Scientific research article / https://doi.org/10.15446/rcciquifa.v53n3.119213

Chemical-pharmaceutical application of carnitine palmitoyltransferase-2 (CPT-II) through regulating mitochondrial against cancer tumoric cells

Majid Monajjemi[1]*, Fatemeh Mollaamin[2], Samira Mohammadi[1], Sara Shahriari[3]

[1] Department of Chemical Engineering, Central Tehran Branch, Islamic Azad University, Tehran 1477893855, Iran

[2] Department of Biomedical Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu 37150, Turkey

[3] Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran 1477893855, Iran

*Correspondence: E-mail address: m_monajjemi@yahoo.com; maj.monajjemi@iauctb.ac.ir

Received: March 16, 2024
Corrected: April 28, 2024
Accepted: June 4, 2024

Summary

Background: Carnitine palmitoyltransferase II deficiency is an inherited disorder of long-chain fatty acid oxidation characterized by hypoketotic hypoglycemia, cardiomyopathy, seizures, muscle pain and weakness, and myoglobin. Individuals with carnitine palmitoyltransferase II deficiency have a defect in the production of the enzyme carnitine palmitoyltransferase-II, which plays an important role in fatty acid oxidation. Signs and symptoms of carnitine palmitoyltransferase II deficiency are due to the buildup of fatty acids and long-chain acyl-carnitine as well as reduced energy production in cells. Carnitine palmitoyltransferase II deficiency is an autosomal recessive disease caused by mutations in the CPT2 gene. During changing Nonalcoholic fatty liver disease (NAFLD) to the cirrhosis, the probability of cancer is high that should be considered as a dangerous situation. **Methods**: SDS-PAGE system of polyacrylamide gel electrophoresis through analytical method for separating charged molecules in mitochondrial mixtures according to their molecular mass in the presence of electrical fields was used. The Invitrogen® Bright Imaging (IBI) system provides was applied for the imaging and analysis of protein imprints. **Results**: Since currently no effective treatment for CPT-II deficiency, prevention of liver failure is a proper way of treatment through controlling mitochondria without affecting CPT-II potency. We discussed about the severe infantile hepatocardiac

muscular position of CPT II deficiency affects the liver heart, and muscles. **Conclusions:** Through this work, we discussed and characterized the pathophysiological function in several tissues such as liver, Kidney cancers.

Keywords: Liver cancer; Kidny failure; Hepatocellular carcinoma; Nonalcoholic fatty liver disease; CPT-I; CPT-II; SDS-PAGE system.

Resumen

Aplicación químico-farmacéutica de la carnitina palmitoiltransferasa-2 (CPT-II) mediante la regulación mitocondrial frente a células tumorales cancerosas

Antecedentes: La deficiencia de carnitina palmitoiltransferasa II es un trastorno hereditario de la oxidación de ácidos grasos de cadena larga caracterizado por hipoglucemia hipocetósica, miocardiopatía, convulsiones, dolor y debilidad muscular y mioglobina. Las personas con deficiencia de carnitina palmitoiltransferasa II tienen un defecto en la producción de la enzima carnitina palmitoiltransferasa-II, que desempeña un papel importante en la oxidación de los ácidos grasos. Los signos y síntomas de la deficiencia de carnitina palmitoiltransferasa II se deben a la acumulación de ácidos grasos y acilcarnitina de cadena larga, así como a la reducción de la producción de energía en las células. La deficiencia de carnitina palmitoiltransferasa II es una enfermedad autosómica recesiva causada por mutaciones en el gen CPT2. Durante el cambio de la enfermedad del hígado graso no alcohólico (NAFLD) a la cirrosis, la probabilidad de cáncer es alta y debe considerarse una situación peligrosa. **Métodos:** Se utilizó el sistema SDS-PAGE de electroforesis en gel de poliacrilamida como método analítico para separar moléculas cargadas en mezclas mitocondriales según su masa molar en presencia de campos eléctricos. El sistema Invitrogen® Bright Imaging (IBI) se utilizó para la obtención de imágenes y el análisis de huellas de proteínas. **Resultados:** Dado que actualmente no existe un tratamiento eficaz para la deficiencia de CPT-II, la prevención de la insuficiencia hepática es una forma adecuada de tratamiento mediante el control de las mitocondrias sin afectar la potencia de CPT-II. Aquí se discute acerca de la posición muscular hepatocardíaca

infantil grave a causa de la deficiencia de CPT II que afecta el hígado, el corazón y los músculos. **Conclusiones:** En este trabajo, se discute y caracteriza la función fisiopatológica en varios tejidos como el cáncer de hígado y riñón.

Palabras clave: Cáncer de hígado; Insuficiencia renal; Carcinoma hepatocelular; Enfermedad del hígado graso no alcohólico; CPT-I; CPT-II; Sistema SDS-PAGE.

Resumo

Aplicação químico-farmacêutica da carnitina palmitoiltransferase-2 (CPT-II) através da regulação mitocondrial contra células tumorais cancerígenas

Antecedentes: A deficiência de carnitina palmitoiltransferase II é um distúrbio hereditário da oxidação de ácidos graxos de cadeia longa, caracterizado por hipoglicemia hipocetótica, cardiomiopatia, convulsões, dor e fraqueza muscular e mioglobina. Indivíduos com deficiência de carnitina palmitoiltransferase II apresentam um defeito na produção da enzima carnitina palmitoiltransferase-II, que desempenha um papel importante na oxidação de ácidos graxos. Os sinais e sintomas da deficiência de carnitina palmitoiltransferase II são devidos ao acúmulo de ácidos graxos e acil-carnitina de cadeia longa, bem como à redução da produção de energia nas células. A deficiência de carnitina palmitoiltransferase II é uma doença autossômica recessiva causada por mutações no gene CPT2. Durante a mudança da doença hepática gordurosa não alcoólica (DHGNA) para cirrose, a probabilidade de câncer é alta, o que deve ser considerado uma situação perigosa. **Métodos:** Foi utilizado o sistema SDS-PAGE de eletroforese em gel de poliacrilamida através de método analítico para separação de moléculas carregadas em misturas mitocondriais de acordo com sua massa molecular na presença de campos elétricos. O sistema Invitrogen® Bright Imaging (IBI) fornecido foi aplicado para a geração de imagens e análise de impressões de proteínas. **Resultados:** Como atualmente não há tratamento eficaz para a deficiência de CPT-II, a prevenção da insuficiência hepática é uma forma adequada de tratamento através do controle das mitocôndrias sem afetar a potência do CPT-II. Discutimos sobre a posição muscular hepatocardíaca infantil grave da deficiência de CPT II que afeta o fígado, o coração e os músculos. **Conclusões:** Através deste trabalho, discutimos e caracterizamos a função fisiopatológica em diversos tecidos, como câncer de fígado e rim.

Palavras-chave: Câncer de fígado; Falência renal; carcinoma hepatocelular; Doença hepática gordurosa não alcoólica; CPT-I; CPT-II; Sistema SDS-PAGE.

Introduction

Carnitine palmitoyltransferase specification (CPT)

