REVIEW ARTICLE

Choline deprivation: An overview of the major hepatic metabolic response pathways

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Abstract
Choline (Ch) is an important nutrient that is involved in many physiological functions. Deprivation of Ch (CD) may lead to hepatocellular modifications and/or even hepatic tumorigenesis and it can be a frequent problem in clinical settings; it can accompany various common pathological (alcoholism and malnutrition) or physiological states (pregnancy and lactation). The aim of this review is to provide an up-to-date overview of the major metabolic pathways involved in the hepatic response toward the experimentally or clinically induced CD, and to shed more light on the implicated (and probably interrelated) mechanisms responsible for the observed hepatocellular modifications and/or carcinogenesis.

Key Words: carcinogenesis, choline, choline deficiency, fatty liver, fibrosis, liver, metabolism, methionine, review, steatosis

Introduction
Choline (Ch) is a quaternary compound (trimethyl-β-hydroxy-ethylammonium) that was discovered and synthesized in 1860 [1]. It is now considered as an essential nutrient for both humans [2] and animals [3,4], and is a well-studied precursor for the synthesis of phospholipids’ phosphatidylcholine (PC), of the neurotransmitter acetylcholine (ACh), as well as of other Ch metabolites such as the platelet-activating factor and sphingomyelin [4,5]. Besides of being involved in many physiological functions (such as the metabolism of methyl groups and lipid transport) [6–8], Ch also plays a critical role in generating second messengers for cell membrane signal transduction [6,9]. It is known that Ch as well as other substances such as methionine (Met), folic acid, and vitamin B12 (Vit. B12) prevent the hepatic deposition of fat and, thus, are known as “lipotropes”; a disturbance in folate- or Met-related metabolism results in changes in the metabolism of Ch and vice versa [10]. Moreover, Ch seems to have a significant impact on the hepatic function and on the systemic lipid metabolism in both humans and animals [11,12]. In fact, deprivation of Ch (CD) can be a frequent problem in clinical settings, since it can accompany various common pathological (alcoholism and malnutrition) or physiological states (pregnancy and lactation) and can lead to significant hepatocellular modifications [13] and/or even hepatic tumorigenesis [14].

The aim of this review is to provide an up-to-date overview of the major metabolic pathways involved in the hepatic response toward the experimentally or clinically induced CD, and to shed more light on the implicated (and probably interrelated) mechanisms responsible for the observed hepatocellular modifications and/or carcinogenesis. A better understanding of the metabolic, inflammatory, hormonal, and oxidative pathways involved in liver response following CD, as well as the consideration of the potential role of other contributing factors (such as age, sex, length of exposure, and others) leading to destructive, fibrotic, regenerative, and morphologically altered liver, might both be precious tools for any
researcher/clinician toward the adoption of more targeted approaches in developing therapeutics aiming to overcome any irreversibility of CD-induced cirrhosis as well as in re-evaluating preventive therapeutic measurements.

Absorption and transportation of Ch

Digested Ch is absorbed from the small intestine through transporter proteins located in the intestinal cells, toward a 10−50 μM physiological plasma level [15]. The life time of Ch in the blood and its distribution within the body, as well as its transport into the cells are critical steps in the metabolic fate of Ch.

The transport of Ch is the rate-limiting step for the synthesis of PC [16]. The Ch transporters belong to the group of organic cation transporters [17,18]. There are several transporter genes encoding for the Ch transporters such as the CHT1 gene (which is mainly expressed in cholinergic neurons) [19], CTLs genes (which can be found throughout the body), and OCT1, OCT2, and OCT3 genes (which are electrogenic organic cation transporters that operate independently from Na+, Cl−, and H+ ions) [20]. In species tested so far, OCT1 was found to be mainly expressed in the liver, OCT2 in the kidney, and OCT3 expression was relatively broad (in skeletal muscles, liver, placenta, kidney, and heart). It should also be noted that Ch may also cross the plasma membrane through a diffusion-like mechanism (as found for normal and cancerous epithelial mammary cells) [21].

