/00365521.2015.1030687]

83 **Jun DW**, Cho WK, Jun JH, Kwon HJ, Jang KS, Kim HJ, Jeon HJ, Lee KN, Lee HL, Lee OY, Yoon BC, Choi HS, Hahm JS, Lee MH. Prevention of free fatty acid-induced hepatic lipotoxicity by carnitine *via* reversal of mitochondrial dysfunction. *Liver Int* 2011; **31**: 1315-1324 [PMID: 22093454 DOI: 10.1111/j.1478-3231.2011.02602.x]

84 **Amato A**, Caldara GF, Nuzzo D, Baldassano S, Picone P, Rizzo M, Mulè F, Di Carlo M. NAFLD and Atherosclerosis Are Prevented by a Natural Dietary Supplement Containing Curcumin, Silymarin, Guggul, Chlorogenic Acid and Inulin in Mice Fed a High-Fat Diet. *Nutrients* 2017; **9** [PMID: 28505074 DOI: 10.3390/nu9050492]

85 **Lee Y**, Kim BR, Kang GH, Lee GJ, Park YJ, Kim H, Jang HC, Choi SH. The Effects of PPAR Agonists on Atherosclerosis and Nonalcoholic Fatty Liver Disease in ApoE-/-FXR-/- Mice. *Endocrinol Metab (Seoul)* 2021; **36**: 1243-1253 [PMID: 34986301 DOI: 10.3803/EnM.2021.1100]

86 **Kim JH**, Lee BR, Choi ES, Lee KM, Choi SK, Cho JH, Jeon WB, Kim E. Reverse Expression of Aging-Associated Molecules through Transfection of miRNAs to Aged Mice. *Mol Ther Nucleic Acids* 2017; **6**: 106-115 [PMID: 28325277 DOI: 10.1016/j.omtn.2016.11.005]

87 **Schulien I**, Hasselblatt P. Diethylnitrosamine-induced liver tumorigenesis in mice. *Methods Cell Biol* 2021; **163**: 137-152 [PMID: 33785162 DOI: 10.1016/bs.mcb.2020.08.006]

88 **Attia YM**, Tawfiq RA, Gibriel AA, Ali AA, Kassem DH, Hammam OA, Elmazar MM. Activation of FXR modulates SOCS3/Jak2/STAT3 signaling axis in a NASH-dependent hepatocellular carcinoma animal model. *Biochem Pharmacol* 2021; **186**: 114497 [PMID: 33675775 DOI: 10.1016/j.bcp.2021.114497]

89 **Zhou M**, Mok MT, Sun H, Chan AW, Huang Y, Cheng AS, Xu G. The anti-diabetic drug exenatide, a glucagon-like peptide-1 receptor agonist, counteracts hepatocarcinogenesis through cAMP-PKA-EGFR-STAT3 axis. *Oncogene* 2017; **36**: 4135-4149 [PMID: 28319060 DOI: 10.1038/onc.2017.38]

90 **Khan H**, Ullah H, Nabavi SM. Mechanistic insights of hepatoprotective effects of curcumin: Therapeutic updates and future prospects. *Food Chem Toxicol* 2019; **124**: 182-191 [PMID: 30529260 DOI: 10.1016/j.fct.2018.12.002]

91 **Holczbauer Á**, Wangensteen KJ, Shin S. Cellular origins of regenerating liver and hepatocellular carcinoma. *JHEP Rep* 2022; **4**: 100416 [PMID: 35243280 DOI: 10.1016/j.jhepr.2021.100416]

92 **Brown ZJ**, Fu Q, Ma C, Kruhlak M, Zhang H, Luo J, Heinrich B, Yu SJ, Zhang Q, Wilson A, Shi ZD, Swenson R, Greten TF. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces $CD4^+$ T cell apoptosis promoting HCC development. *Cell Death Dis* 2018; **9**: 620 [PMID: 29795111 DOI: 10.1038/s41419-018-0687-6]

93 **Paul B**, Lewinska M, Andersen JB. Lipid alterations in chronic liver disease and liver cancer. *JHEP Rep* 2022; **4**: 100479 [PMID: 35469167 DOI: 10.1016/j.jhepr.2022.100479]

94 **Ham JR**, Lee HI, Choi RY, Sim MO, Seo KI, Lee MK. Anti-steatotic and anti-inflammatory roles of syringic acid in high-fat diet-induced obese mice. *Food Funct* 2016; **7**: 689-697 [PMID: 26838182 DOI: 10.1039/c5fo01329a]