Carnitine palmitoyltransferase II (CPT II) deficiency is a condition that prevents the body from using certain fats for energy, particularly during periods without food (fasting). There are three main types of CPT II deficiency: a lethal neonatal form, a severe infantile cardio muscular form, and a myopathy form. The lethal neonatal form of CPT II deficiency becomes apparent soon after birth. Infants with this form of the disorder develop respiratory failure, seizures, liver failure, a weakened heart muscle (cardiomyopathy), and an irregular heartbeat (arrhythmia). Affected individuals also have low blood glucose (hypoglycemia) and a low level of ketones, which are produced during the breakdown of fats and used for energy. Together these signs are called hypoketotic hypoglycemia. In many cases, the brain and kidneys are also structurally abnormal. Infants with the lethal neonatal form of CPT II deficiency usually live for a few days to a few months [1-6]. Since there is a mechanism of oxidation of phospholipids in tumors, these disorders may be caused by the regulation of fatty acids in the mitochondrial matrix [7-9]. The outer membrane transferase CPT-I is generally recognized as the main regulatory site of lipid peroxidation in mitochondria, and for this reason, its regulation has been extensively studied, as has the regulation of the outer membrane transferase CPT-II. Problems related to this form of CPT II deficiency can be triggered by periods of fasting or by illnesses such as viral infections. Individuals with the severe infantile hepato-cardio-muscular form of CPT-II deficiency are at risk for liver failure, nervous system damage, coma, and sudden death. During treatment of the latent status of cancer, several changes occur, such as a striking enhancement in PGE2 concentration [13-15]. It is clear that inhibition of PGE2 reduces tumor growth in both *in vivo* and *in vitro* studies [16-20]. We exhibited the translational activities of CPT-I or CPT-II can also influence the anti-keto genic creation of extra hepatic tumor growth in human liver [10-12]. It is a question whether inhibition of prostaglandin synthesis could impair the tumor growth effects of liver mitochondrial CPTs. CPT-II deficiency is one of the most uncommon inherited traits caused by the lipid chain and also the most common cause of recurrent rhabdomyolysis in adults. It is noteable CPT-I or CPT-II are proteins that help transport fatty chains from the cytoplasm to the mitochondria during beta oxidation of fatty acids for energy production. Fatty acid oxidation (FAO) (Figure 1) is suitable for producing energy during stress, strong sports, long time exercise, fasting, and also cold climate. Consequently through a huge stress, the concentration of CPT-II will be decreased due to transition of acyl-carnitine across the inner mitochondrial membrane as a result oxidation is reduced.

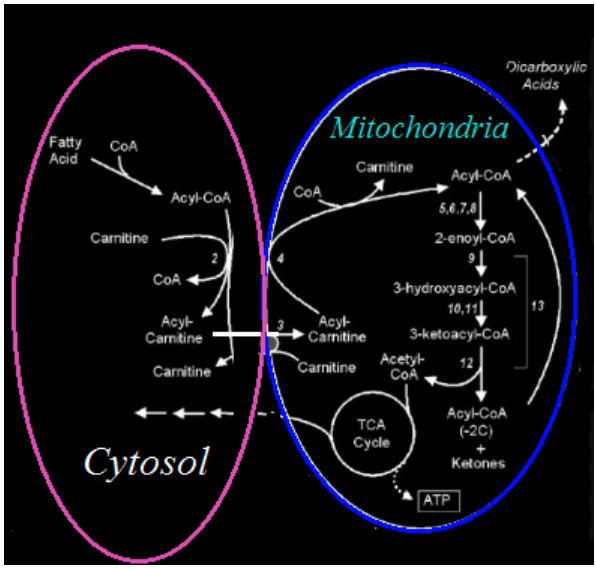

Figure 1. Esterification of fatty acids across mitochondrial.

The lethal neonatal form of CPT II deficiency becomes apparent soon after birth. Infants with this form of the disorder develop respiratory failure, seizures, liver failure, a weakened heart muscle (cardiomyopathy), and an irregular heartbeat (arrhythmia). Affected individuals also have low blood glucose (hypoglycemia) and a low level of ketones, which are produced during the breakdown of fats and used for energy. Together these signs are called hypoketotic hypoglycemia. In many cases, the brain and kidneys are also structurally abnormal. Infants with the lethal neonatal form of CPT II deficiency usually live for a few days to a few months. CPT-II is divided into three main phenotypes, two of which cause hypoglycemia due to hypoglycemia and are fatal in utero or in the first days of life. The third type, which attacks until puberty, is the typical muscle type, characterized by muscle pain, muscle weakness, and rhabdomyolysis due to vigorous exercise. Although for some sports its effect is hidden, in some other sports myalgia appears with frequent attacks of rhabdomyolysis. Mutations in the CPT2 gene cause CPT II deficiency. This gene provides instructions for making an enzyme called carnitine palmitoyltransferase 2. This enzyme is essential for fatty acid oxidation, which is the multistep process that breaks down (metabolizes) fats and converts them into energy. Fatty acid oxidation takes place within mitochondria, which are the energy-producing centers in cells. A group of fats called long-chain fatty acids must be attached to a substance known as carnitine to enter mitochondria. Once these fatty acids are inside mitochondria, carnitine palmitoyltransferase 2 removes the carni-

tine and prepares them for fatty acid oxidation. Fatty acids are a major source of energy for the heart and muscles. During periods of fasting, fatty acids are also an important energy source for the liver and other tissues

Decreasing HCC under NAFLD *in vivo*

HCC often arises in patients with liver cirrhosis caused by chronic hepatitis B or C virus infection. However, recent epidemiology studies found that non-alcoholic fatty liver disease (NAFLD) is also a high-risk factor for HCC . In humans, adoptive transfer of tumor-specific CD4+ T cells caused a complete tumor eradication in a patient bearing cholangiocarcinoma, another primary liver cancer. Furthermore, immunotherapy is becoming standard of care for the treatment of advanced HCC [21,22]. To address this question, a better understanding of the influences of fatty liver environment on T-cell metabolism is required. This may also shed light on the design of a targeted therapy and potentially a combined immunotherapy for HCC. [23–25]. Most humans have no explicit signs and cannot be recognized before liver cirrhosis or hepatocellular carcinoma (HCC) ,because the effect of early clinical screening is negligible [26]. During changing NAFLD to the cirrhosis, the probability of reverse situation has high risk that HCC should be considered as a dangerous position. As a result, many systemic diseases appear in humans by HCC, such as cardiovascular diseases, chronic alcoholic diseases, and colon and rectal tumors, all of which threaten human health [27-29]. As CPT1a up regulation leads to greater ROS and CD4+ T cell apoptosis, we sought to test whether blocking CPT-1 affects CD4+ T cells and HCC development in the context of NAFLD. As we found the CPT inhibitor perhexiline rescued murine CD4+ T cell and Jurkat cell apoptosis when cultured with C18:2 *in vitro*. Liver-specific inducible MYC oncogene transgenic mice, which spontaneously develops HCC after turning on MYC gene (MYC-ON), were fed with the MCD diet and injected with perhexiline. The liver is a central organ for lipid metabolism. With the prevalence of NAFLD in Western countries and the significant risk of NAFLD patients to develop HCC, critical components in lipid metabolism could be potential targets for the treatment of NAFLD-induced HCC. PPARs and CPT proteins are among those potential targets. However, the exact role of PPAR-α in HCC has been controversial on whether PPAR-α promotes or suppresses tumor growth [30]. NAFLD and another type known as nonalcoholic steatohepatitis (NASH) are associated with liver cancer, which causes fatty particles to build up in liver cells. The epidemic of NAFLD is rapidly increasing with the prevalence of diabetes and appears to be present in a quarter of the world's population [31,32]. In addition, this issue has been evaluated in 2011 and diagnosed that from each one per five liver cancers worldwide one of them is related to diabetes. NAFLD can be considered a dire and dangerous world subjects; nevertheless, there is no useful treatment and no mechanism of NAFLD decreasing for this disease up

to now. There are huge information indicating that metabolic conversions in the cancer tumors might be converted to anti-immune metabolism and consequently ruin antitumor immunity [33-35]. Although the anti-tumor effects of CD4 + T cells in various forms of cancers such as liver are the main target for diagnosis by researchers [36], there is no further research except using the diethylnitrosamine (DEN)-primed murine HCC model. Working on a mouse model, it was found that CD4+ T cells are able to prevent tumor initiation and remove malignant liver cells [37-40]. There are 3 particular subunits of CPT-I known as a, b and c [33,34]. CPT-Ia is the first subunit in lymphocytes, liver, kidney, spleen, lung, intestine, and pancreas. CPT-Ib is mostly translated in muscles, heart, and adipose tissue, while CPT-II is mainly translated in the brain [41-42]. Peroxisome proliferator-activated receptor (PPARs) is a category of phospholipids of RNA transcription agents that affects to metabolic systems. They can be separated into three subunits including: alpha, gamma and beta/delta .Although PPAR-α and PPAR-γ is translated in lymphocytes, finally PPAR-α is a major part in the liver for attaching to fatty acids. It has been exhibited CPT-I trough activating PPAR-α15 is considerably affected by fatty acids, , moreover several works confirm the role of both PPAR-α and PPAR-γ as activated receptors to adjustment the transcription of the CPT-Ia gene directly [43-45].