Overview of the main hepatic metabolic pathways for Ch

In 1932, Best and Huntsman discovered that CD results in fatty liver when insufficient Ch is provided in the diet of rodents [3]. It is now known that the daily requirements for Ch in humans are 550 mg for men and 435 mg for women, and are depending on the amount needed to prevent liver dysfunction [22]. There is also a significant variation in the daily dietary requirement for Ch that can be explained by common genetic polymorphisms [23]. Despite the fact that Ch is found in a wide variety of foods [24], the only source of Ch other than diet is from the \textit{de novo} biosynthesis of PC catalyzed by phosphatidylethanolamine-N-methyltransferase (PEMT) [5], through the methylation of phosphatidylethanolamine to PC [25].

There are two main metabolic routes for Ch in the body: the exogenous and the endogenous. In both pathways, Ch is converted into PC, while, in turn, PC can be converted back into Ch through the “phospholipase pathway” (Figure 1). It should be noted that PC is an essential phospholipid, which accounts for about 50% of all phospholipids in the mammalian membranes and, thereby, affects signaling and transport across the membranes [5]. Furthermore, PC accounts for about 95% of the total Ch pool in the mammalian tissues; the remaining 5% include Ch, phosphoryl-choline, glycerophospho-choline, cytidine-5’-diphosphocholine (CDP-choline), and ACh [8,26].

The PEMT/phospholipase reactions comprise the only known endogenous pathway for Ch biosynthesis in animals [27]. In animals, PEMT is quantitatively significant only in the liver [28] and accounts for about 30% of the hepatic PC biosynthesis in rodents; the remaining 70% of the hepatic PC is produced via the Ch pathway [29].

The biosynthesis of PC from exogenous Ch (on the cytosolic side of the endoplasmic reticulum membrane) is achieved by three enzymes (Figure 1): (a) Ch kinase [30], (b) CTP:phosphocholine cytidylyltransferase (PCT) (rate-limiting step) [31,32], and (c) CDP-choline:1,2-diacylglycerol cholinephosphotransferase [31]. Some Ch is metabolized in the gut to betaine (trimethylglycine), in addition to betaine that derives from the oxidation of Ch into the liver’s and the kidneys’ mitochondria. It then enters the one-carbon cycle and serves as a methyl donor in the re-methylation of homocysteine to Met, to ultimately generate the methylation agent S-adenosylmethionine [33].

It is well established that PC biosynthesis is required for the normal secretion of very-low density lipoprotein (VLDL) by the hepatocytes. In an \textit{in vitro} study, the elimination of Ch and Met (two precursors of PC synthesis) from hepatocytes reduced VLDL secretion [34]. If, however, Ch itself was the only precursor to be removed, VLDL secretion was not impaired due to the fact that PC synthesis was not reduced [35]. It seemed as if the requirement for Ch was specific, while Ch could not be replaced by dimethylethanolamine, monomethylethanolamine, or ethanolamine [36].

In rodents, CD markedly reduces plasma levels of apolipoprotein B, a major component of VLDL [37]. The Ch pathway for PC biosynthesis was shown to be required for normal VLDL secretion in mice that lacked hepatocellular PCT [38]. Moreover, it has been observed that in PCT-deficient hepatocytes, there is an almost twofold increase in PEMT activity [39].

It is known that PC, as a primary cell membrane lipid and a precursor of many signaling molecules, can be converted to phosphatidylserine and sphingomyelin or can be degraded by phospholipases. In the liver, the major fates of PC are: (a) secretion into the bile, (b) secretion into VLDL, and (c) utilization for the formation of high-density lipoproteins in the plasma.
Approximately 95% of the biliary PC is reabsorbed by the intestine, but only about 40% of this PC is returned into the liver [40,41]. Thus, for each 100 molecules of PC secreted into the bile, only 40 molecules of PC return to the liver, 5 are excreted, and 55 are utilized by other tissues. Therefore, any changes in the level of PC (such as CD and its sequels) will affect both the cell membrane integrity and the optimal cellular function.