95 **Liu Q**, Pan R, Ding L, Zhang F, Hu L, Ding B, Zhu L, Xia Y, Dou X. Rutin exhibits hepatoprotective effects in a mouse model of non-alcoholic fatty liver disease by reducing hepatic lipid levels and mitigating lipid-induced oxidative injuries. *Int Immunopharmacol* 2017; **49**: 132-141 [PMID: 28577437 DOI: 10.1016/j.intimp.2017.05.026]

96 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]

97 **Patouraux S**, Rousseau D, Bonnafous S, Lebeaupin C, Luci C, Canivet CM, Schneck AS, Bertola A, Saint-Paul MC, Iannelli A, Gugenheim J, Anty R, Tran A, Bailly-Maitre B,

Gual P. CD44 is a key player in non-alcoholic steatohepatitis. *J Hepatol* 2017; **67**: 328-338 [PMID: 28323124 DOI: 10.1016/j.jhep.2017.03.003]

98 **Egan CE**, Daugherity EK, Rogers AB, Abi Abdallah DS, Denkers EY, Maurer KJ. CCR2 and CD44 promote inflammatory cell recruitment during fatty liver formation in a lithogenic diet fed mouse model. *PLoS One* 2013; **8**: e65247 [PMID: 23762326 DOI: 10.1371/journal.pone.0065247]

99 **Fang M**, Yao M, Yang J, Zheng WJ, Wang L, Yao DF. Abnormal CD44 activation of hepatocytes with nonalcoholic fatty accumulation in rat hepatocarcinogenesis. *World J Gastrointest Oncol* 2020; **12**: 66-76 [PMID: 31966914 DOI: 10.4251/wjgo.v12.i1.66]

100 **Yao M**, Yao DF, Bian YZ, Zhang CG, Qiu LW, Wu W, Sai WL, Yang JL, Zhang HJ. Oncofetal antigen glypican-3 as a promising early diagnostic marker for hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 289-294 [PMID: 21669573 DOI: 10.1016/S1499-3872(11)60048-9]

101 **Yao M**, Yang JL, Wang DF, Wang L, Chen Y, Yao DF. Encouraging specific biomarkers-based therapeutic strategies for hepatocellular carcinoma. *World J Clin Cases* 2022; **10**: 3321-3333 [PMID: 35611205 DOI: 10.12998/wjcc.v10.i11.3321]

102 **Wu XF**, Sha CX, Yang JL, Liu Y, Zhou P, Yao DF, Yao M. [Abnormal expression of CD44 aggravates liver disease progression in patients with non-alcoholic fatty liver disease accompanied with hepatitis B virus replication]. *Zhonghua Gan Zang Bing Za Zhi* 2021; **29**: 1083-1088 [PMID: 34933427 DOI: 10.3760/cma.j.cn501113-20210713-00338]

103 **Anstee QM**, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 411-428 [PMID: 31028350 DOI: 10.1038/s41575-019-0145-7]

104 **Meroni M**, Longo M, Rustichelli A, Dongiovanni P. Nutrition and Genetics in NAFLD: The Perfect Binomium. *Int J Mol Sci* 2020; **21** [PMID: 32340286 DOI: 10.3390/ijms21082986]

105 **Singal AG**, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: New trends. *J Hepatol* 2020; **72**: 250-261 [PMID: 31954490 DOI: 10.1016/j.jhep.2019.08.025]

106 **Geh D**, Anstee QM, Reeves HL. NAFLD-Associated HCC: Progress and Opportunities. *J Hepatocell Carcinoma* 2021; **8**: 223-239 [PMID: 33854987 DOI: 10.2147/JHC.S272213]

107 **Harris PS**, Hansen RM, Gray ME, Massoud OI, McGuire BM, Shoreibah MG. Hepatocellular carcinoma surveillance: An evidence-based approach. *World J Gastroenterol* 2019; **25**: 1550-1559 [PMID: 30983815 DOI: 10.3748/wjg.v25.i13.1550]

108 **Fujiwara N**, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J Hepatol* 2018; **68**: 526-549 [PMID: 28989095 DOI: 10.1016/j.jhep.2017.09.016]

109 **Márquez-Quiroga LV**, Arellanes-Robledo J, Vásquez-Garzón VR, Villa-Treviño S, Muriel P. Models of nonalcoholic steatohepatitis potentiated by chemical inducers leading to hepatocellular carcinoma. *Biochem Pharmacol* 2022; **195**: 114845 [PMID: 34801522 DOI: 10.1016/j.bcp.2021.114845]