Although these changes can be tested from liver CD4+ T cells through nutrition rat with linoleic acid or NAFLD diets, more research exhibited that PPAR-α is the reason the turbulent of CPTs genes. Introduction of CPTs genes can increase ROS and lead to apoptosis of CD4 + T cells. *In vivo* treatment of mice with CPTs inhibitor in addition to reducing apoptosis of hepatic CD4 + T cells and controlling HCC growth in NAFLD system provides useful data, such as identify genes for CPTs such as liver cancer therapy promoted by NAFLD [45-50].

CPT-II genes

The carnitine palmitoyltransferase (CPT) system is responsible for transporting long-chain fatty acids from the cytoplasm into the mitochondria where the fatty acids undergo β-oxidation. Transport of long-chain fatty acids into the mitochondrial matrix requires both CPT1 and CPT2, with CPT1 being the rate limiting step. This CPT system contains two separate proteins localized in the outer (CPT1) and the inner (CPT2) mitochondrial membrane. The most suitable action of the CPTs might be mentioned as an ability for helping fatty acids to enter inside the mitochondria for any further oxidation. For this purpose, trans-membrane protein as known CPT-I is placed in OMM, as well as, CPT-II genome is located in IMM. CPT-II genome appears on chromosomes1 (1p32) and consist of 3092 nucleotides in five exons that

can encode the enzyme chain containing 660 amino acids (table 1)[50]. The expression of CPT1b and CPT2, suggesting that other lipids may also contribute to the induction of CPT genes in NAFLD.

Table 1. Structure of CP-II genome

Exson Size (bp)	Nucleotides	Amino acids	Amino acid substitution in mutations of five exons
106	756-857	80-112	Cys84Arg; Ala101Val; Ser113Leu
1303	858-2163	113-549	Met120Cys; Arg121Gln; Asn124Gln; Arg124Ter; Asn146Thr; Ala151Gln; Arg151Gly; Arg161Trp; Ile164Ter; Arg167Gln; Asp173Ser; Glu174Lys; Tyr210Ala; Asp213Trp; Met214Arg; Gln216Arg; Pro227Leu; Ala231Ser; Arg247Trp; Gln274Met; Arg296Ser; Arg296Leu; Ala296Ter; Gly310Gly;Cys326Tyr; Lys328Gly; Met342Asp; Phe352Met; Val368Ile; His369Arg; Arg382Lys; Phe383Tyr;Gln413Gln; Phe448Ala; Arg450Ter; Gln451Glu; Gln454Ter; Lys457Trp; Tyr479Phe; Tyr479Cys;Glu480Arg; Gln487Lys; Gly497Ser; Ile502Thr; Arg503Ser; Phe504Leu; Asn516Ser; Gln545Ala
934	2164-3092	550-660	Ala560Gln; Leu575Pro; Arg576Gly; Ser588Gln; Ser590Asn; Gly600Met; Pro604Ser; Val605Leu;Asp608Gly; Tyr628Ser; Arg631Cys; Lys644Ser
667	1-669	1-50	Pro41Leu; Pro50His
80	669-750	51-79	Pro55Arg; Ala67Gly

Intermediate role of PPAR-α for CPT gene

Previously, it was reported that PPAR-α can directly up regulate CPT1a expression [50, 51], so the hypothesized that the induction of CPT genes observed in CD4+ T cells treatment is mediated by PPAR-α. To test this, we used the PPAR-α agonist bezafibrate and monitored CPT gene expression .CPT-I and CPT-II contributed to the oxidation of long-chain fatty acids in mitochondria as well as their transport across the mitochondrial membrane for β-oxidation [51]. From a genetic concept point of view, CPT-II is identified by seventy percent of mutant alleles, which plays an important role in fatty acid entry into mitochondrial fatty acid oxidation as well as cellular metabolic homeostasis [52]. Oxaliplatin as an effective anti-cancer drug is used for increasing the CPT-II activation in cancer tumors and enhancing the catabolism of fatty acids. Structural amino acids evaluation from Exon 4 and Exon 5 and also its

activities indicating, which CPT-II might be became instable due to heterogeneous mutations , exogenous carcinogens, endogenous perturbation and huge mutation phenomenon [53]. Since the liver cells should control their metabolic systems in any usage of variety of feeds and ATP necessities [52-55], CPT-II must be well catalyzes Trans esterified acylcarnitine's transferred from cytosol into the inter-membrane space (IMS) [55-57]. CPTs act in the oxidation of LCFAs containing CPT-I and CPT-II in the OMM, catalyze fatty acids to form fatty acids with the participation of ATP and CoA, and then transport long-chain acyl-CoA into the system via the delivery system. Mitochondria many genes encoding CPT-II are known to be recessive genetic defects and clinical situations of associated diseases such as hypoglycemia, cardiomyopathy, arrhythmias, and rhabdomyolysis can be considered [58-68].

Materials and methods

Naphthylene was obtained from Aldrich Chemical Co; PPO, Palmitoyl CoA, protein G, carnitine, POPOP, sepharose-protein G, FMP, Indomethacin was prepared from Sigma, and dioxan from Brazil. [3H]-methylcarnitine, nitrocellulose (Highbong extra), Hypercassette and Hyperscreen were purchased from Amersham (UK). Mice were divided into four groups: 1-tumor-bearing group (TB), 2-control group (C), 3-control treated with indomethacin, and 4-tumor-bearing group treated with indomethacin. The livers of rat embryos (gestational days 10-18) were divided into pieces in Dulbecco's modified essential medium (DMEM). The pieces were placed in 0.25% collagenase solution for half hour at 37 °C. Again it centrifuged by speed of 1500 r/min for 8 minutes. Collagen enzyme potential was neutralized by adding 25% fetal serum to the mixture. They were suspended in DMEM and 12% fetal serum and cultured in a plastic thermos at -70°C. This method is based on the method of reference 63 [63] and after killing the mice, their livers were cleaned and homogenized with a buffer solution (250 mM mannitol, 70 mM sucrose, 3 mM HEPES, 0.3 mM EDTA, pH 7.2). In the same buffer, the homogenate is filtered and centrifuged twice at 1200 rpm for 10 minutes. Finally, the above part of the mixture was centrifuged three times at 8000 rpm for 15 minutes (Sorvall RC2B centrifuge). The maximum activity of CPT-I and CPT-II was measured in isolated mitochondria using detergents according to number 64 [64]. Finally, they were re-suspended in buffer containing 0.12 mM KC1, 5.0 mM Tris-HC1 (pH 7.3) and centrifuged (15,000 rpm, 10 min). They were then re-suspended in 15 mM phosphate buffer (pH 7.3), frozen in liquid nitrogen, and then thawed at room temperature. They were again ultra-centrifuged.

PCR for RNA application

RNA was extracted from cell pellets or frozen tissue with QIAshredder (Qiagen) and RNeasy MiniKit . The sequence of primers used for quantitative RT-PCR have been estimated from RNA isolation . All reactions of quantitative RT-PCR were run in triplicates using iQTM SYBR Green Super mix and performed on the ViiATM Real-Time PCR System.

Mitochondrial coloring

Mitochondrial-associated ROS was detected by mitoSOXstaining according to the manufacturer's protocol. Briefly, treated cells were stained with 3 μM mitoSOX for half hour in a CO2 incubator at 38 °C. After washing twice, the cells were processed.

Fluorescent coloring

Fibroblast cells were seeded several times by NuncTM Lab-TekTM Chambered Cover glass wells. After half day, cells were washed with phosphate-buffered saline (PBS) three times and incubated with 2 μM BODIPY probe with 10 nM MitoTracker Deep Red in PBS at 38 °C for half hour min. Cells cleaned by PBS and was kept for imaging. Live cells were imaged using a Zeiss LSM880 laser (Figure 2).

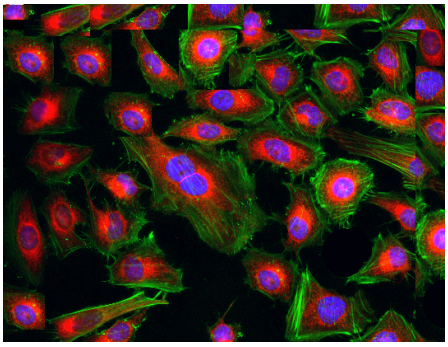

Figure 2. Mitochondrion fluorescent coloring.