Metabolic implications due to CD

The body levels of Ch and its metabolites are balanced by two “Ch-acquisition pathways”: (a) the dietary intake of Ch and (b) the PEMT pathway, as well as by two “Ch-depletion pathways”: (a) Ch oxidation and (b) the biliary PC secretion (Figure 1). If either the acquisition of Ch or its disposal is perturbed, Ch imbalance occurs [42,43].

Healthy individuals meet or exceed the adequate Ch-intake levels [44]; however, certain population groups (including pregnant and lactating women, infants, cirrhosis patients, as well as patients depending on parenteral nutrition) [45] are at risk of Ch deficiency. Precipitating factors, such as dietary fat content and fat origin (animal or plant) as well as other factors, affect the sensitivity and severity of Ch deficiency [46], while susceptibility of animals to CD declines rapidly with age [47].

Dietary fat composition is a major contributor to the development of fatty liver during a Ch-deficiency state. Specifically, the fat composition of Ch-deficient diets (CDDs) is often augmented up to 20% of the diet, whereas standard laboratory chows contain about 5% fat. Moreover, as already stated above, variations in the fatty liver development are observed depending on whether the fat is derived from animal or plant origins [47,48].

In humans, when excess Met and folate are not available in the diet, it is certainly clear that inadequate Ch intake can result into fatty liver development [49] and liver damage [10]. Since normal diets contain

![Figure 1. Synoptic representation of the major choline (Ch) metabolic pathways, including the endogenous and the exogenous pathways of Ch synthesis, methylation, and their important relation to fat metabolism. The major involved enzymes are highlighted in red color. ADP = adenosine diphosphate; ATP = adenosine triphosphate; CDP-choline = cytidine 5'-diphosphocholine; CK = choline kinase; CPT = CDP-choline:1,2-diacylglycerol cholinephosphotransferase; CMP = cytidine monophosphate; CTP = cytidine triphosphate; DAG = diacylglycerol; Dgat = DAG-O-acyltransferase; PC = phosphatidylyceroline; PCT = CTP:phosphocholine cytidylyltransferase; PE = phosphatidylethanolamine; PEMT = phosphatidylethanolamine-N-methyltransferase; SAH = S-adenosyl-L-Methionine; SAM = S-adenosylmethionine; TG = triacylglycerol; Vit. B_{12} = vitamin B_{12}; VLDL = very-low density lipoproteins.](image-url)
sufficient Ch concentrations [8,50,51], insufficiency of Ch is considered to be rare in humans and is manifested only during pregnancy, lactation, or starvation/fasting; however, the non-alcoholic steatohepatitis (NASH) that develops in patients receiving total parenteral nutrition has been attributed to CD [52].

Non-alcoholic fatty liver diseases (NAFLDs) and the Ch-deficient state are giving the most similar picture, while insulin resistance is considered as a key factor in the development of both NAFLD and NASH. The resistance to insulin-induced inhibition of lipolysis in the adipose tissue, as well as the continuous stimulation of lipoprotein lipase-mediated hydrolysis of fats, ultimately lead to an increased free fatty-acid (FFA) flux into the liver. Since glucose uptake in the liver is insulin-independent, increased glucose concentrations in the blood lead to the shunting of glucose from the liver [53].