110 **Clutton-Brock TH**, Iason GR. Sex ratio variation in mammals. *Q Rev Biol* 1986; **61**: 339-374 [PMID: 3532167 DOI: 10.1186/s12885-022-09433-3]

78313_Auto_Edited-check.docx

ORIGINALITY REPORT

13%
SIMILARITY INDEX

PRIMARY SOURCES

1	pubmed.ncbi.nlm.nih.gov Internet	102 words — 2%
2	www.ncbi.nlm.nih.gov Internet	86 words — 2%
3	search.bvsalud.org Internet	44 words — 1%
4	www.dovepress.com Internet	36 words — 1%
5	utsouthwestern.influuent.utsystem.edu Internet	33 words — 1%
6	academic.oup.com Internet	30 words — 1%
7	www.mdpi.com Internet	29 words — 1%
8	G. JOGL. "Structure and Function of Carnitine Acyltransferases", Annals of the New York Academy of Sciences, 11/01/2004 Crossref	26 words — 1%
9	pub2.db.tokushima-u.ac.jp Internet	25 words — < 1%

10	Simona M. Ceccarelli, Odile Chomienne, Marcel Gubler, Arduino Arduini. "Carnitine Palmitoyltransferase (CPT) Modulators: A Medicinal Chemistry Perspective on 35 Years of Research", Journal of Medicinal Chemistry, 2011 Crossref	22 words — < 1%
11	Yu-Shan Hsiao, Gerwald Jogl, Liang Tong. "Crystal Structures of Murine Carnitine Acetyltransferase in Ternary Complexes with Its Substrates", Journal of Biological Chemistry, 2006 Crossref	22 words — < 1%
12	Fuqiang Liu, Xiaoqing Li, Han Yan, Jiao Wu, Yichun Yang, Jin He, Jun Chen, Zhongxiang Jiang, Fan Wu, Zheng Jiang. "Downregulation of CPT2 promotes proliferation and inhibits apoptosis through p53 pathway in colorectal cancer", Cellular Signalling, 2022 Crossref	21 words — < 1%
13	Y. Chen, H. Mizuguchi, D. Yao, M. Ide, Y. Kuroda, Y. Shigematsu, S. Yamaguchi, M. Yamaguchi, M. Kinoshita, H. Kido. "Thermolabile phenotype of carnitine palmitoyltransferase II variations as a predisposing factor for influenza-associated encephalopathy", FEBS Letters, 2005 Crossref	20 words — < 1%
14	link.springer.com Internet	18 words — < 1%
15	www.researchgate.net Internet	18 words — < 1%
16	scholars.houstonmethodist.org Internet	16 words — < 1%

17	Naoto Fujiwara, Hayato Nakagawa, Kenichiro Enooku, Yotaro Kudo et al. "CPT2 downregulation adapts HCC to lipid-rich environment and promotes carcinogenesis via acylcarnitine accumulation in obesity", Gut, 2018 Crossref	15 words —	< 1%
18	Francisco González-Romero, Daniela Mestre, Igor Aurrekoetxea, Colm J. O'Rourke et al. "E2F1 and E2F2-Mediated Repression of CPT2 Establishes a Lipid-Rich Tumor-Promoting Environment", Cancer Research, 2021 Crossref	14 words —	< 1%
19	www.frontiersin.org Internet	14 words —	< 1%
20	www.wjgnet.com Internet	14 words —	< 1%
21	Kompare, M.. "Mitochondrial Fatty-Acid Oxidation Disorders", Seminars in Pediatric Neurology, 200809 Crossref	13 words —	< 1%
22	Jeffrey V. Lazarus, Henry E. Mark, Quentin M. Anstee, Juan Pablo Arab et al. "Advancing the global public health agenda for NAFLD: a consensus statement", Nature Reviews Gastroenterology & Hepatology, 2021 Crossref	12 words —	< 1%
23	Ebru S. Selen, Joseph Choi, Michael J. Wolfgang. "Discordant hepatic fatty acid oxidation and triglyceride hydrolysis leads to liver disease", JCI Insight, 2021 Crossref	11 words —	< 1%
24	id.nii.ac.jp		

Internet

11 words — < 1%

25 www.intechopen.com
Internet

11 words — < 1%

26 Claudio Bruno. "Lipid storage myopathies", Current Opinion in Neurology, 10/2008
Crossref

10 words — < 1%

27 pt.scribd.com
Internet

10 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 10 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 10 WORDS