Results

We found that intrahepatic CD4+ T cells are an indispensable component of antitumor surveillance in NAFLD, and linoleic acid (C18:2) causes CD4+ T cell apoptosis by impairing electron transport chain (ETC) function and generating ROS8. During this process, CPT1 catalyzes the transfer of the acyl group of a long chain fatty

acyl-CoA from coenzyme A to carnitine so the resulting acylcarnitine can cross the mitochondrial outer membrane, while CPT2 reverses this reaction inside mitochondria. Table 2 exhibits the optimal potentials and activities of CPT-I and CPT-II in mitochondria provided by the livers of TB and control mice. For CPT-I, no significant differences emerged between the two groups. However, the CPT-II potential in liver mitochondria of TB-infected rats was 55% lower than that of control rats.

Table 2. Enzymatic potential

	Liver	
Samples	**CPT-I**	**CPT-II**
C (n=15)	3.25	2.22
TB (n=6)	3.85	1.01
	Tumor	
TB (n=8)	2.12	3.43
TB-ind (n=15)	3.09	5.21

Figure 3 exhibits the schematic of proteins separated by SDS-PAGE of mitochondrial samples obtained from tuberculosis and control mice. The only distinct difference from the more abundant proteins was a small translation of the corresponding proteins into the mitochondria of TB mice.

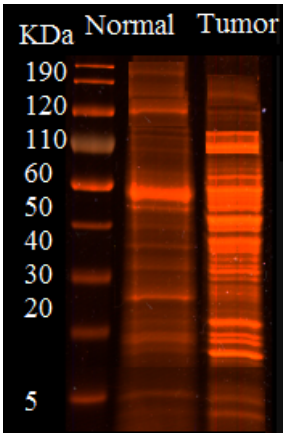

Figure 3. SDS/PAGE segregation of mitochondrial from the rats, including normal and tumors.

In Figure 4, Western blot positions for CPT-I and CPT-II immunoreactivity in mitochondria from control mice and *M. tuberculosis* are shown. Although the difference between these two patterns in CPT-I amounts in mitochondria isolated from control

as well as TB mice was not significant, TB mitochondria showed little CPT-II activity at 69,000. In addition, in these mitochondria, a second band was also detected by the rat liver CPT-II antibody exhibited.

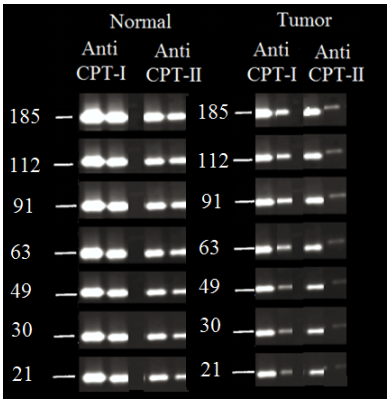

Figure 4. Western blot detection of immunoreactive CPT-I and CPT-II proteins in liver mitochondria isolated.

These effects were not found *in vitro* when embryonic hepatocytes were cultured in the presence of 12 g of indomethacin because there was no induction of CPT-I or CPT-II in indomethacin-treated cells compared with control cells (Table 3).

Table 3. Effects of indomethacin *in vivo* and *in vitro* on CPT I and CPT II from liver mitochondria.

Enzymatic potential (nmol/min.mg)		
Items	CPT-I	CPT-II
control (n=4)	2.99	3.12
control –ind (n=6)	5.85	3.11
TB (n=3)	3.02	1.12
TB-ind (N=12)	4.15	4.05
Cultured hepatocytes (n=5)	3.21	4.41
Cultured hepatocytes –ind (n=5)	3.22	4.21

Discussion

In vivo feeding and *in vitro* culture experiments showed that induced CPT gene up regulation. Using either an agonist or antagonist, it confirms that PPAR-α mediates the induction of CPT genes, as well as targeting of CPT inhibits HCC development in the context of NAFLD. Since fatty acid diffusion to cytoplasm does not stop in

the livers of TB rats, it can be interpreted that the metabolic destroying creates during the transport of fatty acids into the mitochondrial matrix. Contrary to any previous hypothesis and prediction, CPT-I is capable of controlling FAO in all metabolic states. To date, neither the activity of this enzyme nor the translation of the corresponding protein has been affected in the liver mitochondria of TB mice. However, CPT-II potency was halved, and Western blotting using an anti-CPT-II polyclonal antibody revealed the source of the additional reactive enzyme in liver mitochondria obtained from TB mice. These considerations add to our knowledge that the less likely isoform of CPT-II is translated either through transcription of different genes or through post-transcriptional mRNA labeling (*in vivo*) and proteolysis processing in the liver. It is also possible that the CPT-II isoform acts as a master receptor regulator. The results of this work exhibited that treatment with prostaglandin inhibitors was able to reverse the effects of cachexia on hepatic CPT-II activities. The similarity between the potential obtained in mitochondria isolated from control cultured hepatocytes and those cultured with indomethacin predicts that PGE2 modulation of the CPT-II reduction potential is not the result of eicosanoid production by the hepatocytes themselves, but is likely due to the action of prostaglandins. In other wise, the *in vivo* situation is related to the large amounts of PGE2 production by the tumor and of course our data of this work needs more investigation regarding the role of CPT-II and the control of its translation for FAo mechanism in the pathophysiological positions. It is notable, liver is a central organ for lipid metabolism, therefor With the prevalence of NAFLD in Western countries and the significant risk of NAFLD patients to develop HCC, critical components in lipid metabolism could be potential targets for the treatment of NAFLD-induced HCC. PPARs and CPT proteins are among those potential targets. However, the exact role of PPAR-α in HCC has been controversial on whether PPAR-α promotes or suppresses tumor growth.

Conclusions

In conclusion, we demonstrated that by inhibiting CPT1 we can rescue CD4+ T cells and prevent HCC development. Our results provide useful information that CPT1 may be a potential target for NAFLD -promoted HCC therapy. Through this work, we have focused on the partial application of lipid metabolic pathways in disease pathogenesis and considered the modulating cases and clinical treatments of lipid systems in different tissues. Furthermore, through this work, we have characterized the pathophysiological function of both CPT-I and CPT-II systems in liver disease compared to several other chronic infections as well as cancer (Table 4).

Table 4. The role of CPT in inflammatory disease.

Goals	Associated patients	CPTs	Effects	References
Liver	injurey	CPT-I; CPT-II	Decreased in intrahepatic cholestasis model	[68, 69]
Liver	NAFLD	CPT-I	Improved the symptoms of the disease	[70]
Liver	HCC	CPT-I; CPT-II	Upregulated CPT elevated apoptosis of CD4+ T cells and promoted HCC formation in NAFLD	[71-73]
Cardiovascular	Cardiac dysfunction	CPT-I; CPT-II	Downregulated CPT induced cardiac injury during endotoxemia	[74]
Cardiovascular	Cardiac dysfunction	CPT-I	Downregulated CPT1 induced the injury	[75]
Pulmonary	Asthma	CPT2	Increased in asthmatic bronchial SMC	[76]
Pulmonary	Asthma	CPT-IB	Decreased CPT1B increased mortality; increased expression and decreased activity in aged ALI mice	[77]
Kidney	Kidney fibrosis	CPT-IA	Decreased during the disease	[78]
Kidney	Kidney fibrosis	CPT-IA	Overexpression of CPT1A showed protective effects	[79]
Colon	Colorectal cancer	CPT-IA	Exposure to adipocytes or FA upregulated CPT1A	[80]
Colon	Colorectal cancer	CPT-IA	Low expression in primary tumor tissues while high expression in CAFs	[81]
Colon	CAC	CPT-I	Suppressed CPT1 inhibited NLRP3 assembly in macrophages	[82]
Breast	Breast cance	CPT-IA	Upregulated in patients; a new biomarker for the diagnosis	[83]
Breast	Breast cance	CPT-IA	Increased in doxorubicin-treated tumours *in vivo*	[84]
Blood	AML	CPT-IA	Overexpression predicted poor clinical outcome	[85]
Pancreas	Pancreatic cancer	CPT-IA	Downregulated CPT1C inducted tumor senescence	[86]