In healthy individuals, elevated lipid concentrations in the liver lead to increased VLDL production and secretion, whereas the impairments in lipid export via VLDL secretion, β-oxidation of FFA, or other metabolic pathways observed in patients with NAFLD result in an inability to maintain fat balance and prevent fatty liver. The current “two-hit hypothesis” of NASH onset states that the lipid accumulation in the liver constitutes the first hit [54], whereas oxidative stress from mitochondrial reactive oxygen species (ROS) with lipid peroxidation would produce the second hit (thus, ultimately leading to the secretion of pro-inflammatory cytokines and hepatic stellate cell (HSC) activation). Other mechanisms may also be implicated and include inflammation, while the increased secretion of tumor necrosis factor-alpha (TNF-α) and other pro-inflammatory cytokines may also be originating from adipocytes and infiltrating macrophages (as suggested by the resulting systemic inflammation as well as by the obesity-associated insulin resistance) [53].

Gut microbes in insulin-resistant mice were found to catalobize Ch into methylamines that led to the development of Ch deficiency and NAFLD [55]. These observations support the view that Ch absorption is important in maintaining normal Ch homeostasis. Moreover, CDP-choline (an intermediate in PC biosynthesis) is thought to protect cell membranes from damage by stimulating PC production [56–58]. Deletion of the PEMT gene in Pemt–/– mice caused low levels of PC in the liver, but these animals exhibited no obvious phenotype; only when fed a with a CDD did they rapidly develop steatohepatitis and a drastic reduction in the liver PC content combined with extremely low concentrations of triacylglycerol (TG) and cholesterol in plasma [59].

Chronic alcohol consumption also impairs PC formation via the PEMT pathway, with a reduction of VLDL secretion in the steatotic liver of rats [60]. Moreover, in knockout mouse models lacking liver PCT (which is the enzyme responsible on the rate-limiting step in PC synthesis), hepatic PC levels are found decreased and triglycerides accumulated; a fact that is associated at the same time with a reduction of the plasma lipids as well as of the plasma lipoprotein content [38].

Aspects of the hepatic metabolic response to CD and possible compensatory mechanisms

The liver is distinguished from most other tissues in that it has an active alternate pathway for Ch synthesis (the so-called “endogenous pathway” or “PEMT pathway”) [61]. It is now becoming apparent that compensatory mechanisms exist to restore Ch balance in the Ch-deficiency state. For example, the expression of PEMT is enhanced by ~2-fold in the livers of rats fed with a CDD [42,43], while organ dysfunction as a consequence of CD is less common in premenopausal women (where the up-regulation of endogenous PC synthesis by estrogens is the most probable protective factor) [62]. In regards to the later example, it should also be noted that the incidence of NAFLD is lower in premenopausal women than in men or postmenopausal women, which could as well be related to a protective effect from the estrogen-induced expression of PEMT [63].

Unlike the storage of the fatty acids into TG and glucose into glycogen, there is no significant storage pool of Ch in mammalian cells. PC might be considered to be a long-term storage form of Ch, but this was not the case under the conditions of complete CD achieved in Pemt–/– mice fed with CDD; mobilization of Ch from extra-hepatic tissues appears to be an acute response to severe CD, attempting a Ch redistribution. Moreover, Ch imbalance can also be reversed by modifications of the hepatic Ch-related metabolism. The liver (being probably the most active organ with regards to Ch metabolism) deals with the stress of complete CD via the enhancement of Ch recycling [64,65]. Other important donors of Ch have been identified in the kidneys, the lungs, and the intestine.

The specific hepatic pathological sequel under CD

The liver pathology due to CD is composed of different pathological aspects, ranging from fatty changes, inflammatory and oxidative damage, as well as cytokines’ release to more complicated and
irreversible changes (such as progressive fibrosis, cirrhosis, and cancer formation).