(*Continued*)

Table 4. *Continuation.*

Goals	Associated patients	CPTs	Effects	References
Skin	Melanoma	CPT-IA	Inhibited CPT1A led to apoptosis in MAPKi-treated cells	[87]
Muscle	Muscle dysfunction	CPT-II	A conceptual overview on CPT2 deficiency	[88]
Gastric	Gastric Cancer	CPT-IC	Associated with poor prognosis; promoted proliferation of cancer cells	[89, 90]

Abbreviations

C: control rats; CH: cultured hepatocytes; CH-ind: hepatocytes cultured with indomethacin; C-ind: control rats submitted to indomethacin treatment; CPT: carnitine palmitoyltransferase; ETC: electron transport chain; FAMR: fatty acid metabolic regulation; FAO: fatty acid oxidation; FMP: fatty milk powder; IMM: inner mitochondrial membrane; LCFAs: long-chain fatty acids; NEFA: non esterification fatty acids; OMM: outer mitochondrial membrane; PGE2: prostaglandin E2; POPOP:

1,4-bis (5-phenyl-2-oxazolyl)-benzene; PPO: 2,5-diphenyloxazol; ROS: reactive oxygen species; TB: tumor-bearing rats; TB-ind: turnout-bearing rats given indomethacin; TNF: tumor necrosis factor;

Conflict of interest

All authors report that they do not have any conflicts of interest.

References

1. K.C. Fearon, M.J. Tisdale, T. Preston, J.A. Plumb, K.C. Calman, Failure of systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats, *British Journal of Cancer*, **52(1)**, 87-92 (1985). Doi: https://doi.org/10.1038/bjc.1985.153

2. M. Monajjemi, F. Mollaamin, S. Shojaei, An overview on coronaviruses family from past to Covid-19: Introduce some inhibitors as antiviruses from Gillan's plants, *Biointerface Research in Applied Chemistry*, **10**(3), 5575-5585 (2020). Doi: https://doi.org/10.33263/briac103.575585

3. D.H. Lawson, A. Richmord, D.W. Nixon, D. Rudman, Metabolic approaches to cancer cachexia, *Annual Review of Nutrition*, **2**, 277-301 (1982). Doi: https://doi.org/10.1146/annurev.nu.02.070182.001425

4. J.F. Williams, R.A. Siddiqui, Biochemistry of cancer cachexia: Review of results, a new hypothesis and a proposal for treatment, *Medical Science Research*, **18**, 3-10 (1990).

5. S. Shahriari, M. Monajjemi, F. Mollaamin, Determination of proteins specification with SARS- COVID-19 based ligand designing, *Journal of the Chilean Chemical Society*, **67**(2), 5468-5476 (2022). Doi: https://doi.org/10.4067/S0717-97072022000205468

6. F. Mollaamin, S. Shahriari, M. Monajjemi, Treating omicron BA.4 & BA.5 via herbal antioxidant asafoetida: A DFT study of carbon nanocarrier in drug delivery, *Journal of the Chilean Chemical Society*, **68**(1), 5781-5786 (2023). URL: https://www.scielo.cl/pdf/jcchems/v68n1/0717-9707-jcchems-68-01-5781.pdf

7. H. Langstein, J.A. Norton, Mechanisms of cancer cachexia, *Hematology/ Oncology Clinics of North America*, **5**(1), 103-123 (1991).

8. H.D. Mulligan, M.J. Tisdale, Lipogenesis in tumour and host tissues in mice bearing colonic adenocarcinomas, *British Journal of Cancer*, **63**(5), 719-722 (1991). Doi: https://doi.org/10.1038/bjc.1991.162

9. R.A. Siddiqui, J.F. Williams, The regulation of fatty acid and branched-chain amino acid oxidation in cancer cachectic rats: a proposed role for a cytokine, eicosanoid, and hormone trilogy, *Biochemical Medicine and Metabolic Biology*, **42**(1), 71-86 (1989). Doi: https://doi.org/10.1016/0885-4505(89)90043-1

10. M.P. Thompson, J.E. Koons, E.T. Tan, M.R. Grigor, Modified lipoprotein lipase activities, rates of lipogenesis, and lipolysis as factors leading to lipid depletion in C57BL mice bearing the preputial gland tumor, ESR-586, *Cancer Research*, **41**(8), 3228-3232 (1981).

11. J.D. McGarry, D.W. Foster, Regulation of hepatic fatty acid oxidation and ketone body production, *Annual Review of Biochemistry*, **49**, 395-420 (1980). Doi: https://doi.org/10.1146/annurev.bi.49.070180.002143

12. F. Mollaamin, A. Ilkhani, N. Sakhaei, B. Bonsakhteh, A. Faridchehr, S. Tohidi, M. Monajjemi, Thermodynamic and solvent effect on dynamic structures of nano bilayer-cell membrane: Hydrogen bonding study, *Journal of Computational and Thoretical Nanoscience*, **12**(10), 3148-3154 (2015). Doi: https://doi.org/10.1166/jctn.2015.4092

13. A. Guaitani, M. Recchia, M. Carli, M. Rocchetti, I. Bartosek, S. Garatinni, Walker carcinoma 256: A model for studies on tumor-induced anorexia and cachexia, *Oncology*, **39**(3), 173-178 (1982). Doi: https://doi.org/10.1159/000225631

14. L.C. Femandes, U.F. Machado, C.R. Nogueira, A.R. Carpinelli, R. Curi, Insulin secretion in Walker 256 tumor cachexia, *American Journal of Physiology*, **258**(6 Pt 1), E1033-E1036 (1991). Doi: https://doi.org/10.1152/ajpendo.1990.258.6.E1033

15. S. Shahriari, M. Monajjemi, K. Zare, Penetrating to cell membrane bacteria by the effiency of various antibiotics (clindamycin, metronidazole, azithromycin, sulfamethoxazole, baxdela, ticarcillin, and clavulanic acid) using S-NICS theory, *Biointerface Research in Applied Chemistry*, **8**(3), 3219-3223 (2018). URL: https://biointerfaceresearch.com/?page_id=2421

16. J.M. Argilés, J. Azcón-Bieto, The metabolic environment of cancer, *Molecular and Cellular Biochemistry*, **81**, 3-17 (1988). Doi: https://doi.org/10.1007/BF00225648

17. R.A. Karamali, J. Marsh, C. Fuchs, Effect of omega-3 fatty acids on growth of a rat mammary tumor, *Journal of the National Cancer Institute*, **73**(2), 457-461 (1984). Doi: https://doi.org/10.1093/jnci/73.2.457

18. M.G. Vecchia, S. Arizawa, R. Curi, E.A. Newsholme, Propionate inhibits cell proliferation in culture, *Cancer Research, Therapy and Control*, **3**, 15-21 (1992).

19. J. Gelin, C. Andersson, K. Lundholm, Effects of indomethacin, cytokines, and cyclosporin A on tumor growth and the subsequent development of cancer cachexia, *Cancer Research*, **51**(3), 880-885 (1991). URL: https://aacrjournals.org/cancerres/article/51/3/880/497387/Effects-of-Indomethacin-Cytokines-and-Cyclosporin

20. L. Tessitore, P. Costelli, F.M. Baccino, Humoral mediation for cachexia in tumour-bearing rats, *British Journal of Cancer*, **67**(1), 15-23 (1993). Doi: https://doi.org/10.1038/bjc.1993.4

21. F. Mollaamin, M. Monajjemi, Thermodynamic research on the inhibitors of coronavirus through drug delivery method, *Journal of the Chilean Chemical Society*, **66**(2), 5195-5205 (2021). Doi: http://doi.org/10.4067/S0717-97072021000205195

22. N. Stefan, K.A. Cusi, A global view of the interplay between non-alcoholic fatty liver disease and diabetes, *The Lancet: Diabetes & Endocrinology*, **10**(4), 284-296 (2022). Doi: https://doi.org/10.1016/S2213-8587(22)00003-1

23. F. Mollaamin, Physicochemical investigation of anti-COVID19 drugs using several medicinal plants, *Journal of the Chilean Chemical Society*, **67**(2), 5537-5546 (2022). Doi: https://doi.org/10.4067/S0717-97072022000205537

24. N.d.l.A. Segura-Azuara, C.D. Varela-Chinchilla, P.A. Trinidad-Calderón, MAFLD/NAFLD biopsy-free scoring systems for hepatic steatosis, NASH, and fibrosis diagnosis, *Frontiers in Medicine* (Lausanne), **8**, 774079 (2021). Doi: https://doi.org/10.3389/fmed.2021.774079

25. T.V. Rohm, D.T. Meier, J.M. Olefsky, M.Y. Donath, Inflammation in obesity, diabetes, and related disorders, *Immunity*, **55**(1), 31-55 (2022). Doi: https://doi.org/10.1016/j.immuni.2021.12.013

26. N. Tamaki, V. Ajmera, R. Loomba, Non-invasive methods for imaging hepatic steatosis and their clinical importance in NAFLD, *Nature Reviews Endocrinology*, **18**, 55-66 (2022). Doi: https://doi.org/10.1038/s41574-021-00584-0

27. E. Scorletti, R.M. Carr, A new perspective on NAFLD: Focusing on lipid droplets, *Journal of Hepatology*, **76**(4), 934-945 (2022). Doi: https://doi.org/10.1016/j.jhep.2021.11.009

28. F. Foerster, S.J. Gairing, L. Müller, P.R. Galle, NAFLD-driven HCC: Safety and efficacy of current and emerging treatment options, *Journal of Hepatology*, **76**(2), 446-457 (2022). Doi: https://doi.org/10.1016/j.jhep.2021.09.007

29. J.-P. Bonnefont, F. Djouadi, C. Prip-Buus, S. Gobin, A. Munnich, J. Bastin, Carnitine palmitoyltransferases 1 and 2: Biochemical, molecular and medical aspects, *Molecular Aspects of Medicine*, **25**(5-6), 495-520 (2004). Doi: https://doi.org/10.1016/j.mam.2004.06.004

30. J.M. Llovet, R.K. Kelley, A. Villanueva, A.G. Singal, E. Pikarsky, S. Roayaie, R. Lencioni, K. Koike, J. Zucman-Rossi, R.S. Finn, Hepatocellular carcinoma, *Nature Reviews Disease Primers*, **7**, 6 (2021). Doi: https://doi.org/10.1038/s41572-020-00240-3

31. M.A.A. Zadeh, H. Lari, L. Kharghanian, E. Balali, R. Khadivi, H. Yahyaei, F. Mollaamin, M. Monajjemi, Density functional theory study and anti-cancer properties of shyshaq plant: In view point of nano biotechnology, *Journal of Computational and Thoretical Nanoscience*, **12**(11), 4358-4367 (2015). Doi: https://doi.org/10.1166/jctn.2015.4366

32. Z.M. Younossi, A.B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, M. Wymer, Global epidemiology of nonalcoholic fatty liver diseaseMeta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology*, **64**(1), 73-84 (2016). Doi: https://doi.org/10.1002/hep.28431

33. Z. Younossi, Q.M. Anstee, M. Marietti, T. Hardy, L. Henry, M. Eslam, J. George, E. Bugianesi, Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention, *Nature Reviews Gastroenterology & Hepatology*, **15**(1), 11-20 (2017). Doi: https://doi.org/10.1038/nrgastro.2017.109

34. M. Monajjemi, M. Noei, F. Mollaamin, Design of fMet-tRNA and calculation of its bonding properties by quantum mechanics, *Nucleosides, Nucleotides & Nucleic Acids*, **29**(10), 676-683 (2010). Doi: https://doi.org/10.1080/15257771003781642

35. A. Sugiura, J.C. Rathmell, Metabolic barriers to T cell function in tumors, *The Journal of Immunology*, **200**(2), 400-407 (2018). Doi: https://doi.org/10.4049/jimmunol.1701041

36. C. Ma, A.H. Kesarwala, T. Eggert, J. Medina-Echeverz, D.E. Kleiner, P. Jin, D.F. Stroncek, M. Terabe, V. Kapoor, M. ElGindi, *et al.*, NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis, *Nature*, **531**(7593), 253-257 (2016). Doi: https://doi.org/10.1038/nature16969

37. T.-W. Kang, T. Yevsa, N. Woller, L. Hoenicke, T. Wuestefeld, D. Dauch, A. Hohmeyer, M. Gereke, R. Rudalska, A. Potapova, *et al.*, Senescence surveillance of pre-malignant hepatocytes limits liver cancer development, *Nature*, **479**(7374), 547-551 (2011). Doi: https://doi.org/10.1038/nature10599

38. C. Schneider, A. Teufel, T. Yevsa, F. Staib, A. Hohmeyer, G. Walenda, H.W. Zimmermann, M. Vucur, S. Huss, N. Gassler, *et al.*, Adaptive immunity suppresses formation and progression of diethylnitrosamine-induced liver cancer, *Gut*, **61**(12), 1733-1743 (2012). Doi: https://doi.org/10.1136/gutjnl-2011-301116

39. E. Tran, S. Turcotte, A. Gros, P.F. Robbins, Y.-C. Lu, M.E. Dudley, J.R. Wunderlich, R.P. Somerville, K. Hogan, C.S. Hinrichs, *et al.*, Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer, *Science*, **344**(6184), 641-645 (2014). Doi: https://doi.org/10.1126/science.1251102

40. B. Khalili-Hadad, F. Mollaamin, M. Monajjemi, Biophysical chemistry of macrocycles for drug delivery: A theoretical study, *Russian Chemical Bulletin*, **60**, 238-241 (2011). Doi: https://doi.org/10.1007/s11172-011-0039-5

41. J.-P. Bonnefont, F. Djouadi, C. Prip-Buus, S. Gobin, A. Munnich, J. Bastin, Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects, *Molecular Aspects of Medicine*, **25**(5-6), 495-520 (2004). Doi: https://doi.org/10.1016/j.mam.2004.06.004

42. H.E. Xu, M.H. Lambert, V.G. Montana, D.J. Parks, S.G. Blanchard, P.J. Brown, D.D. Sternbach, J.M. Lehmann, G.B. Wisely, T.M. Willson, *et al.*, Molecular

recognition of fatty acids by peroxisome proliferatoractivated receptors, *Molecular Cell*, **3**(3), 397-403 (1999). Doi: https://doi.org/10.1016/S1097-2765(00)80467-0

43. J.K. Reddy, T. Hashimoto, Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system, *Annual Review of Nutrition*, **21**, 193-230 (2001). Doi: https://doi.org/10.1146/annurev.nutr.21.1.193

44. S. Song, R.R. Attia, S. Connaughton, M.I. Niesen, G.C. Ness, M.B. Elam, R.T. Hori, G.A. Cook, E.A. Park, Peroxisome proliferator activated receptor alpha (PPARalpha) and PPAR gamma coactivator (PGC-1alpha) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements, *Molecular and Cellular Endocrinology*, **325**(1-2), 54-63 (2010). Doi: https://doi.org/10.1016/j.mce.2010.05.019

45. P. Sadana, Y. Zhang, S. Song, G.A. Cook, M.B. Elam, E.A. Park, Regulation of carnitine palmitoyltransferase I (CPT-Ialpha) gene expression by the peroxisome proliferator activated receptor gamma coactivator (PGC-1) isoforms, *Molecular and Cellular Endocrinology*, **267**(1-2), 6-16 (2007). Doi: https://doi.org/10.1016/j.mce.2006.11.012

46. T. Kurokawa, Y. Shimomura, G. Bajotto, K. Kotake, T. Arikawa, N. Ito, A. Yasuda, H. Nagata, T. Nonami, K. Masuko, Peroxisome proliferator-activated receptor alpha (PPARalpha) mRNA expression in human hepatocellular carcinoma tissue and noncancerous liver tissue, *World Journal of Surgical Oncology*, **9**, 167 (2011). Doi: https://doi.org/10.1186/1477-7819-9-167

47. H.L. Petrick, G.P. Holloway, Cytosolic reverse CrAT activity in cardiac tissue: potential importance for fuel selection, *Biochemical Journal*, **475**(7), 1267-1269 (2018). Doi: https://doi.org/10.1042/BCJ20180121