Steatosis

Fatty liver (hepatosteatosis) is common in humans and animal models of Ch deficiency [49,50]. It is also one of the features of liver diseases such as NAFLD, NASH, and other nutritional and metabolic defects, as well as the histopathological hallmark of Ch deficiency in rodents. During CD, extremely large amounts of lipids (mainly TG) can accumulate in the liver in the form of micro- or macro-vesicular steatosis (Figure 2), eventually filling up the entire hepatocyte; the accumulation of TG within hepatocytes begins within hours after rats are started on a CDD, and then diminishes as liver gradually becomes fibrotic [46]. Since TG must be packaged as VLDL in order to be exported from the liver, PC is an essential component of VLDL, while other phospholipids cannot substitute [34,36,37]; one should remind that hepatocytes, isolated from Ch-deficient rats, were unable to export VLDL until Ch or Met was available [34].

There are many factors contributing to this pathology: increased fatty acid availability, increased fatty acid synthesis, and conversion into TGs (within the liver) as well as decreased export of TGs (from the liver) in the form of lipoproteins. It is now known that both methionine–choline-deficient diet (MCDD) and CDD reliably induce a fatty liver in animals, but through different mechanisms (Table I)3. The prementioned steatotic pathology was significant in animals fed with MCDD [66], but in those fed with CDD, Ch could be biosynthesized via Met. Therefore, CDD alone did not impair the hepatic VLDL excretion [67], due to a compensatory activation of the methylation pathway in order to maintain adequate PC synthesis and VLDL excretion [35]. Moreover, both PEMT expression and activity were also increased [35,43,68], while the enzymes involved in FFA esterification to TG presented without changes in the expression of those involved in the de novo lipogenesis or fatty acid oxidation and without changes in the major transcription factors that regulate these processes. Therefore unlike MCDD, CDD increases liver fat content without affecting the body weight or peripheral fat pad weight in mice [69].

On the other way, CDD-fed rats may develop steatotic features similar to the human metabolic syndrome characterized by insulin resistance, dyslipidemia, and obesity [70]. As already mentioned, insulin resistance is probably due to a combination of factors determining hepatocyte sensitivity to insulin such as increased TNF-α and hypertriglyceridemia. The increased TNF-α reduces the expression of GLUT-4 (an insulin-dependent glucose transporter) and decreases the phosphorylation of insulin-receptor substrate-1 (IRS-1) [71]. Moreover, hypertriglyceridemia potentially contributes to the observed insulin resistance, as TGs directly inhibit peripheral glucose uptake via IRS-1 down-regulation [72]. In contrast, MCDD-fed rats presented no insulin resistance, even though they did present extensive lipid peroxidation and fibrinogenesis.

3The ingredients of CDD used in our experiments are [13]: casein 10%, α-protein 10%, sucrose 51%, alphacel 5%, lard 20%, manganese sulfate H2O 150 mg kg-1, zinc chloride 20 mg kg-1, chromium potassium sulfate 12H2O 35 mg kg-1, sodium selenite 1 mg kg-1, and salt mixture Wesson 4% (calcium carbonate 21%, copper sulfate 5H2O 0.0039%, ferric phosphate 1.470%, manganese sulfate H2O 0.02%, magnesium sulfate 7H2O 9%, potassium aluminum sulfate 0.009%, potassium chloride 12%, potassium dihydrogen phosphate 31%, potassium iodide 0.005%, sodium chloride 10.5%, sodium fluoride 0.057%, tricalcium phosphate 14.9%, plus ICN vitamin diet fortification mixture except Ch chloride). An experimentally suitable matched MCDD would be an isocaloric diet that would also be deprived of Met. Readers should bear in mind that diets differ from reference to reference, depending upon the manufacturers’ formulas.
Raubenheimer et al. [69] suggested that the hepatic insulin-sensitizing effects of a CDD-induced CD may result from the shunting of FFA into metabolically innocuous TG stores due to the stimulatory effects of CDD on the enzymes involved in FFA esterification to TG.