48. Y. Wang, J.-H. Lu, F. Wang, Y.-N. Wang, M.-M. He, Q.-N. Wu, Y.-X. Lu, H.-E. Yu, Z.-H. Chen, Q. Zhao, *et al.*, Inhibition of fatty acid catabolism augments the efficacy of oxaliplatin-based chemotherapy in gastrointestinal cancers, *Cancer Letters*, **473**, 74-89 (2020). Doi: https://doi.org/10.1016/j.canlet.2019.12.036

49. J.-J. Gu, M. Yao, J. Yang, Y. Cai, W.-J. Zheng, L. Wang, D.-B. Yao, D.-F. Yao, Mitochondrial carnitine palmitoyl transferase-II inactivity aggravates lipid accumulation in rat hepatocarcinogenesis, *World Journal of Gastroenterology*, **23**(2), 256-264 (2017). Doi: https://doi.org/10.3748/wjg.v23.i2.256

50. A.C. Rufer, R. Thoma, M. Hennig, Structural insight into function and regulation of carnitine palmitoyltransferase, *Cellular and Molecular Life Science*, **66**, 2489-2501 (2009). Doi: https://doi.org/10.1007/s00018-009-0035-1

51. S. Song, R.R. Attia, S. Connaughton, M.I. Niesen, G.C. Ness, M.B. Elam, R.T. Hori, G.A. Cook, E.A. Park, Peroxisome proliferator activated receptor alpha (PPARalpha) and PPAR gamma coactivator (PGC-1alpha) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements, *Molecular and Cellular Endocrinology*, **325**(1-2), 54-63 (2010). Doi: https://doi.org/10.1016/j.mce.2010.05.019

52. A.C. Rufer, R. Thoma, J. Benz, M. Stihle, B. Gsell, E. De Roo, D.W. Banner, F. Mueller, O. Chomienne, M. Hennig, The crystal structure of carnitine palmitoyltransferase 2 and implications for diabetes treatment, *Structure*, **14**(4), 713-723 (2006). Doi: https://doi.org/10.1016/j.str.2006.01.008

53. S. Han, R. Wei, X. Zhang, N. Jiang, M. Fan, J.H. Huang, B. Xie, L. Zhang, W. Miao, A.C. Butler, *et al.*, CPT1A/2-mediated FAO enhancement-A metabolic target in radioresistant breast cancer, *Frontiers in Oncology*, **9**, 1201 (2019). Doi: https://doi.org/10.3389/fonc.2019.01201

54. M. de Carvalho-Ribeiro, G. Szabo, Role of the inflammasome in liver disease, *Annual Review of Pathology: Mechanisms of Disease*, **17**, 345-365 (2022). Doi: https://doi.org/10.1146/annurev-pathmechdis-032521-102529

55. A.C. Rufer, A. Lomize, J. Benz, O. Chomienne, R. Thoma, M. Hennig, Carnitine palmitoyltransferase 2: analysis of membrane association and complex structure with a substrate analog, *FEBS Letters*, **581**(17), 3247-3252 (2007). Doi: https://doi.org/10.1016/j.febslet.2007.05.080

56. A. Song, Y. Park, B. Kim, S.G. Lee, Modulation of lipid metabolism by trans-anethole in hepatocytes, *Molecules*, **25**(21), 4946 (2020). Doi: https://doi.org/10.3390/molecules25214946

57. F. Mollaamin, M. Monajjemi, S. Salemi, M.T. Baei, A dielectric effect on normal mode analysis and symmetry of BNNT nanotube, *Fullerenes, Nanotubes and Carbon Nanostructures*, **19**(3), 182-196 (2011). Doi: https://doi.org/10.1080/15363831003782932

58. J. Wang, H. Xiang, Y. Lu, T. Wu, G. Ji, The role and therapeutic implication of CPTs in fatty acid oxidation and cancers progression, *American Journal of Cancer Research*, **11**(6), 2477-2494 (2021). URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8263643/pdf/ajcr0011-2477.pdf

59. M. Yao, M. Cai, D. Yao, X. Xu, R. Yang, Y. Li, Y. Zhang, H. Kido, D. Yao, Abbreviated half-lives and impaired fuel utilization in carnitine palmitoyltransferase II variant fibroblasts, *PLoS One*, **10**(3), e0119936 (2015). Doi: https://doi.org/10.1371/journal.pone.0119936

60. M. Yao, D. Yao, M. Yamaguchi, J. Chida, D. Yao, H. Kido, Bezafibrate upregulates carnitine palmitoyltransferase II expression and promotes mitochondrial energy crisis dissipation in fibroblasts of patients with influenza-associated encephalopathy, *Molecular Genetics and Metabolism*, **104**(3), 265-272 (2011). Doi: https://doi.org/10.1016/j.ymgme.2011.07.009

61. F. Mollaamin, S. Shahriari, M. Monajjemi, Monkeypox disease treatment by tecovirimat adsorbed onto single-walled carbon nanotube through drug delivery method, *Journal of the Chilean Chemical Society*, **68**(1), 5796-5801 (2023). Doi: http://doi.org/10.4067/S0717-97072023000105796

62. F. Mollaamin, S. Shahriari, M. Monajjemi, Drug design of medicinal plants as a treatment of omicron variant (COVID-19 variant B.1.1.529), *Journal of the Chilean Chemical Society*, **67**(3), 5562-5470 (2022). Doi: https://doi.org/10.4067/S0717-97072022000305562

63. A. Tahan, F. Mollaamin, M. Monajjemi, Thermochemistry and NBO analysis of peptide bond: Investigation of basis sets and binding energy, *Russian Journal of Physical Chemistry A*, **83**(4), 587-597 (2009). DOI: https://doi.org/10.1134/S003602440904013X

64. M. Monajjemi, M. Khaleghian, N. Tadayonpour, F. Mollaamin, The effect of different solvents and temperatures on stability of single-walled carbon nanotube: A QM/MD study, *International Journal of Nanoscience*, **9**(5), 517-529 (2010). Doi: https://doi.org/10.1142/S0219581X10007071

65. M. Khaleghian, M. Zahmatkesh, F. Mollaamin, M. Monajjemi, Investigation of solvent effects on armchair single-walled carbon nanotubes: A QM/MD study, *Fullerenes, Nanotubes and Carbon Nanostructures*, **19**(4), 251-261 (2011). Doi: https://doi.org/10.1080/15363831003721757

66. K. Bakhshi, F. Mollaamin, M. Monajjemi, Exchange and correlation effect of hydrogen chemisorption on nano V(100) surface: A DFT study by generalized gradient approximation (GGA), *Journal of Computational and Theoretical Nanoscience*, **8**(4), 763-768 (2011). Doi: https://doi.org/10.1166/jctn.2011.1750

67. E.M. Sarasia, S. Afsharnezhad, B. Honarparvar, F. Mollaamin, M. Monajjemi, Theoretical study of solvent effect on NMR shielding tensors of luciferin derivatives, *Physics and Chemistry of Liquids*, **49**(5), 561-571 (2011). Doi: https://doi.org/10.1080/00319101003698992

68. M. Monajjemi, M.T. Baie, F. Mollaamin, Interaction between threonine and cadmium cation in [Cd(Thr)] (n = 1-3) complexes: Density functional calculations, *Russian Chemical Bulletin*, **59**, 886-889 (2010). Doi: https://doi.org/10.1007/s11172-010-0181-5

69. Q. Zhao, R. Yang, J. Wang, D.D. Hu, F. Li, PPARα activation protects against cholestatic liver injury, *Scientific Reports*, **7**(1), 9967 (2017). Doi: https://doi.org/10.1038/s41598-017-10524-6

70. C.-J. Liou, C.-H. Wei, Y.-L. Chen, C.-Y. Cheng, C.-L. Wang, W.-C. Huang, Fisetin protects against hepatic steatosis through regulation of the Sirt1/AMPK and fatty acid β-oxidation signaling pathway in high-fat diet induced obese mice, *Cellular Physiology and Biochemistry*, **49**(5), 1870-1884 (2018). Doi: https://doi.org/10.1159/000493650

71. N.F. Brown, J.K. Hill, V. Esser, J.L. Kirkland, B.E. Corkey, D.W. Foster, J.D. McGarry, Mouse white adipocytes and 3T3-L1 cells display an anomalous pattern of carnitine palmitoyltransferase (CPT) I isoform expression during differentiation. Inter-tissue and inter-species expression of CPT I and CPT II enzymes, *Biochemical Journal*, **327**(1), 225-231 (1997). Doi: https://doi.org/10.1042/bj3270225

72. N.F. Brown, B. Weis, J.E. Husti, D.W. Foster, J.D. McGarry, Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart, *Journal of Biological Chemistry*, **270**(15), 8952-8957 (1995), Doi: https://doi.org/10.1074/jbc.270.15.8952

73. Z.J. Brown, Q. Fu, C. Ma, M. Kruhlak, H. Zhang, J. Luo, B. Heinrich, S.J. Yu, Q. Zhang, A. Wilson, *et al.*, Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4+ T cell apoptosis promoting HCC development, *Cell Death & Disease*, **9**, 620 (2018). Doi: https://doi.org/10.1038/s41419-018-0687-6

74. M. Makrecka-Kuka, S. Korzh, M. Videja, R. Vilskersts, E. Sevostjanovs, O. Zharkova-Malkova, P. Arsenyan, J. Kuka, M. Dambrova, E. Liepinsh, Inhibition of CPT2 exacerbates cardiac dysfunction and inflammation in experimental endotoxaemia, *Journal of Cellular and Molecular Medicine*, **24**(20), 11903-11911 (2020). Doi: https://doi.org/10.1111/jcmm.15809

75. T.-I. Lee, Y.-H. Kao, L. Baigalmaa, T.-W. Lee, Y.-Y. Lu, Y.-C. Chen, T.-F. Chao, Y.-J. Chen, Sodium hydrosulphide restores tumour necrosis factor-α-induced mitochondrial dysfunction and metabolic dysregulation in HL-1 cells, *Journal of Cellular and Molecular Medicine*, **23**(11), 7641-7650 (2019). Doi: https://doi.org/10.1111/jcmm.14637

76. P. Esteves, L. Blanc, A. Celle, I. Dupin, E. Maurat, N. Amoedo, G. Cardouat, O. Ousova, L. Gales, F. Bellvert, *et al.*, Crucial role of fatty acid oxidation in asthmatic bronchial smooth muscle remodeling, *European Respiratory Journal*, **58**, 2004252 (2021). Doi: https://doi.org/10.1183/13993003.04252-2020

77. K.W. Gibbs, C.-C.C. Key, L. Belfield, J. Krall, L. Purcell, C. Liu, D.C. Files, Aging influences the metabolic and inflammatory phenotype in an experimental mouse model of acute lung injury, *The Journals of Gerontology: Series A*, **76**(5), 770-777 (2021). Doi: https://doi.org/10.1093/gerona/glaa248

78. Y.H. Xie, Y. Xiao, Q. Huang, X.F. Hu, Z.C. Gong, J. Du, Role of the CTRP6/AMPK pathway in kidney fibrosis through the promotion of fatty acid oxidation, *European Journal of Pharmacology*, **892**, 173755 (2021). Doi: https://doi.org/10.1016/j.ejphar.2020.173755

79. V. Miguel, J. Tituaña, J.I. Herrero, L. Herrero, D. Serra, P. Cuevas, C. Barbas, D. Rodríguez-Puyol, L. Márquez-Expósito, M. Ruiz-Ortega, *et al.*, Renal tubule Cpt1a overexpression protects from kidney fibrosis by restoring mitochondrial homeostasis, *The Journal of Clinical Investigation*, **131**, e140695 (2021). Doi: https://doi.org/10.1172/JCI140695

80. X. Xiong, Y.-A. Wen, R. Fairchild, Y.Y. Zaytseva, H.L. Weiss, B.M. Evers, T. Gao, Upregulation of CPT1A is essential for the tumor-promoting effect of adipocytes in colon cancer, *Cell Death & Disease*, **11**, 736 (2020). Doi: https://doi.org/10.1038/s41419-020-02936-6

81. S. Peng, D. Chen, J. Cai, Z. Yuan, B. Huang, Y. Li, H. Wang, Q. Luo, Y. Kuang, W. Liang, *et al.*, Enhancing cancer-associated fibroblast fatty acid catabolism within a metabolically challenging tumor microenvironment drives colon cancer peritoneal metastasis, *Molecular Oncology*, **15**(5), 1391-1411 (2021). Doi: https://doi.org/10.1002/1878-0261.12917

82. S. Qiao, C. Lv, Y. Tao, Y. Miao, Y. Zhu, W. Zhang, D. Sun, X. Yun, Y. Xia, Z. Wei, *et al.*, Arctigenin disrupts NLRP3 inflammasome assembly in colonic macrophages via downregulating fatty acid oxidation to prevent colitis-associated cancer, *Cancer Letters*, **491**, 162-179 (2020). Doi: https://doi.org/10.1016/j.canlet.2020.08.033

83. Z. Tan, Y. Zou, M. Zhu, Z. Luo, T. Wu, C. Zheng, A. Xie, H. Wang, S. Fang, S. Liu, Y. Li, Z. Lu, Carnitine palmitoyl transferase 1A is a novel diagnostic and predictive biomarker for breast cancer, *BMC Cancer*, **21**, 409 (2021). Doi: https://doi.org/10.1186/s12885-021-08134-7

84. G. Petóvári, T. Dankó, A.-M. Tókés, E. Vetlényi, I. Krencz, R. Raffay, M. Hajdu, D. Sztankovics, K. Németh, K. Vellai-Takács, *et al.*, *In situ* metabolic characterisation of breast cancer and its potential impact on therapy, *Cancers*, **12**(9), 2492 (2020). Doi: https://doi.org/10.3390/cancers12092492

85. S. Mao, Q. Ling, J. Pan, F. Li, S. Huang, W. Ye, W. Wei, X. Lin, Y. Qian, Y. Wang, *et al.*, Inhibition of CPT1a as a prognostic marker can synergistically enhance the antileukemic activity of ABT199, *Journal of Translational Medicine*, **19**, 181 (2021). Doi: https://doi.org/10.1186/s12967-021-02848-9

86. L. Guan, Y. Chen, Y. Wang, H. Zhang, S. Fan, Y. Gao, T. Jiao, K. Fu, J. Sun, A. Yu, *et al.*, Effects of carnitine palmitoyltransferases on cancer cellular senescence, *Journal of Cellular Physiology*, **234**(2), 1707-1719 (2019). Doi: https://doi.org/10.1002/jcp.27042

87. A. Aloia, D. Müllhaupt, C.D. Chabbert, T. Eberhart, S. Flückiger-Mangual, A. Vukolic, O. Eichhoff, A. Irmisch, L.T. Alexander, E. Scibona, *et al.*, A fatty acid oxidation-dependent metabolic shift regulates the adaptation of *BRAF*-mutated melanoma to MAPK inhibitors, *Clinical Cancer Research*, **25**(22), 6852-6867 (2019). Doi: https://doi.org/10.1158/1078-0432.CCR-19-0253

88. P.R. Joshi, S. Zierz, Muscle carnitine palmitoyltransferase II (CPT II) deficiency: A conceptual approach, *Molecules*, **25**(8), 1784 (2020). Doi: https://doi.org/10.3390/molecules25081784

89. H. Chen, Z. Li, L. Dong, Y. Wu, H. Shen, Z. Chen, Lipid metabolism in chronic obstructive pulmonary disease, *International Journal of Chronic Obstructive Pulmonary Disease*, **14**, 1009-1018 (2019). Doi: https://doi.org/10.2147/copd.s196210

90. T. Chen, G. Wu, H. Hu, C. Wu, Enhanced fatty acid oxidation mediated by CPT1C promotes gastric cancer progression, *Journal of Gastrointestinal Oncology*, **11**(4), 695-707 (2020). Doi: https://doi.org/10.21037/jgo-20-157

How to cite this article

M. Monajjemi, F. Mollaamin, S. Mohammadi, S. Shahriari, Chemical-pharmaceutical application of carnitine palmitoyltransferase-2 (CPT-II) through regulating mitochondrial against cancer tumoric cells, *Rev. Colomb. Cienc. Quim. Farm.*, **53**(3), 777-803 (2024). Doi: https://doi.org/10.15446/rcciquifa.v53n3.119213