In relation to the link between the fat metabolic pathway and the Ch pathway (Figure 1), 1,2-sn-diacylglycerol (1,2-DAG) is one of the lipid metabolites that was found to be overexpressed under both CDD and MCDD. Due to its role in the interrelation of the PC- and TG-synthesis pathways (since 1,2-DAG is an important intermediate for the biosynthesis of TG and membrane phospholipids, see Figure 1), Ch-containing phospholipids are one of the important sources for 1,2-DAG release during transmembrane signaling [74]. Furthermore, enzymes for both pathways are subject to common transcriptional regulation, such as the lipogenic transcriptional factor (sterol regulatory element-binding protein) [75–78]. It might be that specific activation of DAG synthesis is a compensatory mechanism itself, acting together with the up-regulation of PCT expression in order to preserve PC synthesis during dietary-induced CD. Therefore and due to CD, one pathway could lead to accelerated and increased intrahepatic TG stores, but in the presence of lower intracellular FFA levels [69].

In obesity, increased hepatocellular FFA concentrations result from increased de novo lipogenesis, decreased β-oxidation, and increased FFAs’ influx from the diet and the adipose tissue. Enhancing the removal of FFAs by their esterification into TGs might reduce their negative impact on insulin-sensitivity. Listenberger et al. [79] have reported that accumulation of excess fatty acids in cellular TG in CHO cells protects against lipotoxicity, whereas impaired synthesis of TG in cells from Dgat1-null mice leads to lipotoxicity. Moreover, the fatty liver of a CDD-fed animal, unlike that of an MCDD-fed one, does not lead to marked hepatitis or cirrhosis but does lead to the development of hepatocellular carcinoma [80,81].

<table>
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<tr>
<th>Parameters</th>
<th>MCDD-induced liver damage</th>
<th>CDD-induced liver damage</th>
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<tr>
<td>Fatty liver state</td>
<td>Severe steatosis</td>
<td>Mild steatosis</td>
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<td>Synthesis of PC</td>
<td>Impairment</td>
<td>Slightly impaired</td>
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<td>Serum levels of VLDL</td>
<td>Reduced</td>
<td>Not affected</td>
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<td>Weight</td>
<td>Significant weight loss</td>
<td>Not affected</td>
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<td>Peripheral fat</td>
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<td>Not affected</td>
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<td>Hepatic TG</td>
<td>Increased</td>
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<td>Inflammation</td>
<td>Severe inflammatory response</td>
<td>Weak to mild response</td>
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<td>Oxidative reaction and lipid peroxidation</td>
<td>Severe reaction and damage</td>
<td>Mild reaction</td>
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<td>Steatohepatitis</td>
<td>Significant</td>
<td>Slightly significant</td>
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<td>Enzymatic activity of PCT</td>
<td>Stimulated</td>
<td>Stimulated</td>
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<td>Activity of the FFA esterification enzyme to TG</td>
<td>Stimulated</td>
<td>Selectively stimulated</td>
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<td>Insulin resistance</td>
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<td>More similar to metabolic syndrome</td>
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<td>Enzymatic activity of PEMT</td>
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<td>DAG intermediate</td>
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<td>Enzymatic activity of PKC</td>
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<td>Apoptosis</td>
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<td>Liver tissue damage</td>
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<td>Initiated and promoted</td>
<td>Initiated and promoted</td>
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<td>Fibrosis and cirrhosis</td>
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<td>Protective effect against lipotoxicity</td>
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Abbreviations: MCDD = methionine-choline-deficient diet; CDD = choline-deficient diet; PC = phosphatidylcholine; VLDL = very-low density lipoproteins; TG = triacylglycerol; PCT = CTP:phosphocholine cytidylyltransferase; FFA = free fatty acid; PEMT = phosphotidylethanolamine-N-methyltransferase; DAG = diacylglycerol; PKC = protein kinase C.

**Inflammatory and oxidative reactions**

The association of CD with leaky mitochondria, leakage of free radicals, and DNA damage is well established [82,83]. A possible mechanism of both the CDD-induced steatohepatitis in rats and of the MCDD-induced steatohepatitis in mice is through the generation of oxygen-free radicals and oxidative...
stress, a mechanism similar to that proposed in alcoholic steatohepatitis (ASH) and NASH of humans [84,85]. This is the reason why CD can induce changes in the liver that are reproducible, rapid, and similar to those observed in human NASH and ASH, which in turn are characterized by both steatosis and necro-inflammation [86], leading to: (a) liver DNA and parenchymal damage and, ultimately, (b) hepatic dysfunction (that can be evaluated by leakage of liver enzymes into the serum, cell membrane defects, pro-inflammatory reaction responses, cytokines' release, apoptosis, necrosis, and accompanying oxidative reactions) [23,87,88].

Generally, hepatic fatty change, irrespectively of the cause, leads to lipid peroxidation [89], to a large extent due to the induction of microsomal lipoygenases (which serve as a source of ROS) [90]. End products of lipid peroxidation cause the activation of pro-inflammatory cytokines' release and of HSCs, which lead to necro-inflammatory changes and the development of fibrosis [91]. Therefore, the clinically relevant pathological features such as increased oxidative stress and the Kupffer cell-mediated inflammatory response contributing to the development of steatohepatitis, as attributed to the feeding of MCDD, are not necessarily similarly induced by the CDD [86].

In the MCDD-fed rats, increased oxidative stress and decreased antioxidant response were observed in the presence of steatohepatitis (activation of HSCs and collagen deposition), but these features were significantly less prominent in the CDD-fed rats. Both oxidative stress and lipid peroxidation are generally considered as the most pathogenic mechanisms of NASH [92]. For the prevention of oxidative stress, a balance between ROS production and antioxidant response is essential. The important sources for oxidative stress are intracellular fatty acids via direct toxicity or via the induction of cytochrome P-450 microsomal lipoygenase 2E1 (CYP2E1) and peroximal β-oxydation [93,94]. CYP2E1 has been found significantly increased in patients with ASH [95–97] and NASH [98], as well as in experimental models of steatohepatitis induced by MCDD [84]. More specifically, CYP2E1 and β-oxydation generate free radicals from endogenous metabolites and dietary N-nitrosamines, causing lipid peroxidation and promoting cell membrane damage. The aldehyde end products of lipid peroxidation, malondialdehyde (MDA) and 4-hydroxyenonenal (HNE), are important pro-inflammatory mediators (Figure 3); MDA causes further structural changes in the mitochondrial matrix, leading to mitochondrial dysfunction [99,100]. Mitochondrial dysfunction further activates the pro-inflammatory cytokine release from Kupffer and HSCs, leading to necro-inflammatory changes in the hepatic parenchyma [99,101]. Other studies focusing on the CYP2E1-induction have reported the possible stimulatory (or modulatory) effects of CD on the metabolism and toxicity of certain chemical compounds in the fatty liver; most studies indicate ASH as a potent inhibitor of the hepatotoxicity induced by carbon tetrachloride (CCl4) [102], or by phenobarbital and acetalaminophen [13]. Indeed, in the MCDD-fed rats, the observed increased lipid peroxidation was accompanied by marked hepatocellular damage and increased hepatic TNF-α levels, while in the CDD-fed rats, the antioxidant scavenging of ROS was apparently sufficient, since less lipid peroxidation and hepatocellular damage were observed [103]. However, although decreased antioxidant content and increased lipid peroxidation are earlier biochemical alterations that precede and lead to histological cell death by necrosis in CD, it is also clear that the process of lipid peroxidation is preceded by a decrease in hydrophilic and hydrophobic antioxidants [104].

Fibrosis

Hepatic fibrosis is a wound-healing response or “scarring” of the liver, due to chronic liver injury. It is a multistep process involving inflammation and fibrogenesis, resulting from the chronic effect of noxious elements of different nature (viral infection, alcohol consumption, ischemia, toxic effects of drugs, and natural substances) [105,106], accompanied by the release of cytokines and other soluble mediators (Figure 3). Fibrosis can develop into cirrhosis (representing the end stage of many different liver disorders, such as alcoholism and chronic hepatitis). Liver cirrhosis is the final stage of the disruption of the normal liver architecture that follows liver fibrosis, leading to subsequent liver dysfunction and portal hypertension.

The mechanisms that participate in the induction of the fibrotic process are fairly constant, including necrosis, apoptosis [107], inflammatory reactions [108], and the activation of HSCs [109]. Inflammatory cells in the liver move into the injured areas (in addition to the damaged and regenerating hepatocytes), and release inflammatory cytokines that “activate” the HSCs. The injured liver’s HSCs are the key cells in fibrosis, since they constitute the major source of the extracellular matrix proteins expressed in the fibrotic liver [110–112]. HSCs are usually quiescent, with a low proliferation rate [113]. Transdifferentiation of HSCs from a quiescent state into myofibroblast-like cells (usually with high proliferative capacity) enables the appearance of smooth muscle-α-actin and leads to the loss of their cellular vitamin A storage [110]. Activated HSCs secrete fibrillar collagens (thus
producing an increase in extracellular matrix in the space of Disse) and become sensitive to the powerful vasoconstrictor endothelin. Their accumulation will lead to the impairment of the hepatic function.

The earliest changes observed during HSC activation affect sinusoidal endothelium, hepatocytes, and platelets. Hepatocytes are a potent source of fibrogenic ROS, generated by membrane injury and lipid peroxidation [114]. Hepatocyte apoptosis following injury also promotes HSC initiation through a process mediated in part by Fas; this process may involve the TNF-related apoptosis-inducing ligand (TRAIL) [107,115]. Kupffer cell infiltration and activation also contribute to HSC activation [116]. Kupffer cells stimulate matrix synthesis, cell proliferation, and release of retinoids by HSCs through the actions of cytokines (especially of transforming growth factor-1 (TGFβ-1)) and of ROS [117].

Endothelial cells are also likely to participate in the conversion of TGFβ from the latent to active profibrogenic form, while they also produce a cellular isoform of fibronectin that provokes early HSC activation [118]. However, platelets provide paracrine stimulation through the platelet-derived growth factor (PDGF), the TGFβ-1, and the endothelial growth factor [119].

Carcinogenesis

Hepatocellular turnover is greatly increased during Ch deficiency [48]. Dietary-induced CD is hepatocarcinogenic in rodents and promotes liver tumor formation following initiation by a chemical carcinogen; in fact, it causes spontaneous carcinoma of the liver and generally increases sensitivity to carcinogens [8]. It is the only type of nutritional deficiency known to have such a deleterious effect (Figure 3) [120]. In contrast, dietary supplementation of Ch, with or without Met, reduces liver tumor incidence in carcinogen-treated mice [121,122].

The mechanisms by which CD is thought to be carcinogenic include liver cell damage, regeneration...
shown that Ch-deficiency affects the expression of 1,000 genes in neural precursor cells, with one-third of these genes being involved in cell proliferation, differentiation, methyl-group metabolism, and apoptosis [134], thus demonstrating the essentiality of Ch for proper cell function.

Conclusion

The hepatic pathogenesis due to CD is a combination of different pathological pathways’ crosstalk. Different degrees of CD produce a variety of reversible and irreversible phenomena in liver pathology that could provide a basis for the development of non-liver-associated diseases (including diabetes, cardiovascular disorders, etc.). The deeper understanding of the pathophysiology linked to the hepatic injury caused by Ch deficiency could provide important tools for the understanding, the prevention, and/or the treatment of many metabolic and non-metabolic disorders associated with different extents of CD.

Acknowledgements

This work was supported by the State Scholarships Foundation of the Hellenic Republic (in terms of a scholarship to Dr. Hussam Al-Humadi), as well as by the National and Kapodistrian University of Athens.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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